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EFFECT OF OXIDATION-REDUCTION POTENTIAL ON HEMOCHROME
FORMATION AND RESULTANT PINK COLOR DEFECT
OF COOKED TURKEY ROLLS

by

Farzaneh Vahabzadeh

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

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ABSTRACT

Effect of Oxidation-Reduction Potential on Hemochrome
Formation and Resultant Pink Color Defect
of Cooked Turkey Rolls

by

Farzaneh Vahabzadeh, Doctor of Philosophy

Utah State University, 1986

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Department: Nutrition and Food Sciences

A pink color defect is commonly observed in freshly cut surfaces of cooked turkey rolls and fades rapidly upon exposure to air. The non uniform pink color makes the product appear undercooked, and the product must be discounted. The oxidation-reduction potential of the meat is important in development of pink defect. A pink color similar to that of commercial product was observed when the cooked meat was treated with either sodium nitrite or sodium dithionite. The pink color in nitrite treated meat was due to nitroso pigment formation, but in samples treated with dithionite the pink color was due to formation of a hemochrome complex. Pink color was also observed in turkey rolls formulated with nicotinic acid, nicotinamide or sodium nitrite. Reflectance and absorbance spectrophotometric studies on commercial or laboratory prepared samples having pink defect showed that the responsible pigment was a reduced hemochrome rather than a nitroso pigment. The hemochrome is probably a nicotinamide-denatured globin complex with ferrous iron of the heme molecule. Oxidation-reduction potential measurement of meat systems showed that hemochrome formation is promoted by reducing conditions and prevented

by oxidizing conditions. All constituents necessary for formation of pink defect are present in turkey meat, the variable most affecting its appearance being the redox potential of the meat.

(80 pages)

INTRODUCTION

Pink color defect in cooked turkey rolls is a serious economic problem in the turkey processing industry, because consumers assume the product has not been properly processed. The pink color is sometimes seen in basted or chunked and formed rolls formulated with phosphates and salt. The pink color fades rapidly upon exposure of the sliced meat to air (Anon., 1984a). A persistent pink color is also occasionally observed in cooked beef products, such as thin sliced steak for sandwiches (Anon., 1984a).

The most widely accepted cause for pink defect is nitrite or contamination of the meat, leading to formation of the pink nitrosyl or carboxyl hemochromes. Sources of nitrite include (a) accidental nitrite or nitrate contamination from equipment, water supplies, ingredients, or packaging, (Froning, 1983); (b) leaking of gases such as carbon dioxide, carbon monoxide (CO), or nitric oxide (NO) from compressors, freezers, (Anon., 1984b) or gas-fired grills, (Pool, 1956); (c) green leafy vegetables such as onions or green peppers, when added to items such as meat loaf.

Oxymyoglobin is another pink pigment that has been suggested to cause pink defect in cooked meat products. Schmidt and Trout (1984) have clearly shown that high pH beef remains much more red after processing. The same was true but to a lesser extent for pork and turkey. High cooking temperature also reduced intensity of pink color development. They postulated that at high pH and lower cooking temperature the muscle pigments were less denatured, and thus remain pink after cooking. On the other hand, Howe et al. (1982) studied

development of pink color in cooked pork and concluded that the color problem could not be due to the presence of undenatured oxymyoglobin.

Reduced hemochromes are a third class of pink pigments that may cause pink defect in cooked meats. Brown and Tappel (1957) described a pink pigment in canned tuna with similar characteristics to cooked turkey meat with pink color defect. Brown and Tappel (1957) concluded that the pink pigment of canned tuna was either (1) a nicotinamide hemochrome, (2) a denatured-globin hemochrome, or more likely (3) a mixed nicotinamide denatured-globin hemochrome. A hemochrome is a substance formed by reaction between heme or hematin and nitrogenous compound(s) (Lemberg and Legge, 1949). There are several studies indicating use of nitrogenous substances such as nicotinamide or nicotinic acid either for preserving the color of fresh meat (Hopkins and Sato, 1971), or as a substitute for sodium nitrite in formation of cured pink color (Kemp, 1974).

There have been previous studies regarding pink color defect in cooked turkey meat, (Froning, 1983) but without characterization of the nature of the pigment responsible for this color problem. Therefore, the purpose of this study is to characterize the pigment responsible for pink color defect in cooked turkey rolls and specifically to determine if it is a nitroso pigment or not. Furthermore, factors promoting formation of pink defect will be determined.

REVIEW OF LITERATURE

Pink Color Defect of Turkey Meat

The great increase in use of turkey meat in many products has led the poultry industry to search for an answer for the problem of pink color defect which occasionally is seen in products such as cooked breast of turkey. The incidence of pink color defect is variable but has been characterized as a multimillion dollar problem for turkey processors. Work on the nature of the pink color defect in turkey meat has been reported as early as 1956, and through the years many factors have been studied.

Effect of Preslaughter Conditions

Preslaughter stress and struggle may have an important effect on turkey meat color and its textural characteristics (Froning et al., 1978). Effect of preslaughter environmental temperature in the presence of electrolytes on the quality of turkey meat has been studied by Babji et al. (1982a). The electrolytes at a recommended level were given to all the birds for three days through the drinking water. Administration of electrolytes (0.1% Dawelyte) did not prevent changes in muscle characteristics after birds were exposed to preslaughter temperature stress (Dawelyte is a mixture of several salts such as potassium chloride, and sodium chloride). Birds kept at 38°C for four hr. prior to slaughter had meat with a lower pH, water holding capacity, cooking yield, and a higher shear value. The color of the meat from heat stressed birds was pale to whitish as compared with that either from cold stressed or control birds. The

concentration of total pigment (myoglobin plus hemoglobin) was lower in the heat stressed muscles. Babji et al. (1982a) suggested that the rapid rate of postmortem glycolysis at a high temperature resulted in a fast decrease in pH and color intensity with possible denaturation of myoglobin. This may be the reason for the decrease in total pigment content in heat stressed birds (Babji et al., 1982a).

Muscle from turkeys struggling during slaughter was found to be abnormally red and had high myoglobin content (Ngoka et al., 1982). Froning et al. (1978) found that heat stressing turkeys prior to slaughter at 42°C for one hr. resulted in meat with darker and redder color. This was in conflict with the results reported by Babji et al. (1982a). The time of exposure to heat in these two experiments was different (one hr in the study done by Froning et al. (1978) and four hr in the study done by Babji et al. (1982a)). The pH of the muscle was 6.25 reported by Froning et al. (1978) vs a pH of 5.99 reported by Babji et al. (1982a). The time and temperature of heat stress during the preslaughter period was then suggested as important factors in the study of turkey meat color (Babji et al., 1982a). A cool preslaughter environment appears to produce turkey meat with better carcass quality characteristics (Babji et al., 1982b).

Color changes produced in turkey muscle by excitement and free struggle before and during slaughter were studied by Ngoka and Froning (1982). The muscle from birds which were excited before slaughter and allowed to struggle freely during slaughter was darker in color and had higher level of cytochrome c as opposed to that in the muscle obtained from anesthetized birds. It was then suggested that

cytochrome c may be at least partially responsible for the dark colored muscle as seen in birds allowed free struggle. This heat stable pigment may contribute to pink color problems observed in further processed turkey products. In fact Cornish and Froning (1974) have indicated the importance of both myoglobin and cytochrome c proteins in evaluating color of turkey meat in respect to pink color problems. The effect of age, sex, and strain on the myoglobin concentration of turkey meat and therefore on its color has been studied by Froning et al. (1968a). It was found that with the exception of white meat of female turkeys, myoglobin content in the meat increased significantly with advancing age. Significantly higher concentrations of myoglobin were also found in male than in female birds. Meat from male turkey had a positive correlation between a_L (redness) values and myoglobin content. Pinkness as measured by increasing a_L values is said to be of a greater problem in meat from older birds (Froning et al., 1968a). In contrast, Pool (1956) found that the occurrence of pinkness was more frequent in the small and young birds exposed to CO and NO gas during roasting in a gas-fired grill. Pool (1956) pointed out the skin layer protects the birds from getting pink during cooking. Young birds have thinner skin which is more permeable to NO and CO gasses during cooking.

The effect of exposure to engine exhaust fumes prior to slaughter on color of poultry meat was also studied (Froning et al., 1969a). Birds may become exposed to gasses such as NO or CO from the truck during transfer to slaughter. With the exception of uncooked white chicken, a_L values were increased significantly in all

treatments. The same increase was also noted for visual scores.

The effect of dietary nitrates and nitrites on color of poultry meat was studied by Froning et al. (1967). Uncooked white meat from nitrate-fed chickens was found to have both significantly higher a_L values and visual scores as compared with that from control birds (Froning et al., 1967). The meat from nitrite-fed chickens seemed to have an upward trend of a_L values but the differences were not significant. Visual scores and a_L values were increased significantly for both uncooked and cooked meat obtained from turkeys having either nitrate or nitrite in their diets (Froning et al., 1969b). However, Mugler et al. (1970) reported that various levels of nitrate-nitrogen (75,150,300, and 450 ppm) in the drinking water did not significantly affect the color of turkey meat. Meat from older birds was found to be significantly redder than meat from younger birds. Presence of at least 200 ppm nitrate-nitrogen in chill water was necessary to make turkey meat red. Moreover, grinding cooked turkey meat also significantly reduced redness (Mugler et al., 1970).

Effect of Processing Conditions

Pool (1956) reported that factors such as scalding treatment, and freezing and thawing had little or no effect on development of the pink color defect. However, Baker (1954) found that freezing rate plays an important role in discoloration of poultry carcasses. With each group of poultry carcasses frozen at different temperature, the semi-scalded and air-cooled carcasses had the lightest color while those which were sub-scalded and water-cooled were the darkest. Birds frozen at -20°F in a blast air were found to be light in color and a

gradual darkening of the flesh was noticeable as the freezing rate became slower (Baker, 1954). Berg and Lentz (1958) studied the effects of air velocity, temperature, and boxing on freezing rates and appearance of air blast frozen poultry. Lightness and uniformity of appearance of the poultry was found to be dependent on freezing rate and increased greatly when the velocity of air at -20°F was raised from 0 to 700 feet per minute (ft/min) or air temperature was reduced from $+10^{\circ}\text{F}$ to -20°F at a velocity of 500 ft/min. Bird size had no significant effect on appearance or freezing time of the skin, although it had effect on total freezing time. Moreover, boxing birds before freezing resulted in a dark and non-uniform appearance. Increasing ventilation area in the boxes by increasing spacing between birds was important in obtaining light and uniform skin color (Berg and Lentz, 1958).

The effect of cooking temperature and storage period after cooking on the color of turkey meat has been studied by Helmke and Froning (1971). Lower redness and higher lightness values were associated with raising cooking temperature from 60°F to 82°F . Occurrence of a regenerated pink color within 2 hr. storage at refrigeration temperature was also reported. The absorption spectra of extracted pigment from turkey meat cooked to $60^{\circ} - 77^{\circ}\text{C}$ was similar with that normally reported for oxymoglobin. However, the pigment extracted from the meat cooked to 82°F had quite different spectra. Helmke and Froning (1971) have pointed out the possibility that a portion of the myoglobin in turkey meat was partially denatured during cooking and this may cause the development of the pink in the meat.

during the storage period. Results of the study done by Wierbicki et al. (1957) indicates that heat denaturation of muscle proteins begins at about 40°C and is essentially completed at 70°C. Howe et al. (1982) studied development of pink color in cooked pork. Their results from absorption spectra indicated that most of the oxymyoglobin was denatured by 60°C. Storage and time of storage had no significant effect on absorbance values, indicating that the development of pink color during storage was not a result of oxymyoglobin or undenatured myoglobin.

Additives have been found to affect color of poultry meat. Egg white solids can be used as a binder in preparation of turkey meat products. Spray-dried albumen was found to increase significantly the redness values of the cooked ground turkey meat, but pan dried albumen did not affect these values (Froning et al., 1968b). The pinkness in the meat caused by the spray dried albumen was decreased significantly by allowing the meat to stand 30 minutes in the air. More stable pink color in the meat was produced when the level of the spray dried albumen increased to 10%. This pinkness was present in the samples kept at refrigeration temperature for four or five days. It was suggested that the observed pinkness was possibly caused by either a change in pH and/or formation of an iron-conalbumin complex. The pH of cooked turkey meat did not change greatly upon addition of spray-dried albumen. The pH values for the cooked meat with 0, 2.5, 5 and 10% added spray-dried albumen, with pH range of 7.0 to 8.0, were 6.10, 6.18, 6.18 and 6.23 respectively. The pH range of the pan-dried albumen was 5.0-5.5 due to addition of lactic and citric acids. The

pH of the meat for 0, 2.5, 5.0 and 10% of the added pan-dried albumen was 6.10, 6.08, 5.98 and 5.96 (Froning et al., 1968b).

Schmidt and Trout (1984) have studied the effect of pH on the color of the cooked meat. Beef, pork, and turkey meats were used and the pH of the meat was adjusted to 5.5, 6.0 or 6.5 before cooking. Three different cooking temperatures (63°, 68°, and 74°C) were tested. The results indicated that meat with higher pH and at lower cooking temperature had more pink-red color after cooking. It was suggested that a high pH prevented the formation of a brown cooked meat color. Beef with the highest myoglobin concentration was more red.

There are food additives such as alkaline phosphates which can influence pH of meat. Uncooked chicken meat with 6% added commercial sodium polyphosphates had pH 6.6 while the control sample had pH 6.1 (Froning, 1965). Moreover, the sample had a more bluish white glistening appearance. The color of the cooked chicken meat tended to change much less than that observed in the uncooked sample (Froning, 1965). In a study by Klose et al. (1963), addition of a mixture of two types of polyphosphates to the chilling water had no influence on the color of the cooked chicken but caused a color change in the chilled carcasses (bluish white appearance). Froning (1966) found that using 0.5 or 1% commercial polyphosphates in chicken meat products would seem to be advisable considering color, flavor, and texture properties. The pH of the cooked meat was 6.2, 6.4, 6.5, and 6.7 with 0, 0.5, 1, and 2% added commercial polyphosphates (Froning, 1966).

Chemistry of the Iron Center in Myoglobin
with Respect to Meat Color

As previously pointed out in the introduction, there are three major classes of pink or red pigments; nitrosyl pigments, undenatured oxymyoglobin, and reduced hemochromes. Most of the nitrosyl-related work on pink defect in cooked meats has focused on factors affecting pink color without attempting to identify the nature of the pink pigment. It is important to know the nature of the pink pigment in order to prevent its formation. For instance high cooking temperature will denature oxymyoglobin and destroy pink color, but high cooking temperature enhances formation of denatured globin hemochromes. Thus it is important to know the chemistry of the various pink pigments. These pigments can be distinguished by their different visible reflectance and absorption spectra. Heme iron oxidation state and nature of the ligand (nicotinamide, O_2 , NO, Co, etc.) affect spectral characteristics. In order to identify the pigment responsible for pink color, it is necessary to understand how the spectral characteristics are influenced by heme iron chemistry. Thus, the following sections briefly review heme pigment chemistry, and discuss the influence of heme iron chemistry on reflectance and absorption spectra.

Iron has an atomic number of 26, and is thus one of the nine transition metals of the first transition series in the fourth period of the Periodic Table. Iron has two electrons in the K shell, eight in the L shell, fourteen in its M shell and only two electrons in the N shell. Thus, the electronic structure of iron in the ground state

is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6 4s^2$. Iron exhibits oxidation states of +2, +3, and +6, and the oxidation state relates with incompletely filled 3d subshells. The main consideration is for iron with oxidation state of +2 and +3, the oxidized and reduced state, respectively. Loss of two electrons from elemental iron results in the electronic structure of $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6$ for ferrous iron, and ferric iron with oxidation number of +3 corresponds to loss of three electrons and the electronic structure is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^5$ (Nebergall et al., 1976).

Heme, the nonprotein unit of myoglobin and hemoglobin, consists of an iron atom and an organic part called protoporphyrin which is made up of four pyrrole groups linked by methene bridges to form a tetrapyrrole ring. Four methyl, two vinyl, and two propionate side chains are attached to the latter ring and these substituents can be arranged in fifteen different ways. Only one of these isomers, known as protoporphyrin IX, is present in biological systems (Stryer, 1975).

The iron atom in heme binds to the four nitrogen atoms of the tetrapyrrole ring. There are two additional bonds from the iron, one on either side of the heme plane. These bonding sites are known as the fifth and sixth coordination positions (Stryer, 1975).

The d orbitals, which occur in sets of five, each consist of lobe-shaped regions, and the designated lobes are d_{z^2} , $d_{x^2-y^2}$, d_{xy} , d_{xz} , and d_{zy} . For an octahedral configuration for a metal; i.e., heme iron in myoglobin, the d_{z^2} , and $d_{x^2-y^2}$ orbitals which point toward corners of the octahedral are referred to as antibonding orbitals and the remaining three orbitals, d_{xy} , d_{xz} , and d_{yz} , which point in

between the corners of the octahedron, are called nonbonding orbitals. Any electrons which occupy lobes of the antibonding orbitals will be repelled by the electron pairs belonging to ligand groups located at the corners of the octahedron. On the other hand, electrons in the nonbonding orbitals are not directly affected by the electron pairs of reacting ligands. The energy level of antibonding and nonbonding orbitals is referred to as the e_g and t_{2g} levels respectively. The electrons at the e_g level are at the higher energy level. The difference in energy between these two levels for a given complex is a measure of the energies involved in the movement of electrons from one energy level to the other (referred to as electronic transitions) (Nebergall et al., 1976).

Iron at the oxidized state has five unpaired electrons in its d orbital, and according to crystal field theory, when the iron is coordinated to a ligand producing a weak field, the five electrons remain one in each of the five d orbitals, i.e., three unpaired electrons at the t_{2g} energy level and two unpaired electrons at the e_g level. However, if the bonding ligand produces a strong electrostatic field on the iron, the five unpaired electrons of ferric iron remain at the t_{2g} energy level. The complex of ferric iron with ligands of low field strength is called a high spin compound. The complex of ferric iron with a ligand of high field strength results in a low spin complex. The same terminology exists for the iron at the reduced state. The strong field, low spin complex in this case is the one in which six electrons of the d orbitals all are at the t_{2g} energy level. Weak field, high spin complexes then are those in which four electrons

out of six electrons are at the t_{2g} energy level and the remaining two electrons at the e_g energy level (Nebergall et al., 1976). In general a complex of ferrous iron with ligands producing strong field is more stable than one with ligands forming low field (Nebergall et al., 1976). A compound with no unpaired electron(s) is diamagnetic while a paramagnetism is characteristic of a substance containing one or more unpaired electrons (Bromberg, 1984). The only diamagnetic iron complex is a strong field, low spin ferrous iron i.e. oxymyoglobin (Nebergall et al., 1976; Livingston and Brown, 1981). Complexes of ferric iron (low or strong field) and complexes of low field ferrous iron, all have unpaired electrons and therefore exhibit characteristic of paramagnetic substances (i.e. metmyoglobin, nitric oxide metmyoglobin, and deoxymyoglobin) (Brill and Williams, 1961; Nebergall et al., 1976).

With respect to bonding between the iron center and the ligand at the fifth or sixth coordination positions of heme there are two types of bonding. The sigma bond which is formed by donation of electrons from the ligand to the iron (iron acts as electron acceptor), and pi back-bonding in which the iron gives up electrons back to the ligand (iron acts as electron donor) (Williams, 1956). Ferrous iron has a lower charge on the nucleus and more electrons in its d orbitals as compared to ferric iron. This enables ferrous iron to form strong pi back-bonding with suitable ligands. However, ferric iron does not form strong pi back-bonding (Livingston and Brown, 1981). Ligands, such as oxygen and NO bind to ferrous iron of the heme with strong pi back-bonding. However, the oxygen molecule with its two unpaired

electrons cannot bind to metmyoglobin because ferric iron of the heme in this complex does not engage in a strong pi back-bonding. On the other hand, NO has one unpaired electron which participates in sufficiently strong sigma bonding with metmyoglobin to enable stable bonding (Nebergall et al., 1976; Livingston and Brown, 1981). Ligands producing a high electrostatic field by binding to the sixth position of the iron of heme can stabilize the ferrous iron form toward oxidation. This is important in the case of NO which can stabilize the ligand at the fifth position, the histidine group. During heat denaturation then the histidine ligand is replaced with a second NO ligand. The resulting di-nitrosohemochrome complex is quite stable to oxidation and ligand exchange (Tarladgis, 1962b; Livingston and Brown, 1981).

As mentioned before, there is an energy gap between e_g and t_{2g} energy levels and visible light can excite electrons over this difference in energy level. The exact wavelength absorbed and visible wavelengths which are transmitted are important for color of an iron complex, i.e. the oxymyoglobin complex having red color. The bonding pattern between oxygen and the iron (pi back-bonding) is also important in forming color in the complex (Livingston and Brown, 1981).

Heme-globin interaction influences not only the overall electronic configuration of the heme, but also its ligand binding properties. The globin-histidine imidazole at the fifth position feeds extra electron density into the iron, therefore the iron has more ability to donate electrons to the ligand at the sixth position

via pi back-bonding (Livingston and Brown, 1981). The basicity, or ability to donate electrons, of the ligand at the fifth position can determine the way that other ligands bind to the sixth position (Giddings, 1977). For example, ligands that are less effective bases than the imidazole group in the fifth position of deoxymyoglobin would raise the possibility of metmyoglobin formation instead of oxy-myoglobin formation. On the other hand, a group with a very strong basicity at the fifth position would cause oxygen binding to deoxymyoglobin to become more irreversible. This condition is undesirable from respiratory standpoint, but would increase stability of red color of fresh meat (Giddings, 1977).

Protoporphyrin IX ring characteristics are also important in binding properties of the iron center. Increasing electron-acceptor properties of the substituents in the porphyrin ring results in the iron becoming less electronegative and therefore having more tendency to participate in pi back-bonding with suitable ligands (Williams, 1956).

Myoglobin Oxidation

Oxidation is a process in which an atom or ion loses electron(s) and by this its oxidation number increases. Reduction proceeds with gain of electron(s) by an atom or ion, corresponding to a decrease in the oxidation number. Oxidation and reduction (redox) reactions always occur simultaneously (Nebergall et al., 1976). An oxidizing agent then is the one which increases the oxidation number of an element, and the opposite is true for a reducing compound.

One important reaction in myoglobin chemistry is the autoxidation reaction in which the oxymyoglobin converts to metmyoglobin. As the ferrous iron by loss of one electron becomes oxidized to the ferric iron, the oxygen molecule by gaining electrons becomes reduced (acting as an oxidizing agent) (Nebergall et al., 1976, Livingston and Brown, 1981). One-electron transfer from an iron atom to a strong field ligand (such as oxygen) is thermodynamically unfavorable and does not occur (an overall negative redox potential of $E^\circ - 0.13V$) (Castro, 1971). If reduction of the oxygen molecule were by only one electron which could be provided by the ferrous iron, the binding oxygen to myoglobin would occur only for a very short period of time. However, providing two electrons makes this autoxidation reaction rather slow (Livingston and Brown, 1981). The half-life for autoxidation of bovine myoglobin is 26.5 hr. at pH 6.5 and 22°C (Brown and Mebine, 1969). The sources for two electrons are one from the ferrous iron of oxymyoglobin and other from the deoxymyoglobin complex. That is probably why an oxygen tension which gives a half and half mixture of oxymyoglobin and deoxymyoglobin causes a rapid rate of autoxidation (Livingston and Brown, 1981). Very low or very high oxygen tension prevents the autoxidation by providing more deoxymyoglobin in the former condition and more oxymyoglobin in the latter condition (Landrock and Wallace, 1955; Livingston and Brown, 1981). Moreover, the rate of autoxidation reaction is increased at low pH values. A mechanism has been suggested by Livingston and Brown (1981), in which the oxygen molecule in oxymyoglobin becomes protonated and this protonated oxygen can no longer be considered to be a strong ligand.

Therefore one-electron transfer from the ferrous iron to this weak ligand (HO_2^-) would occur. Autoxidation reactions also occur faster in the presence of metal ions such as ferrous iron. This is due to ferrous iron being a source of electrons for two-electron transfer in the reduction of the oxygen (Livingston and Brown, 1981). There is an interesting point about function of ascorbic acid. This compound is a known reducing agent and widely used in meat products. However, ascorbic acid may also act as an oxidizing agent in a meat system by being a second source of electrons for two-electron transfer in reduction of the oxygen in myoglobin autoxidation reactions (Livingston and Brown, 1981). Ascorbic acid added to hemoglobin solution in absence of nitrite causes a rapid discoloration and greening of the solution indicative of oxidation of hemoglobin. However, pink color characteristic of cured meat was developed when ascorbic acid was added to hemoglobin solution in the presence of nitrite indicating ascorbic acid acts as reducing agent (probably reducing nitrite to nitric oxide) (Watts and Lehmann, 1952). The oxidizing ability of ascorbic acid also has been shown when it was added to pyridine hemochrome complex in the presence of air. Ascorbic acid addition caused no change in the hemochrome complex when atmospheric air was excluded (Lemberg et al., 1938). Moreover, nonenzymatic reduction of metmyoglobin by ascorbic acid under aerobic conditions was found to be catalyzed by nitrogenous compounds, i.e. nonphysiological pyridine derivatives (Fox et al., 1975).

Rate of myoglobin autoxidation is different for myoglobin from different sources. The autoxidation of crystalline turkey myoglobin proceeds at pH 5.7 and 30°C with a rate constant K of 0.56 hr^{-1} (Froning, 1972), while pork myoglobin autoxidizes at pH 6.0 and 30°C with K of 0.119 hr^{-1} (Bemmers and Satterlee, 1975). The K value for crystalline beef myoglobin is 0.22 hr^{-1} at pH 5.7 and 30°C (Snyder and Ayres, 1961), but the native oxymyoglobin from beef has a K value of 0.083 hr^{-1} at pH 6.0 and 25°C (Gotoh and Shikima, 1974). In fact, if the pattern of the autoxidation reaction would be similar for all myoglobins, then the differences in the reaction rate must be due to differences in structure between myoglobins isolated from different sources (Livingston and Brown, 1981).

Heat denaturation of heme proteins is as important as autoxidation, with regard to meat discoloration. The heat denaturation has an enhancing effect on the rate of the autoxidation reaction. Stability of myoglobin can be determined by a heat denaturation process. The thermal denaturation midpoint of myoglobin of tunafish is 85° while the midpoint is 93°C for whale myoglobin (Fosmire and Brown, 1976). Livingston and Brown (1981) pointed out that 78°C as the midpoint for pork myoglobin is an exception from the higher midpoints (more stable myoglobin) of other mammalian myoglobins. Midpoint for thermal denaturation of turkey myoglobin is 78°C (Cornish and Froning, 1974).

Sodium dithionite is an important reducing agent widely used in biochemistry. In a study with oxidants such as hydrogen peroxide and dioxygen the SO_2^- radical was found to be the active reducing agent

not $S_2O_4^{2-}$ (Greatz and Sutin, 1974). The SO_2^- radical also was the active reducing agent for reduction of metmyoglobin derivatives at pH 8.2 and 25°C (Olives et al., 1977). Nitrite ion, cyanide, water, and imidazole were among several species binding to metmyoglobin. The reduction of metmyoglobin in all the cases except for imidazole and cyanide, occurred through dissociation of the metmyoglobin-ligand complex. Then the metmyoglobin reacted with the SO_2^- radical and was reduced to deoxymyoglobin. Reduction in the case of imidazole and cyanide was done directly with the SO_2^- radical. Deoxymyoglobin while bonded to cyanide was found to be intermediate in the reduction of metmyoglobin-cyanide complex (Olives et al., 1977).

Reducing Ability of Meat

The ability of ground beef to enzymatically reduce metmyoglobin has been studied by Stewart et al. (1965). All pigments of the meat were oxidized by the addition of potassium ferricyanide and the reduction of metmyoglobin by the meat in 1 hr. then was measured. Raising pH from 5.1 to 7.1 resulted in increasing metmyoglobin reducing ability of the meat. The same thing was found when storage temperature increased from 3°C to 35°C. Also, when whole cuts of the meat were stored for several days in the refrigerator, this reducing ability of the meat decreases only slightly but the decrease was more rapid in the stored ground meat. Testing various substrates, Saleh and Watts (1968) have demonstrated that the overall rate of reduction of metmyoglobin in ground beef was controlled by the rate of nicotinamide adenine dinucleotide (NAD) reduction. Also, it was shown

that the addition of appropriate substrates to the meat resulted in raising the reducing activity of the meat samples, and from practical point of view, only monosodium glutamate was suggested (Saleh and Watts, 1968). However, the mechanism of the enzymatic reduction of metmyoglobin in meat has not been clearly demonstrated (Hagler et al., 1979).

Brown and Snyder (1969) reported that the reduced form of NAD (NADH) could reduce metmyoglobin in their assay and considerable increase in the reduction rate was observed by using flavins, such as flavinmononucleotide and riboflavin, or methylene blue. Although this type of nonenzymatic reduction has not been suggested to be responsible for metmyoglobin reduction in vivo, it might be capable of doing so when small amounts of ferric proteins are present (Brown and Snyder, 1969). Livingston and Brown (1981) have questioned the physiological importance of the activities of the enzymes isolated from fish and mammalian systems because of use of an artificial electron carrier, e.g., methylene blue, in the assays. However, an NADH-dependent enzyme being able to directly reduce metmyoglobin in vitro, has been isolated from beef heart muscle (Hagler et al., 1979). The reduction rate was much greater than that reported for nonenzymatic reduction. Ferrocyanide ion and NADH were needed and there was no need for methylene blue or dichlorophenolindophenol. The activity of this enzyme was increased as temperature of the mixture increased up to 37°C but no activity was detected at 50°C.

Hemochrome Complexes

Formation of hemochromes has been known for more than half a century (Anson and Mirsky, 1928). Heme, or hematin can react with a great variety of nitrogenous compounds to form hemochromes. Heme does not form any hemochromes with alcohols, aldehydes, carbohydrates, and fatty acids (Keilin, 1960). The iron in hemochromes is bound to the four porphyrin nitrogens and also to two additional nitrogen atoms provided by the nitrogenous substances. One molecule of heme may react with one molecule of each of two different nitrogenous substances. Heme also can combine with denatured proteins, or CO to form a hemochrome (Lemberg and Legge, 1949).

Neither free heme nor free hematin can combine with either hemoglobin or methemoglobin, indicating that all the available heme binding groups in the native globin are occupied. However, globin hemochrome forms upon mild denaturation of hemoglobin (Keilin, 1960). Addition of free heme to this globin hemochrome results in intensifying of the absorption bands of the latter complex, therefore indicating that denaturation of globin increases its heme binding ability (Keilin, 1960). Globin hemochrome like hemoglobin consists of a globin and heme. The difference between these two complexes is that denatured globin and heme make the former compound while native globin and heme are present in the latter complex. Moreover, ten molecules of heme can be converted into hemochrome with only one molecule of denatured globin (Anson and Mirsky, 1928). Carboxyhemochrome also can be formed when a hemochrome complex is treated with CO. One of the two nitrogenous bases, then is replaced by CO (Anson and Mirsky,

1928; Keilin, 1960). Considerable difference exists between nitrogenous bases for the affinity towards heme iron. Heme has very high affinity for the histidine imidazole side chain of denatured globin or cyanide, while the affinity for pyridine or ammonia is intermediate and low respectively (Lemberg and Legge, 1949). Only substances having their nitrogen atoms in an exposed position can combine with the heme iron to give hemochromes (Lemberg and Legge, 1949).

The hemochrome complex is always partially dissociated into its two components, the reduced heme and the nitrogen base. For the reaction of reduced heme and nitrogen base to form a hemochrome, the lower the temperature, the more favorable it is for the equilibrium to exist toward hemochrome formation (Anson and Mirsky, 1928).

All hemochromes react with atmospheric oxygen. The ferrous iron becomes oxidized and the resulting compound is a hemichrome. Speed of this type autoxidation is closely related to the oxidation-reduction potential of the specific hemochrome/hemichrome system (Keilin, 1960). The following compounds are known to bind only to the ferrous iron of heme to form ferrohemochromes: nicotinamide, nicotinic acid, arginine, thiamine, casein and ovalbumin (Akoyunoglou et al., 1963).

One convenient and precise way to differentiate among the various hemochromes is by using the exact position of the sharp alpha-band present in the visible region at 550-560 nm (Anson and Mirsky, 1928; Lemberg and Legge, 1949). A particular hemochrome always has the alpha-band in exactly the same position although that hemochrome complex may be prepared in different ways (Anson and

Mirsky, 1928). There are definite and important relationships between properties of the ligands present on either side of the iron porphyrin complexes and that of the iron as well as the porphyrin ring (Brill and Williams, 1961). Changes in the absorption spectra of the complexes can help in knowing the nature of unknown ligands.

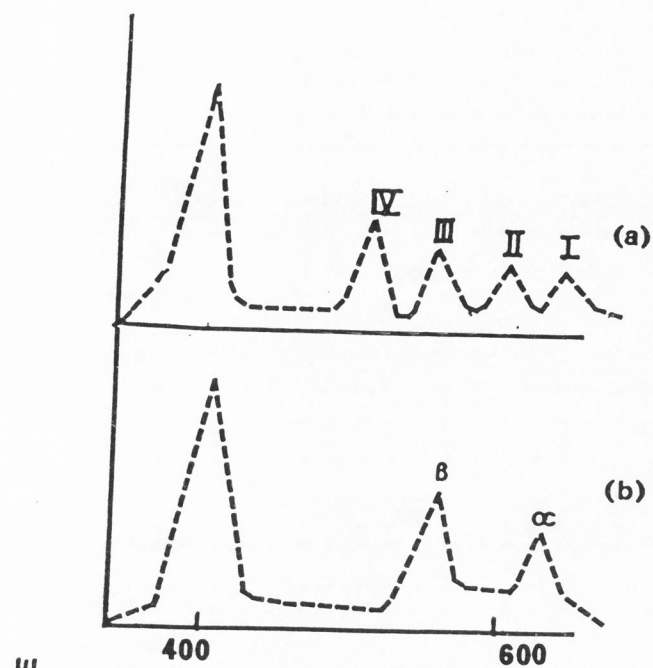
A Brief Interpretation of the Absorption Spectra of Some Porphyrin Complexes

The spectrum of a typical porphyrin complex consists of an intense band at about 400 nm (Soret band) and four weaker bands at the longer wavelengths (Figure 1) (Williams, 1956). The Soret band is due to an electronic transition between two pi orbitals at different energy levels and the other four bands arise in pairs, two from each tautomer, from a second electronic transition between two pi orbitals. Absence of tautomeric forms (compounds with different arrangement of atoms but existing in equilibrium) results in replacement of the four bands by two bands: alpha- and beta bands with the latter being at the shorter wavelength (Williams, 1956). Among fifteen different arrangements for the porphyrin ring, there is only one form in biological systems, i.e., myoglobin and hemoglobin (Stryer, 1975). Therefore, it is expected to have only two bands (alpha- and beta-) besides the Soret band in the visible region. According to Williams (1956), and Livingston and Brown (1981):

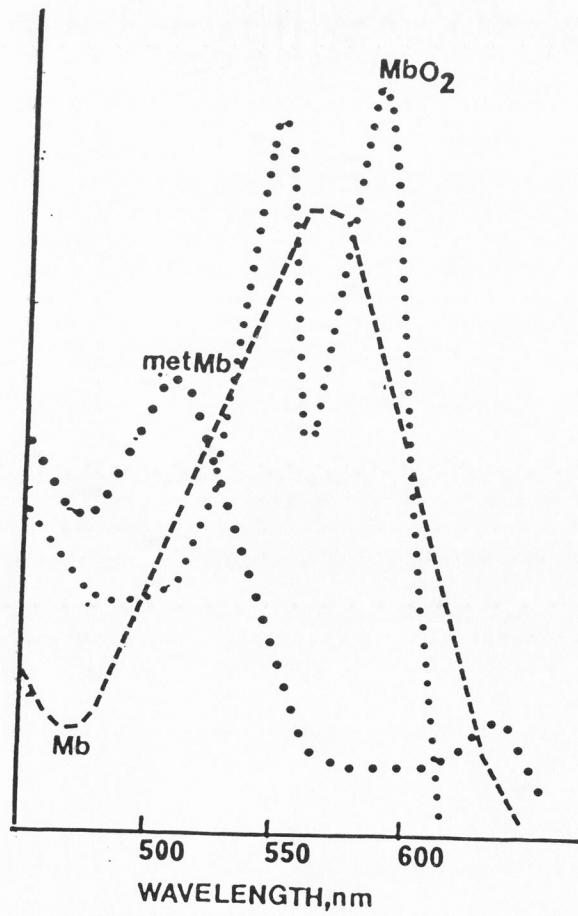
- a) Both the alpha- and beta- bands move to longer wavelengths with increasing electron-acceptor properties of the substituents at the peripheral position of the pyrrole rings.
- b) The shorter the wavelengths of the absorption maxima, the more

Figure 1. Absorption spectra of the porphyrin ring with (a) and without (b) tautomeric forms (from Williams, 1956).

Figure 2. Absorption spectra of myoglobin (Mb), oxymyoglobin (MbO₂), and metmyoglobin (MetMb) (from Fennema, 1976).



ABSORBANCE



electronegative is the metal ion. Absorption maxima of metmyoglobin is at a shorter wavelength, as compared to that of the deoxymyoglobin (Figure 2). Ferric iron has greater electronegativity as compared to ferrous iron. The greater the intensity of the alpha-band, the greater is the electron-donor character of the metal cation.

c) Presence of both alpha- and beta-bands becomes very definite by binding between myoglobin and a ligand. The alpha-band is at the longer wavelength and more intensified in oxymyoglobin as compared to that in deoxymyoglobin (Figure 2).

d) The alpha-band moves to a shorter wavelength and becomes more intense as the ferrous iron of the porphyrin complex binds to nitrogenous bases such as ammonia or pyridine compared to heme complexes with small unsaturated molecules such as CO, NO, or O₂. Ammonia has greater basicity than oxygen or NO, therefore it has greater electron-donor properties. Formation of a strong pi back-bonding is due to binding between the ferrous iron and a ligand with good electron-acceptor property (such as NO or oxygen). Nitrogenous compounds which cannot engage in strong pi back-bonding dissociate rapidly from the ferrous iron with subsequent oxidation of the iron.

Hemochrome Complexes and Meat Color

Coleman et al. in 1949 reported formation of a bright red color in mammalian muscle by using nicotinic acid. Hopkins and Sato (1971) selected several compounds including derivatives of nicotinic acid and nicotinamide in conjunction with ascorbic acid, to preserve the color

of fresh meat. Using fresh ground pork or beef, Kendrick and Watts (1969) reported that hemochromes were not formed in aerobically stored meats with added nicotinamide or nicotinic acid, even in the presence of ascorbic acid. Anaerobic conditions, on the other hand resulted in hemochrome formation but only when higher concentrations of the nitrogenous compounds and lower pHs were used. Nicotinamide, or niacin addition to cured ham was found to stabilize the meat pigment to light irradiation. The effect of nicotinamide was greater than the addition of niacin with or without added sodium ascorbate (Bailey et al., 1964). It has been suggested that nicotinamide may protect NAD in the tissues from destruction by nucleosidase (Bailey et al., 1964; Kendrick and Watts, 1969). Ando (1974) reported that nicotinamide enhanced the decomposition of nitrite with formation of NO in the presence of ascorbate.

Pink color formation in cooked model and meat systems with several organic and inorganic substances have been reported (Dymicky et al., 1975). Pyridine, pyrimidine arginine, and stannous chloride were among the test compounds. Howard et al. (1973) tested many nitrogenous bases to find possible substitutes for sodium nitrite for pigment formation in cured meat products. Among 24 test substances only methyl and hexyl nicotinate, and N,N-di-ethylnicotinamide were found to form stable pink pigments in cooked meat (a mixture of ground pork and beef). These compounds in combination with 10 or 20 ppm sodium nitrite produce stable and long-lasting pink pigment in the cooked meat (storage at 5°C up to 10 weeks). Nicotinamide and many nitrogenous compounds are unsaturated molecules with strong basic

properties (good electron-donor) (Tarladgis, 1962a; Williams, 1956). In searching for a suitable substitute for sodium nitrite, a ligand with acidic (good electron acceptor) properties is needed. This prevents dissociation of the ligand from the ferrous iron and therefore stabilizes the ferrous complex (Livingston and Brown, 1981).

Brown and Tappel (1957) studied the properties of the pigment responsible for the desirable pink color of precooked and canned tuna and it was concluded that the pigment was hemochrome in its nature. Either denatured-globin or nicotinamide or both were found to form this pink color pigment in the tuna. Later, Tappel (1957a) studied the pigments of cooked beef and concluded that the brown pigments were best characterized as mixed denatured-globin nicotinamide hemichromes. The reflectance spectra of the cooked meat pigments have also been studied by Tarladgis (1962a). He concluded that the iron of this brown pigment, had a denatured globin at the fifth position and a water molecule at the sixth position. This complex he concluded to be a high spin ferric iron and named the pigment metmyochromogen. Tarladgis (1962a) argued that presence of nicotinamide, strong base (good electron-donor), as an axial ligand would change the high spin complex to a low spin one. Giddings (1977) points out that presence of an imidazole group, a strong base, at the fifth position of the cooked meat pigment does not change the high spin character of the complex to the low spin one. Therefore, the high spin nature of the cooked meat pigment does not rule out a denatured-globin hemichrome having a nitrogenous base as a ligand at the sixth position (Giddings,

1977). Giddings further points out that spin state of a complex is largely determined by the ligand at the sixth position.

Meat preservation by irradiation causes some changes in myoglobin which can affect meat color. According to Tappel (1957b) irradiation converts the brown pigment of precooked meat, denatured globin hemichrome, to a red pigment. The latter pigment was best characterized as denatured-globin hemochrome. Satterlee (1972) found that the red myoglobin pigment produced by gamma irradiation of metmyoglobin was oxymyoglobin, although he did not describe the exact mechanism by which oxymyoglobin was formed during the irradiation. One possibility that Satterlee (1972) mentioned was the formation of a reducing radical, during gamma irradiation, with the ability to reduce the iron of metmyoglobin. Irradiation can cause formation of oxymyoglobin from metmyoglobin, when there are high amounts of metmyoglobin present initially (Livingston and Brown, 1981). Hansen et al. (1963) reported the development of an objectionable red color in irradiated chicken samples during anaerobic storage at elevated temperature. The irradiated chicken samples, which were partially cooked (68°C for 45 min.), became objectionably red when stored for three months in a nitrogen atmosphere at 38°C. Very little red color developed in the samples stored aerobically, although no oxygen remained in the cans after three months storage (Hansen et al., 1963).

MATERIALS AND METHODS

Turkey Roll Preparation

Turkey breast meat was purchased from a local distributing company. Frozen breast was thawed at 4°-5°C and turkey rolls were made as follows: an emulsion was made with 10% of the meat and sufficient salt to give 2% salt, as a % of the meat weight (1% non-fat dry milk (NFDM) was included in formulation of some of the turkey rolls). Then the emulsion was hand-mixed for 5-7 min with the rest of the meat, which had been previously cut into 1-2" chunks. The mixture was then stuffed in 9.5 cm diameter, pre-soaked fibrous casings (Smith Supply Co., Nampa, ID). Turkey rolls were cooked to desired internal temperature using dry heat, as described in results. Relative humidity of 39-40% was used during cooking for some products. For reflectance spectrophotometry measurements, turkey rolls were made with ground rather than chunked turkey breast, using a 0.95 cm grinder head. For ground product, 10% added water was used in making the emulsion with 10% of the meat. Two percent salt and one of the following test compounds were also added, sodium nitrite (10 or 156 ppm), nicotinamide (2%), or nicotinic acid (3%). This emulsion was then hand-mixed with the rest of the previously ground meat. Commercial turkey breast rolls used in this study were obtained from a midwest turkey processing company. The ingredients as listed on the label were turkey breast, turkey broth, salt, sugar, modified food starch, and sodium phosphates.

Hunter Color Measurements

The Hunter Lab Digital Color Difference Meter (D25D2A) (Hunter Associates Laboratory, Inc., Reston, VA) was standardized with the pink standard plate (L=66.8, a=12.0, b=21.4). Turkey slices 1 cm in thickness and 6 cm in diameter were exposed on each side to fluorescent light at room temperature in air for 45 min to bleach the samples. Then each slice was soaked in a 0.1 M solution of a test compound for 3 min. Hunter color readings were taken immediately after treatment with test solution. For some samples Hunter color readings were taken every 5 min for 90 min after treatment. Test solutions were: sodium dithionite, glutathione, cysteine-HCl, NADH, ascorbic acid, erythorbic acid, sodium nitrite, pyridine, and histidine-HCl, all at 0.1 M concentrations.

Reflectance Spectrophotometry

Slices of cooked turkey rolls were also used for reflectance spectrophotometry. Some slices were left under ordinary light in air for 10-15 min to cause fading of pink color. Pink color was regenerated by soaking the sample for 3-4 min in 0.1 M sodium dithionite solution. The visible spectra from 400 nm to 700 nm was recorded using a Lab Scan II spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA). The reflectance difference spectra was also obtained as the difference between the spectra of each sample and a control sample having normal color (no pink defect).

Hemochrome Characterization

Several nitrogenous substances were used to evaluate formation of hemochromes in solution. Nicotinamide, nicotinic acid, turkey albumin, human globin, arginine-HCl, thiamine-HCl, and non-fat dry milk were the test compounds.

The hematin solution was made up by dissolving 5 mg hemin in 5 ml of 0.1 N NaOH and then diluting to 10 ml with distilled H₂O. Two ml of the test compound was then mixed with 0.1 ml of the hematin solution and diluted to 5 ml final volume with 0.1 M sodium borate, pH 12.5. Human globin solutions were diluted with 0.1 M potassium phosphate, pH 7.5. Sodium dithionite (3-4 mg) was then added to reduce the solution. Hemochrome formation was identified by immediate development of a pink color.

To obtain absorption spectra, test solutions were prepared as follows: nicotinamide (14 mM), nicotinic acid (100 mM), turkey albumin (1 mg/ml), NFDM (2.5 mg/ml), arginine-HCl (400 mM), and thiamine-HCl (200 mM) were prepared in 0.1 M borate, pH 12.5. Human globin (1 mg/ml) was prepared in 0.1 M potassium phosphate, pH 7.5. Final concentration of the test solutions were: nicotinamide (5.6 mM), nicotinic acid (40 mM), turkey albumin (400 ppm), NFDM (1000 ppm), human globin (200 ppm), arginine-HCl (160 mM), and thiamine-HCl (80 mM). These concentrations were found in preliminary tests to produce stable pink color at pH 11.0-12.5. Pink color was obtained at pH 5.5 for nicotinamide, but was less stable. Nicotinic acid produced pink color at pH 8.5, and denatured globin hemochrome was pink at pH 7.5. The other compounds did not produce stable pink color at pH

values below 11.0. Undenatured globin solutions were green, rather than pink. Denatured globin solution was prepared by heating 2 ml of the solution in a water bath for 30 sec at 70°C. Reduced denatured globin solution was pink.

Nitroso hemochrome (nitroso pigment) and total pigment in cured meat were determined by the method of Hornsey (1956), as modified by Kramlich et al. (1973). Reagents were prepared as follows.

1. acetone a. Place 90 ml distilled water in a 1 L volumetric flask. Bring to volume with acetone, then mix.
2. acetone b. Mix water with 20 ml concentrated HCl and bring to 100 ml volume. Transfer the diluted HCl to a 1 L volumetric flask, add acetone, mix, and bring to volume with acetone.

The following steps were conducted in subdued light to lessen pigment fading. Weigh 2.0 g sample into a 50 ml polystyrene centrifuge tube. Add 9.0 ml acetone a, and macerate 2-3 minutes with a glass rod. Stopper the tube with centrifuge tube cover, mix, let stand for 10 min, then filter through 2 Whatman #42 filter papers into a test tube. Transfer the filtrate into a 1 cm cuvette and read the absorbance within 1 hour at 540 nm. Calculate as nitroso pigment.

Prepare another 2.0 g sample with 9.0 ml acetone b. Macerate and let stand for 1 hr before filtering into a test tube. Read the absorbance at 640 nm, and calculate as total pigment. The ppm nitroso pigment = $A_{540} \times 290$. The ppm total pigment = $A_{640} \times 680$.

$$\% \text{ conversion} = \frac{\text{ppm nitroso pigment}}{\text{ppm total pigment}} \times 100$$

Absorption spectra of hemochromes were recorded over the visible region from 400 to 700 nm using a Beckman (Irvine, CA) DU-8B

spectrophotometer equipped with a plotter.

Residual nitrite was determined spectrophotometrically (AOAC, 1980). The following reagents and materials were prepared.

- a) NED REAGENT. Dissolve 0.2 g N-(1-naphthyl) ethylenediamine.2HCl in 150 ml 15% (v/v) acetic acid. Filter if necessary and store in a brown glass bottle.
- b) Sulfanilamide reagent. Dissolve 0.5 g sulfanilamide in 150 ml 15% acetic acid. Filter if necessary and store in a brown glass bottle.
- c) Nitrite standard solutions. Stock solution, 1,000 ppm NaNO_2 . Dissolve 1 g sodium nitrite in water and dilute to 1 L. Intermediate solution, 100 ppm sodium nitrite. Dilute 100 ml of stock solution to 1 liter with water. Working solution, 1 ppm sodium nitrite. Dilute 10 ml of intermediate solution to 1 L with water.
- d) Filter paper. Test for nitrite contamination by analyzing 3-4 sheets at random in the box. Filter about 40 ml of water through each sheet. Add 4 ml sulfanilamide reagent, mix, let stand for 5 minutes, add 4 ml NED reagent, mix, then wait 15 minutes. If any sheets are positive, discard the entire box.

Weigh 5 g finely comminuted and thoroughly mixed sample into a 50 ml beaker. Add about 50 ml of water heated to 80°C. Mix with a glass rod, breaking up all lumps, and transfer to a 500 ml volumetric flask. Wash the beaker and rod with successive portions of the hot water, adding all washings to the flask. Add enough hot water to bring the volume to about 300 ml. Place the flask in a steam bath for 2 hours

with occasional shaking. Cool to room temperature, dilute to volume with water, and remix. Filter through 2 layers of the tested filter paper. Add 2.5 ml sulfanilamide reagent to an aliquot containing 5-50 ug sodium nitrite in a 50 ml volumetric flask, and mix. After 5 minutes, add 2.5 ml NED reagent, mix, dilute to volume, mix, and let color develop for 15 minutes. Transfer a portion of the solution to a photometer cell and determine the absorbance at 540 nm against a blank of 45 ml water, 2.5 ml sulfanilamide reagent, and 2.5 ml of NED reagent.

Determine the nitrite concentration by comparison with a standard curve prepared as follows. Add 10, 20, 30 and 40 ml of the nitrite working solution to 50 ml volumetric flasks, respectively, then add the sulfanilamide and NED reagents as previously described. The standard curve is a straight line to 1 ppm sodium nitrite in the final solution. It is common practice in the meat industry to describe sodium nitrite concentration with the term "nitrite". Thus 5 ppm nitrite is understood to be 5 ppm sodium nitrite. Chemically, this terminology is correct if ppm is interpreted such that 1 ppm sodium nitrite produces 1 ppm NO_2^- ion upon dissociation. If ppm is defined as mg/kg, then 156 ppm nitrite (sodium nitrite) would yield only 104.52 ppm dissociated NO_2^- ion in the product.

Redox Potential Measurements

The oxidation-reduction potential (ORP) of test compounds was determined under N_2 using a Orion pH meter model 601A equipped with a combination electrode (Orion electrode model 96-78 Cambridge, MA)

with a platinum redox and a silver/silver chloride reference electrode. Concentration of the test compounds used for the ORP determination was the same as that used for recording absorption spectra. To measure the potential for meat samples, 20 g meat product was rapidly homogenized with 30 ml of 0.1 M sodium carbonate using a Brinkman (Westbury, NY) polytron-homogenizer. The ORP was then measured under N_2 as described previously.

Pink Color Prevention

The possible beneficial effects of adding an oxidizing agent to prevent pink color defect was investigated in vitro. Meat slurries, consisting of 10% added water or saturated potassium iodate (KIO_3) solution and nicotinamide (2%) were prepared in an Osterizer blender (Milwaukee, WI). Approximately 10 g of each slurry were added to a test tube and cooked in a 71°C water bath for 30 minutes. The tubes were sealed with parafilm and held at 4°C overnight. Treatments were then visually appraised for color and oxidation reduction potential was measured.

RESULTS AND DISCUSSION

Effect of Phosphate, Storage Temperature and Time, and
Cooking Temperature on Incidence of Pink Color Defect

Samples with or without 0.5% sodium tripolyphosphate (STP) were stored at 4.4°C or 10°C for zero or 3 days before cooking and then cooked to an internal temperature of 60° or 71°C, for a total of 16 treatments. In another trial the samples were stored at 10°C for 1, 2, or 3 days before cooking to an internal temperature of 60°, 71°, or 82°C, for a total of 18 treatments. Less pink defect was observed in treatments held at lower temperature and shorter storage time before cooking. Ten treatments had visually significant and spotty pink defect. Nine of the ten were stored at 10°C for 3 days before cooking, and the other pink sample was also stored for 3 days but at 4.4°C. The pink color defect was seen in treatments with or without addition of STP. In another trial pink defect was seen both in rolls stored in vacuum sealed plastic bags, or only in sausage casings. In other trials pink defect was seen in rolls cooked with only dry heat or in a smokehouse at 39-40% humidity. In conclusion, higher storage temperature, and longer storage times before cooking appeared to favor pink defect in cooked turkey rolls.

A larger experiment was then conducted as a 2 X 2 X 3 X 3 with 0 or 0.5% of STP, 24 or 72 hrs. storage before cooking at 0°, 4.4°, or 10°C, and then cooked to 60°, 71°, 82°C internal temperature. In contrast with the previous experiment, no pink defect was observed in

any of these 36 treatments in either of two replicates as judged by an 11 member sensory panel.

There were 3 factors which were found to be different in these two sets of experiments and which may explain the different results; (1) source of meat, (2) absence of 1% non-fat dry milk (NFDM) in the second experiment and (3) grinding of the meat in the second experiment. In the second experiment in which no pink defect was observed, the meat was from a different source. It was forced through a 1" plate before formulation to assure sample homogeneity, and NFDM was eliminated from the formulation since it was not needed for bind.

The most important difference was probably the grinding of the meat used in the second experiment. Enzymatic reduction of metmyoglobin is reported to decrease rapidly in ground beef stored in a refrigerator, while only a slight decrease was found in whole cuts of meat (Stewart et al., 1965). Also the nicotinamide adenine dinucleotide (NAD) level of meat decreases after grinding due to enzymes released during maceration (Severin et al., 1963). The concentration of NAD in meat has been found to affect metmyoglobin reducing ability. In fact, addition of NAD to ground meat increased the metmyoglobin reducing ability (Watts et al., 1966). Addition of 10% spray-dried albumen to turkey meat was found to produce a pink defect in cooked product (Froning et al., 1968b). Thus, NFDM may also play a role in development of pink defect.

In conclusion, grinding appears to prevent pink defect by increasing the incorporation of oxygen and lowering NAD levels, thus raising the oxidation-reduction potential (ORP) of the meat. The ORP

may also be different among different sources of meat. Addition of binding agents such as NFDM may also play a role in pink color defect under certain conditions. Pink defect may be enhanced due to reduced ORP in samples stored at higher temperature and longer time, as oxygen is consumed in the meat.

Effect of Various Reducing Agents and Nitrogenous Compounds on Pink Color Development

Since meat ORP appeared to be important in pink color development a third experiment was conducted to measure the intensity of pink color development in the presence of various reducing agents and nitrogenous compounds.

Turkey rolls for this experiment were cooked to an internal temperature of 52°, 62°, 72°, or 82°C. Slices from each roll were left under fluorescent light in air at room temperature for about 2 hr. No pink defect was seen in these samples. Then each sample was soaked in test solution for 3 minutes. The color of the sample was measured with a Hunter Colorimeter standardized with a pink tile (a=12.0, b=21.4, L=66.8). Hunter color values after treatment of meat slices with various agents are shown in Figure 3a-d. Visually only slices soaked in sodium nitrite or sodium dithionite were pink, and these treatments also had the highest 'a' values for slices cooked to 62°, 72°, or 82°C (Figure 3b-d). At 52°C, all slices had 'a' values of about 4-6, but at higher temperatures only dithionite or nitrite treated samples had 'a' values above 3.5 (commercial processed rolls are cooked to at least 71°C internal temperature). The 'a' values were

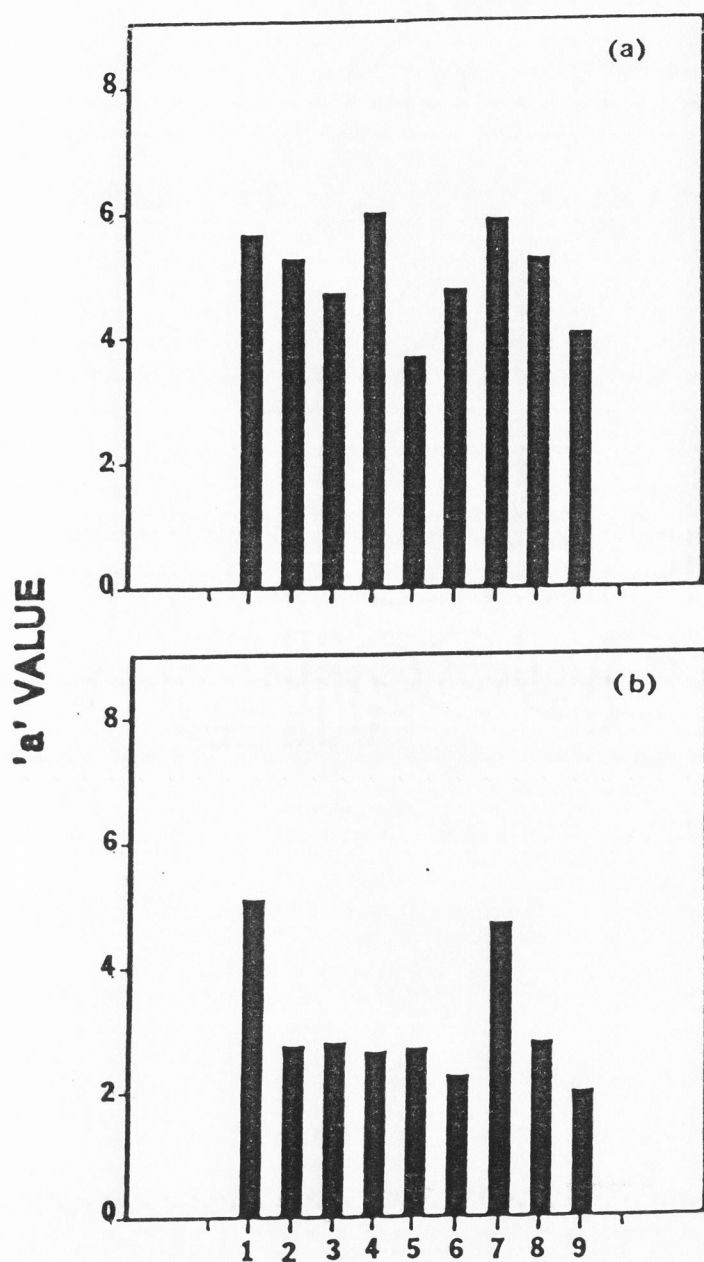


Figure 3. Hunter color 'a' or redness values of turkey rolls cooked to (a) 52°C, (b) 62°C, (c) 72°C or (d) 82°C, sliced, then held in a reducing solution of sodium dithionite and/or various nitrogenous compounds. Each values is the mean of two samples done in duplicate. 1-9 are sodium dithionite, glutathione, cystein-HCL, NADH, ascorbic acid, erythorbic acid, sodium nitrite, pyridine, histidine-HCL.

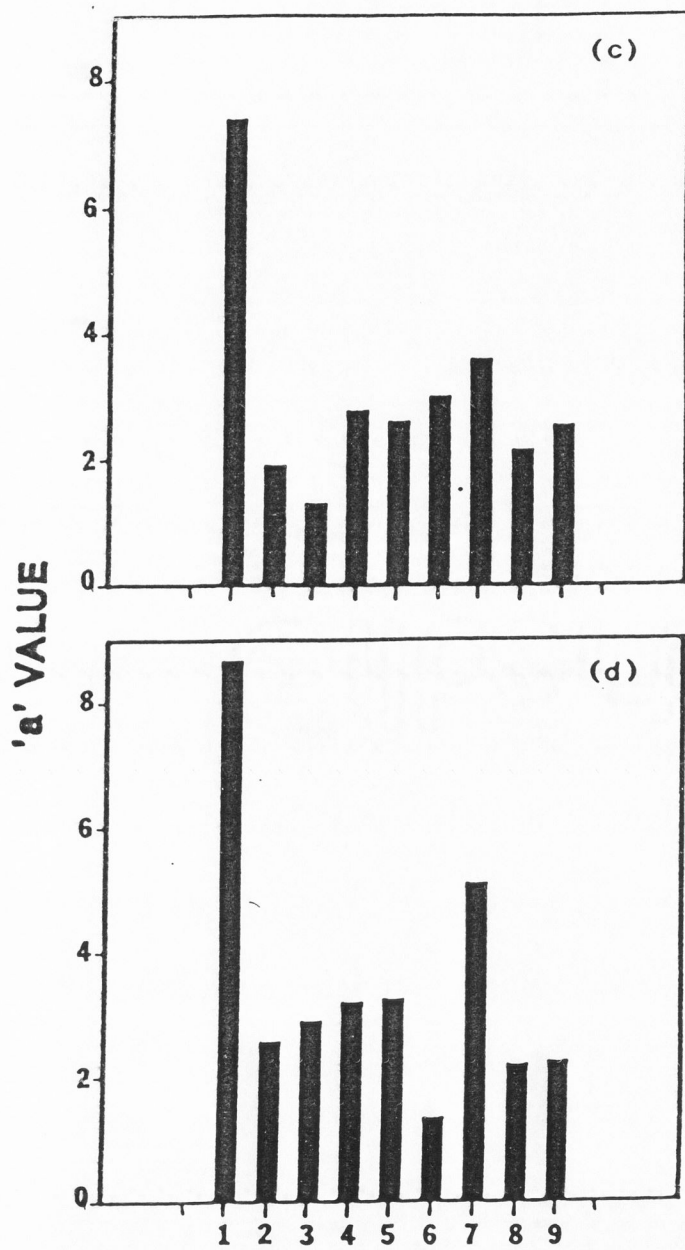


Figure 3. (continued)

probably higher at the lower cooking temperature of 52°C due to less pigment denaturation. Thus the higher level of undenatured oxymyoglobin in these samples led to higher 'a' values. Heat denaturation of muscle proteins starts at about 40°C and is finished at about 70°C (Wierbicki et al., 1957).

The 'a' values for dithionite treated slices increased with increasing cooking temperature (Figure 3a-d). This may be due to increased protein denaturation at higher temperature leading to increased hemochrome formation. It is known that undenatured hemoglobin or methemoglobin cannot form hemochromes (Keilin, 1960). Also, denatured globin has a very high affinity for hemochrome formation (Lemberg and Legge, 1949), and can form a hemochrome complex with up to 10 molecules of heme (Anson and Mirsky, 1928).

Nitroso pigment and residual nitrite were detected in the samples treated with nitrite solution but not in those samples treated with dithionite solution. Results are given in Table 1.

The results in Table 1 indicate that the cooked meat had an adequate level of reducing capability to form nitric oxide from nitrite, allowing nitroso pigment development. Reducing capability was apparently present even in the samples cooked to 82°C.

The ORP value for formation of nitric oxide from nitrous acid is 0.99V (CRC, 1969) and the value for reduction of ferric to ferrous iron in formation of pyridine hemochrome is 0.004V (CRC, 1968). Therefore, reducing capability of the cooked turkey roll was adequate for nitroso pigment formation when it was treated with nitrite solution. However, hemochrome complex formation would not occur until

Table 1. Nitrite and nitroso pigment content of nitrite treated turkey slices.

Temperature (°C)	Nitrite (ug/g)	NO-heme pigment (ug hematin/g)	Total heme pigment (ug hemtin/g)	% Conversion ^a
52	418.09 ^b	9.57	26.52	36.09
62	435.06	10.0	25.84	38.69
72	313.93	8.41	23.12	36.38
82	406.65	7.68	19.72	38.95

^a% conversion = (NO-heme/total heme X 100).

^beach value is the mean of one sample done in triplicate.

the ORP was lower, as with treatment with a strong reducing agent such as dithionite.

Rate of Color Fading in Nitrite Cured or Dithionite Treated Turkey Slices

Turkey rolls were formulated with 10 or 100 ppm sodium nitrite, or an uncured control. Hunter color 'a', 'b', and 'L' values were obtained at various intervals for nitrite treated samples as compared to control samples soaked in 0.1M sodium dithionite solution for 3 minutes.

Both nitrite cured and dithionite treated turkey slices were visually pink initially, and had Hunter color 'a' values of about 5.6 (Figure 4). Dithionite treated samples faded rapidly when exposed to air in either light or dark, and were no longer pink after 5-10 minutes. Nitrite cured samples were noticeably less pink after 45 minutes, but still had 'a' values above 4.0 at 90 minutes. The samples cured with 100 ppm nitrite were indistinguishable from those cured with 10 ppm, and faded at the same rate (data not shown). Uncured control slices soaked in 0.1M sodium nitrite solution for 3 minutes were also pink initially and the pink color was retained much longer than slices soaked in dithionite solution, after exposure to air in light or dark. There was no difference among 'a' values of similar samples exposed to air in light or dark. Apparently, oxygen in air is more responsible for the color fading than is exposure to light. Nitroso pigment is known to be sensitive to light (Erdman and

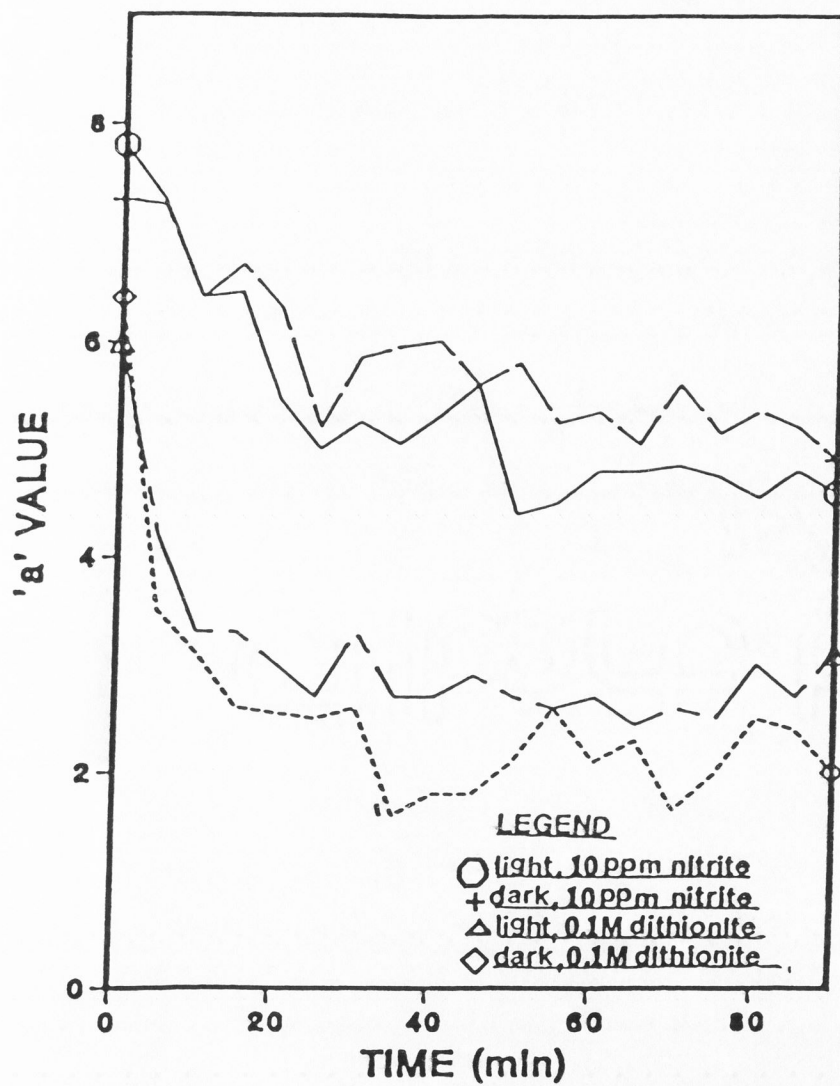


Figure 4. Fading rate of the pink color of cooked turkey slices after treatment with 0.1M sodium dithionite or formulated with 10 ppm sodium nitrite. Samples were kept in air either in dark or under fluorescent light at room temperature. Each point is the mean of two samples done in duplicate.

Watts, 1955), while hemochrome complexes fade rapidly in air (Keilin, 1960).

Comparison of Absorption Spectra of Nitroso Pigment and Pink Exudate from Commercial Samples

The nitroso pigment formed in meat products formulated with nitrite is extractable in acetone solution (Hornsey, 1956). As little as 5 ppm nitrite added to meat is sufficient to produce a satisfactory cured meat color but only for a limited time (Ingram, 1974). Higher levels are required to provide adequate color stability. Samples formulated with 20 or 156 ppm nitrite resulted in almost identical absorption spectra. The spectra shown for samples made with 156 ppm nitrite (Figure 5) has absorption maxima at 417.5, 475.8, 483.3, 535.8, and 564.2 nm. For comparison, the spectra for the extracted pigment from the cooked cured pork has absorption maxima at 576, 535, and 563 nm (Hornsey, 1956).

Samples of cooked turkey breast having either spotty or uniform pink color were received from a midwest turkey processing plant. The pigment responsible for the pink defect in these samples was not extractable in acetone, giving at best a very light yellow or almost colorless extracted solution. Similarly, the pink pigment of canned tuna, identified as a hemochrome complex, was not extractable in several solvents including acetone (Brown and Tappel, 1957). The spectra for the acetone extracted solutions from the commercial samples was totally different from that for nitroso pigment. However, a pink colored exudate was obtained from one of the commercial samples

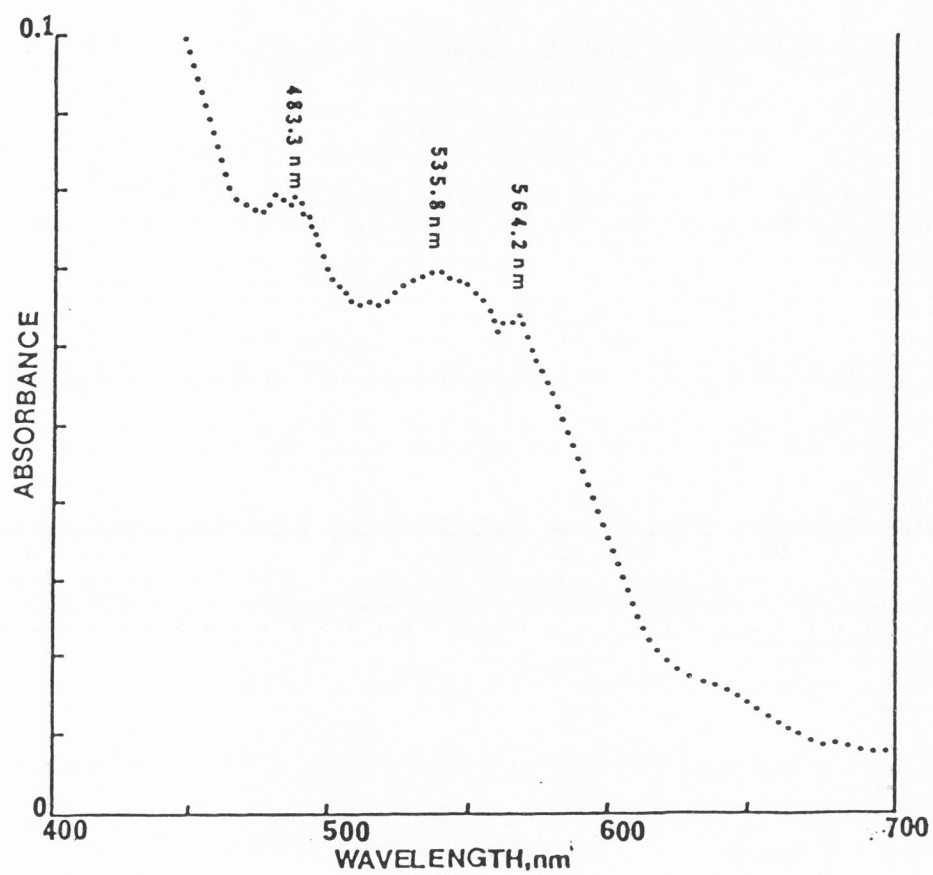


Figure 5. Absorption spectra of nitroso pigment of cooked turkey formulated with 156 ppm sodium nitrite (acetone extract). Curve is representative of two trials.

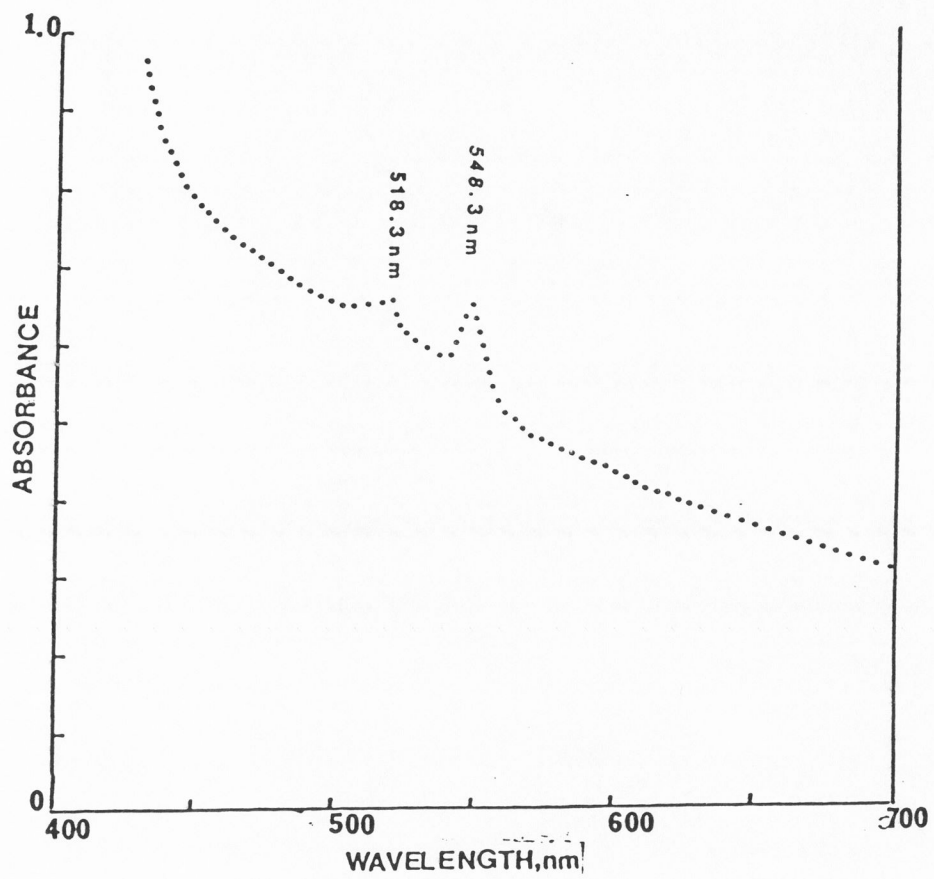


Figure 6. Absorption spectra of pink exudate from a commercial sample with pink defect. Curve is representative of two trials.

with pink defect. The spectra is given in Figure 6. The spectra shows two bands at about 518, and 548 nm.

Absorption Spectra of Test Hemochrome Solutions

The following nitrogenous compounds were used to prepare hemochrome solutions: nicotinamide, nicotinic acid, albumin, denatured globin, thiamine-HCl, arginine-HCl, and NFDM. The absorption spectra of the hemchromes formed by nicotinamide, nicotinic acid, albumin, denatured globin, and NFDM are given in Figures 7, 8, 9, 10, and 11 respectively. All hemochrome absorption spectra have two distinguishable bands in the visible region. The alpha-band by definition is located at longer wavelength than the beta-band (Williams, 1956). For all hemochromes tested the alpha-band was of greater intensity than the beta-band.

Williams (1956) has reviewed many of the characteristics of metal porphyrins. He states that the intensity of the alpha-band is associated with 3d atomic electrons in a promoted d state. Thus ligands with stronger pi back-bonding with the ferrous iron will produce a complex with a more intense alpha-band.

The interaction of a transition metal cation with a ligand consists of two parts: (1) the formation of sigma molecular orbitals in which the cation acts as electron-acceptor and (2) the formation of pi molecular orbitals in which the cation acts as an electron-donor (back-bonding). For back-bonding to occur, the metal must have sufficient electron density for donation to its ligand. Ferrous iron meets this criterion. The relatively low charge on the nucleus leads

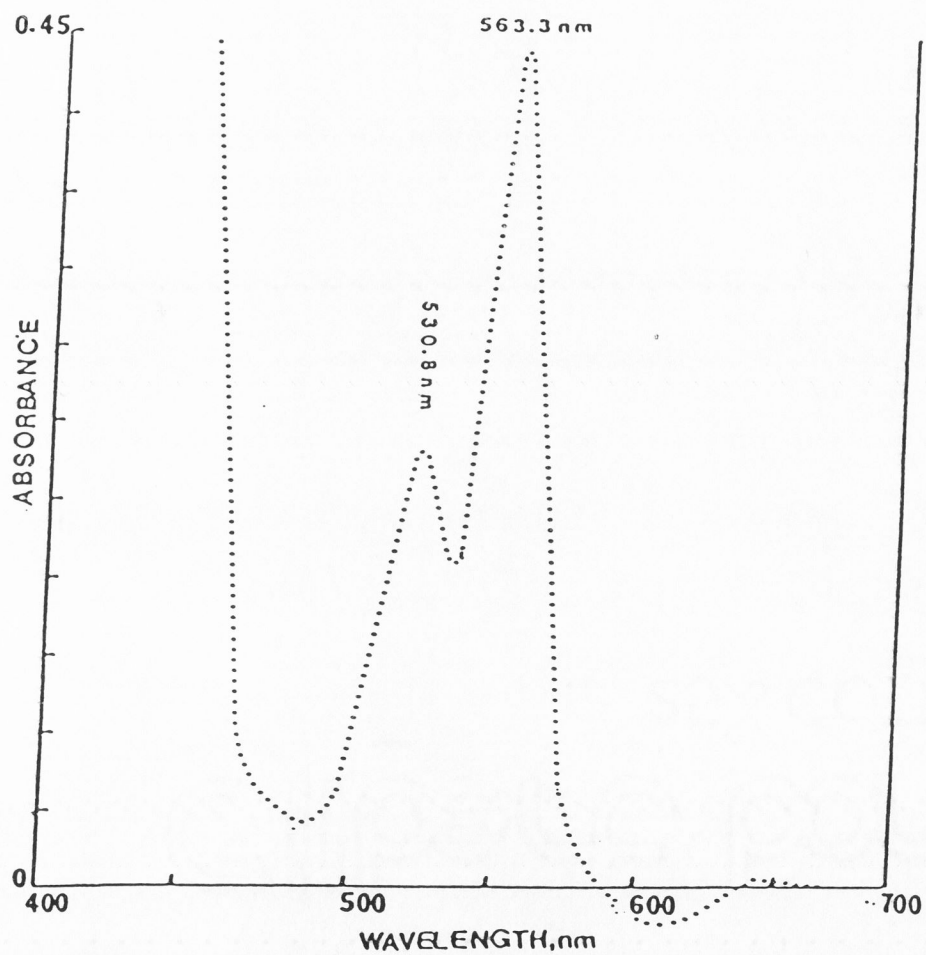


Figure 7. Absorption spectra of the hemochrome formed with nicotinamide (5.6 mM) and hematin (1 ug/ml), pH 12.5. Curve is representative of at least four trials.

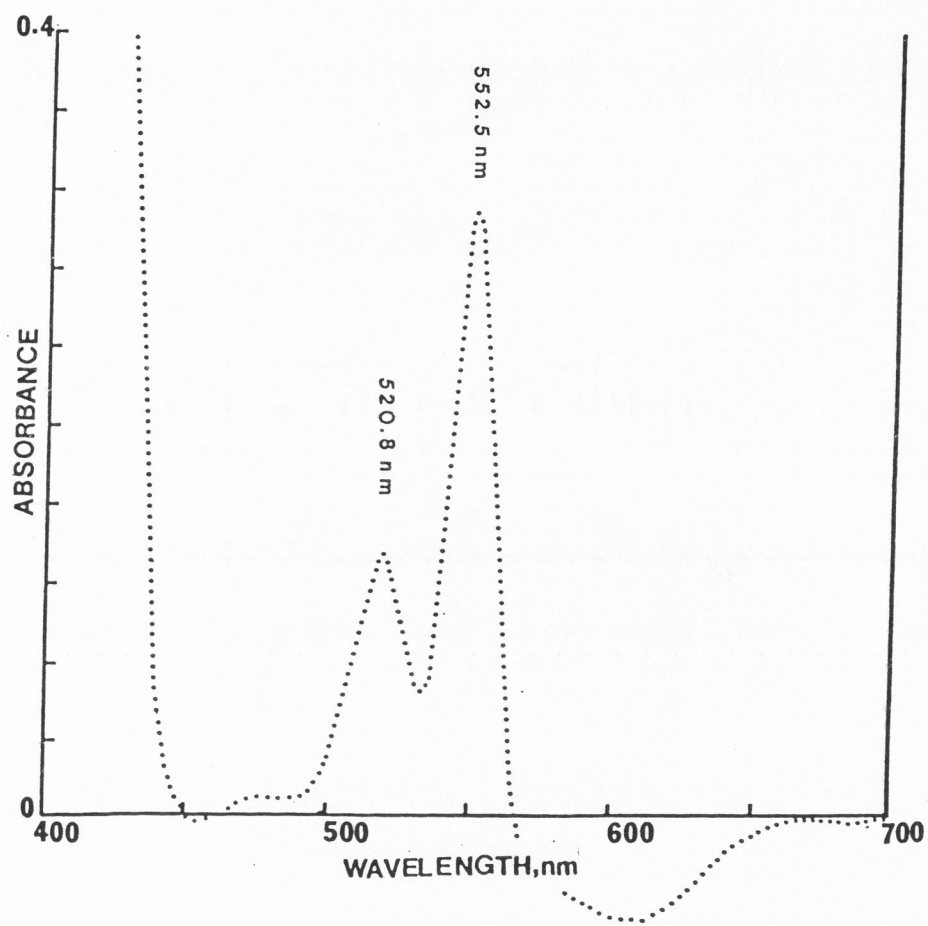


Figure 8. Absorption spectra of the hemochrome formed with nicotinic acid (40 mM) and hematin (1 ug/ml), pH 12.5. Curve is representative of at least four trials.

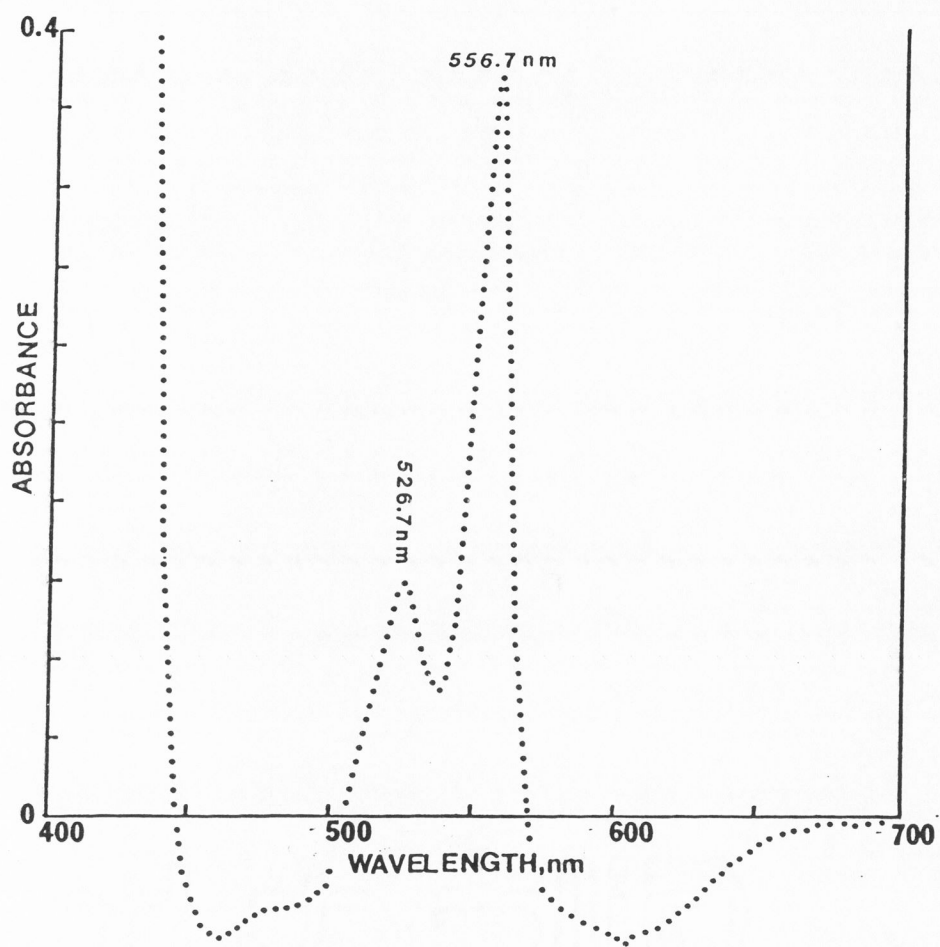


Figure 9. Absorption spectra of the hemochrome formed with turkey albumin (400 ppm) and hematin (1 ug/ml), pH 12.5. The curve is representative of at least four trials.

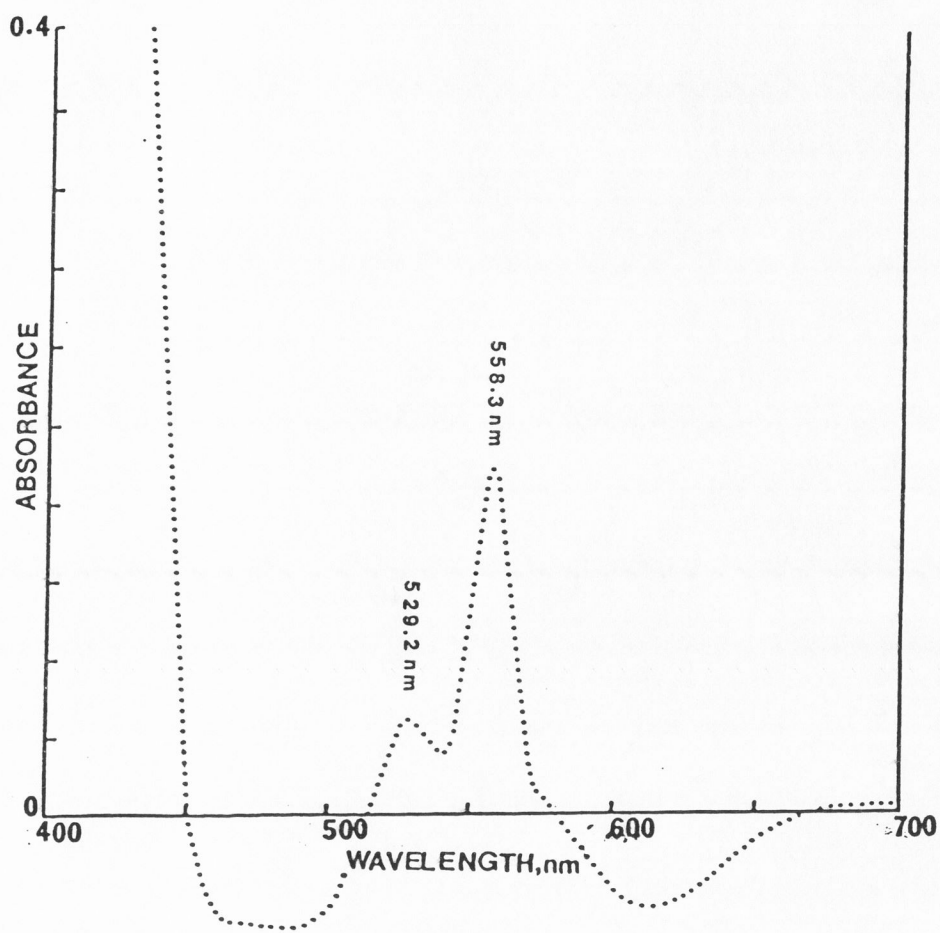


Figure 10. Absorption spectra of hemochrome formed with denatured-globin (200 ppm) and hematin (1 ug/ml), pH 11.0. The curve is representative of at least four trials.

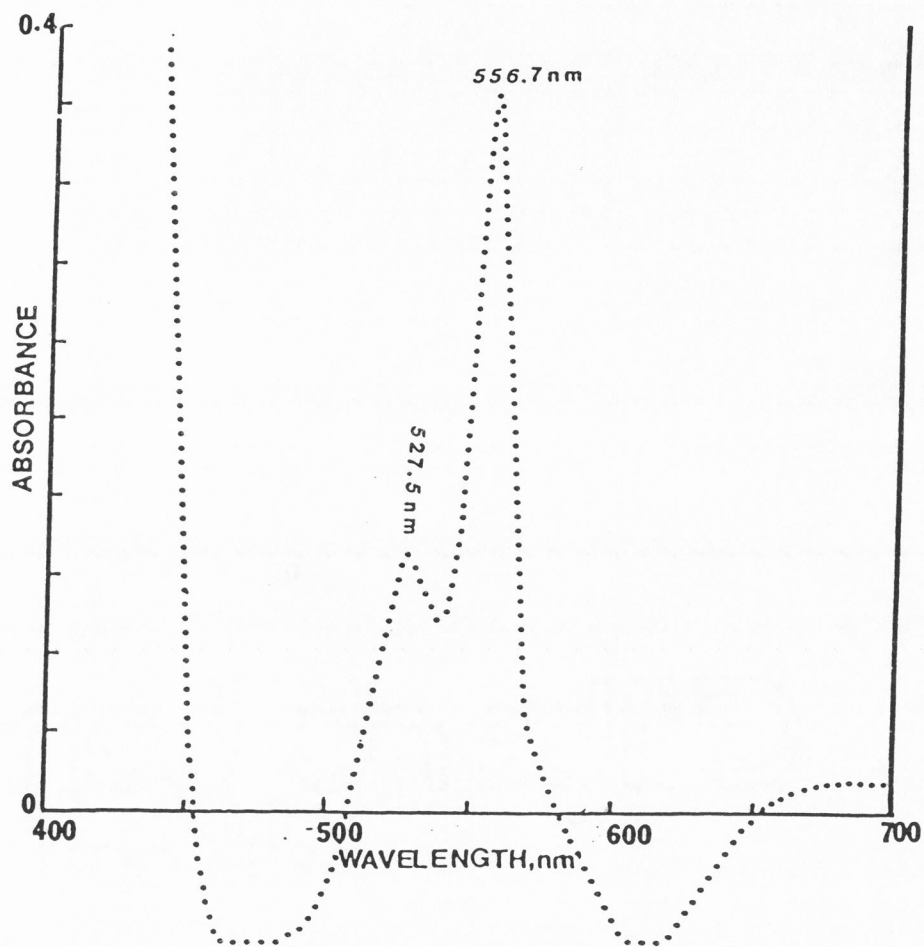


Figure 11. Absorption spectra of the hemochrome formed with non-fat dry milk (1000 ppm) and hematin (1 ug/ml), pH 12.5. Curve is representative of at least four trials.

to expanded d orbitals. This, along with its relatively many d electrons, enables pi back-bonding with suitable ligands. In contrast, ferric iron, with its high nuclear charge, does not engage in strong back-bonding (Livingston and Brown, 1981).

Williams (1956) states that the wavelengths of the absorption maxima are shorter the more electronegative (electron-withdrawing) the cation. Also, the alpha-band intensity is greater with greater electron donor character of the cation (via pi back-bonding). He further states that these same principles hold true for ligand-metal complexes, in which the effective electronegativity is the result of ligand-metal interactions. Electronegativity is made up of two parts: the electron-acceptor properties of the ferrous iron (sigma bonds) and its donor properties (pi back-bonding). He further states that a base such as pyridine moves the absorption bands to shorter wavelength and increases the intensity of the alpha-band to a much larger value than found in the complexes of small, unsaturated molecules such as oxygen, nitric oxide, or carbon monoxide.

Brown and Tappel (1957) found that nitroso hemochrome had diffuse maxima at 540 and 565 nm in water solution. In this study, nicotinic acid, albumin, NFDM, denatured-globin, and nicotinamide had intense alpha-bands at 552.5, 556.7, 556.7, 558.3, and 563.2 nm, respectively. They also had a definite beta-band, which together with the alpha-band constitute the characteristic double peak spectra of hemochromes.

Table 2 shows the effects of pH and concentration on hemochrome formation in test solutions containing 5 mg hemin, and reduced with dithionite. Only nicotinamide at 5.6 mM gave pink color at pH 5.5 or

Table 2. Effects of pH and concentration on hemochrome formation in solution.

Compound	Concentration	pH 5.5	pH 6.0	pH 8.5	pH 12.5
Nicotinic acid	5 mM	-	-	-	-
	10 mM	-	-	+	+
	20 mM	-	-	+	+
	40 mM	-	-	-	+
Nicotinamide	1.4 mM	-	-	+	+
	2.8 mM	-	-	+	+
	5.6 mM	+	+	+	+
Albumin (turkey)	100 ppm	-	-	-	+
	200 ppm	-	-	-	+
	300 ppm	-	-	-	+
	400 ppm	-	-	-	+
Arginine-HCl	10 mM	-	-	-	-
	20 mM	-	-	-	-
	40 mM	-	-	-	-
	80 mM	-	-	-	+
	160 mM	-	-	+	+
Thiamine-HCl	10 mM	-	-	-	+
	20 mM	-	-	-	+
	40 mM	-	-	-	+
	80 mM	-	-	-	+
Non-fat dry milk	200 ppm	-	-	-	-
	1000 ppm	-	-	-	+
	2000 ppm	-	-	-	+

(+ indicates presence of pink color). Each observation was repeated at least once.

6.0. Nicotinic acid at higher concentration (10 mM and 20 mM) gave pink solution at pH 8.5 and 12.5 arginine at 160 mM produced pink color at 8.5. Albumin, thiamine, and NFDM solutions were only pink at pH 12.5.

Incidence of Pink Color in Cooked Turkey Rolls Formulated with Various Nitrogenous Compounds

Pink color was observed in cooked turkey rolls formulated with 10 or 156 ppm (0.001 or 0.0156%) sodium nitrite, 2% nicotinamide, or 3% nicotinic acid but not for rolls formulated with arginine, thiamine, albumin or NFDM (Table 3). In preliminary tests, it was observed that pink development was possible at somewhat lower levels; 1.4% and 2.2% for nicotinamide and nicotinic acid respectively. A high concentration of nicotinamide or nicotinic acid is needed for pink color, compared to nitrite, because these nitrogenous bases dissociate from heme more rapidly, due to weaker pi back-bonding with heme iron, compared to nitric oxide (Livingston and Brown, 1981). This also accounts for the more rapid fading of samples formulated with nicotinamide or nicotinic acid, compared to nitrite. Since nicotinamide or nicotinic acid dissociate more rapidly, molecular oxygen can more rapidly oxidize the heme iron of the dissociated heme-ligand complex.

Reflectance Spectra of Cooked Turkey Rolls

Reflectance spectra of turkey rolls formulated with 10 or 156 ppm sodium nitrite, 2% nicotinamide, or 3% nicotinic acid are shown in

Table 3. Pink color development and pH of the turkey rolls processed with various nitrogenous compounds.

Treatment	Concentration (%)	Intensity of pink color	pH (after cooking)
no additive	-	-	6.15 ^a
sodium nitrite	0.001	+++	6.20
sodium nitrite	0.0156	+++	6.22
nicotinamide	2	++++	6.23
nicotinic acid	3	++++	4.85
arginine-HCl	3	-	6.16
thiamine-HCl	3	-	5.48
albumin	2.4	-	6.26
non-fat dry milk	5	-	6.20

^aEach value is the mean of 1 sample done in duplicate.

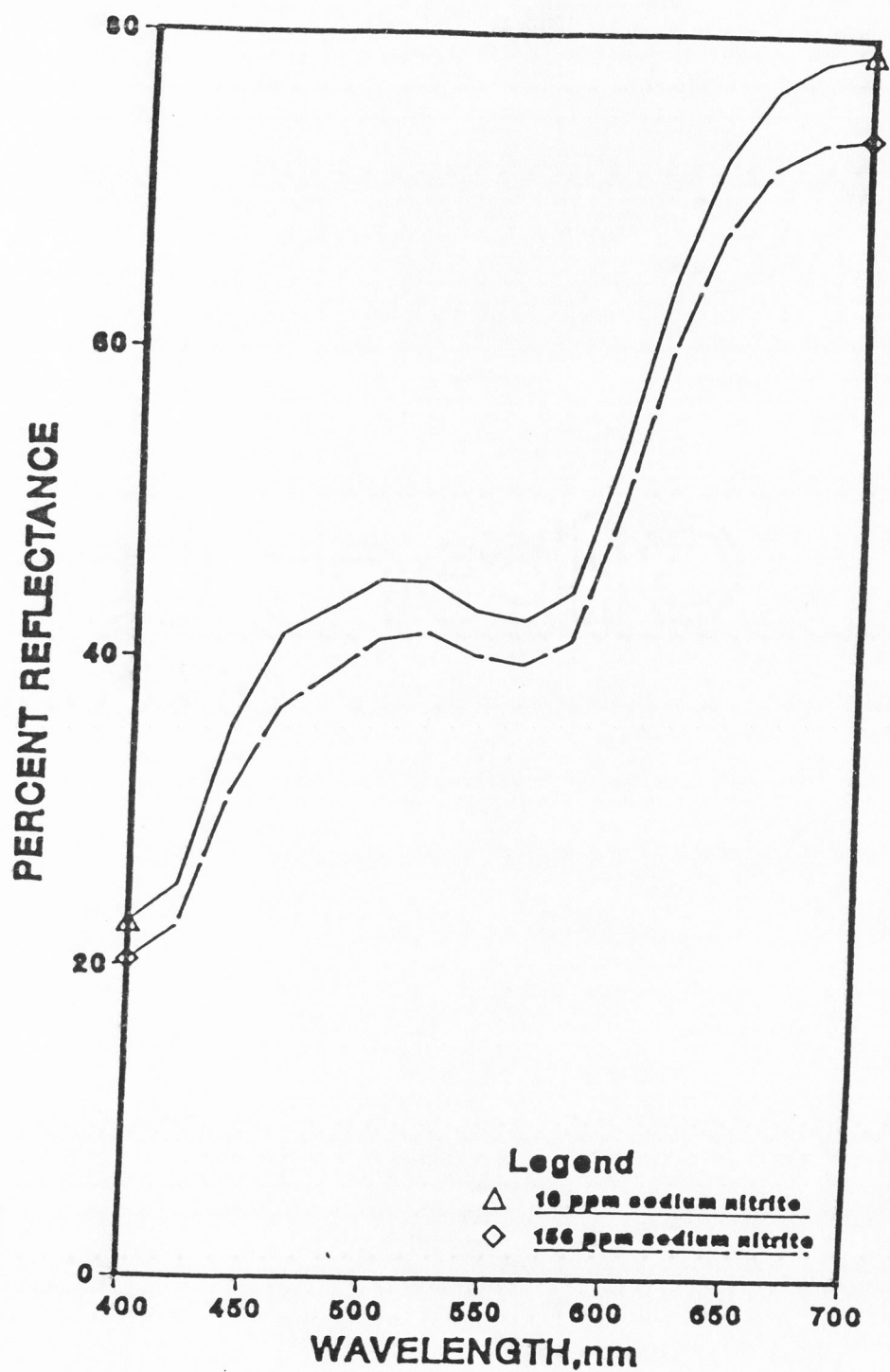


Figure 12. Reflectance spectra of cooked turkey slices formulated with 10 or 156 ppm sodium nitrite. Curves are representative of two samples done in duplicate.

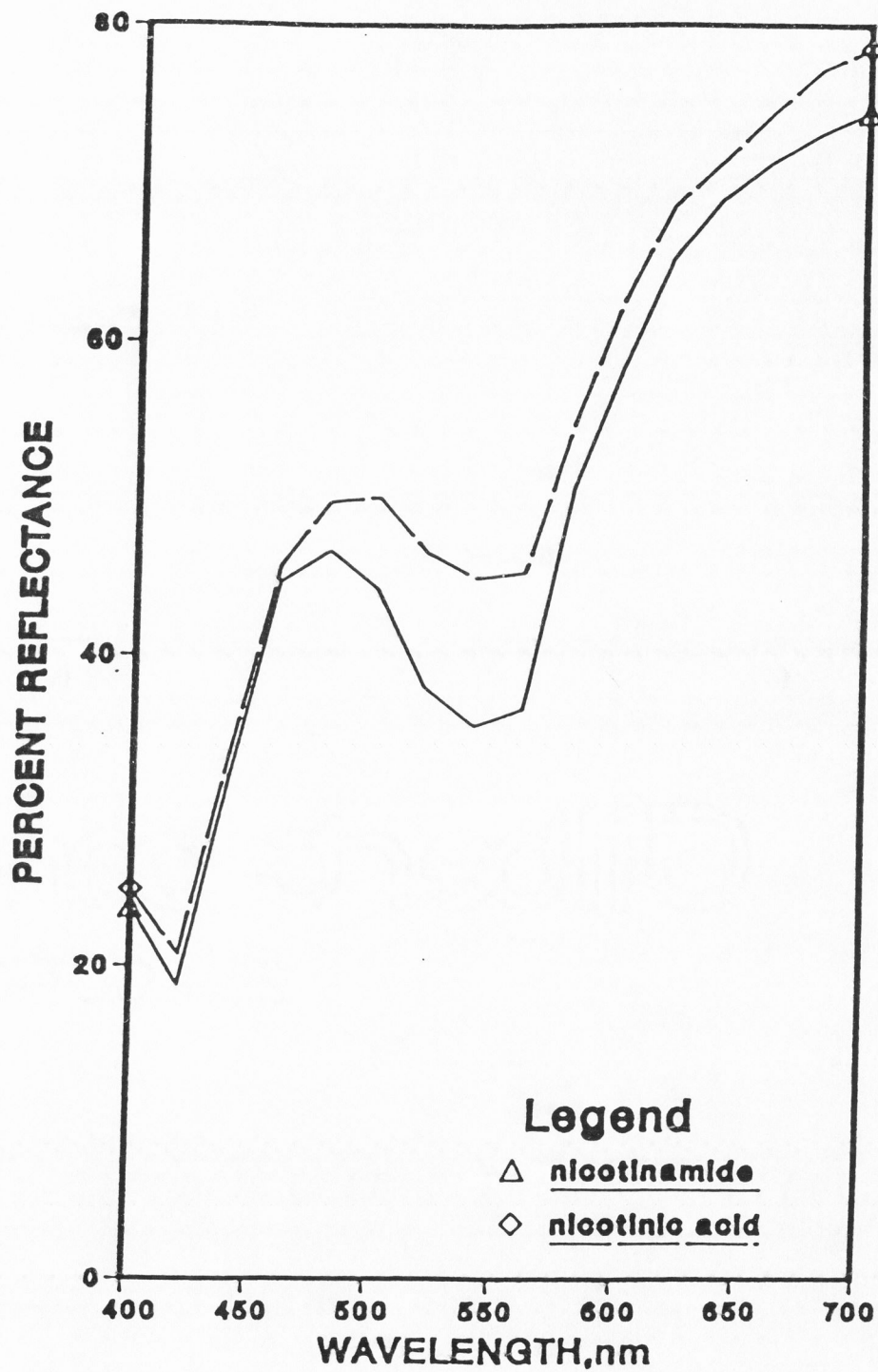


Figure 13. Reflectance spectra of cooked turkey slices formulated with 2% nicotinamide or 3% nicotinic acid. Curves are representative of two samples done in duplicate.

Figures 12 and 13. The spectra of nitrite cured turkey rolls show reflectance minima at 400 and 560 nm which correspond to the absorption maxima of the Soret and alpha-bands in the absorption spectra at similar wavelength, respectively (Figure 12). The reflectance minima in the spectra for nicotinamide and nicotinic acid, however are at 420 and 540 nm.

These spectra agree well with previous work. Tappel (1961) showed that reflectance minima of 575 nm (alpha-band), 553 nm (beta-band), 484 nm, and 405 nm (gamma or Soret band), were characteristic for cooked cured meats. In this study, reflectance minima at 553 and 575 nm were observed as a single minimum at 560 nm, and the 484 nm minimum was not observed. This is due to fact that the Hunter spectrophotometer used in this study scanned the visible spectrum in 20 nm increments, apparently averaging the alpha and beta minima at 575 and 553 nm into a single minimum at 560 nm.

Brown and Tappel (1957) found that reduced (pink) canned tuna exhibited reflectance spectra with minima at 555, 530, and 415 nm. Tappel (1957a) stated that nicotinamide hemochrome had reflectance minima at 556, 524, and 423 nm. In this study reflectance minima were observed at 520-560 nm and at 420 nm (Figure 13). The broad minima from 520-560 nm are again likely due to instrument averaging at the two minima at 524 and 556 nm.

Reflectance spectra for commercial turkey rolls with pink defect are shown in Figure 14. This spectra is virtually identical to that for turkey rolls formulated with nicotinamide (Figure 13), with a broad reflectance minima at 540-560 nm, and also at 420 nm. The

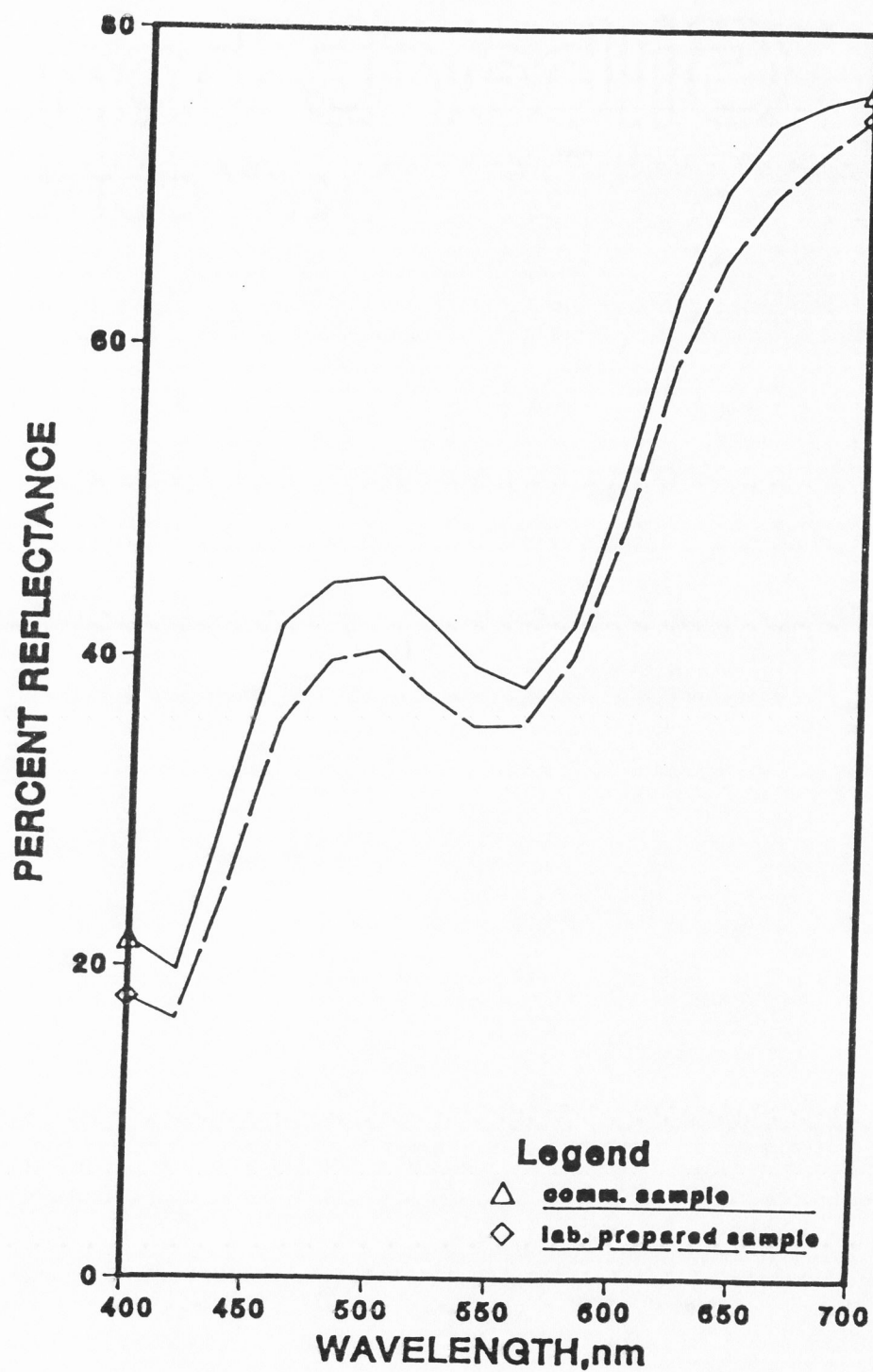


Figure 14. Reflectance spectra of pink colored commercial and laboratory prepared samples. Curves are representative of two samples done in duplicate.

spectra for a laboratory prepared turkey roll having pink defect are also shown in Figure 14 and are very similar to the spectra of the commercial sample. The spectra for a control, non-pink sample and a dithionite-treated control (pink) are shown in Figure 15. Control samples also have reflectance minima at about 540-560 nm and at 420 nm. However, control samples have a much smaller reflectance maxima at 480 nm. Control samples after treating with dithionite turned pink, and again exhibit the typical hemochrome reflectance spectra. These results indicate that the pink pigment of commercial samples with pink defect is a hemochrome.

The reflectance difference spectra (as compared to a non-pink control) are shown in Figure 16 for samples formulated with 2% nicotinamide, 156 ppm sodium nitrite, or commercial product with pink defect (negative values indicate lower reflectance at a particular wavelength than the control). The nicotinamide sample, the commercial sample, and the reduced control were all pink and had similar shaped spectra with minima at about 570-590 nm. In contrast, the nitrite containing sample had a definite peak at about 550 nm. The oxidized nicotinamide sample was not pink, and the resultant difference spectra were flat, indicating essentially no difference between this sample and the control (Figure 16).

Oxidation-Reduction Titration

The potentiometric oxidation-reduction titration curves for nicotinamide hemochrome and denatured-globin hemochrome are presented in Figure 17, and inflection points (equivalence points) in both

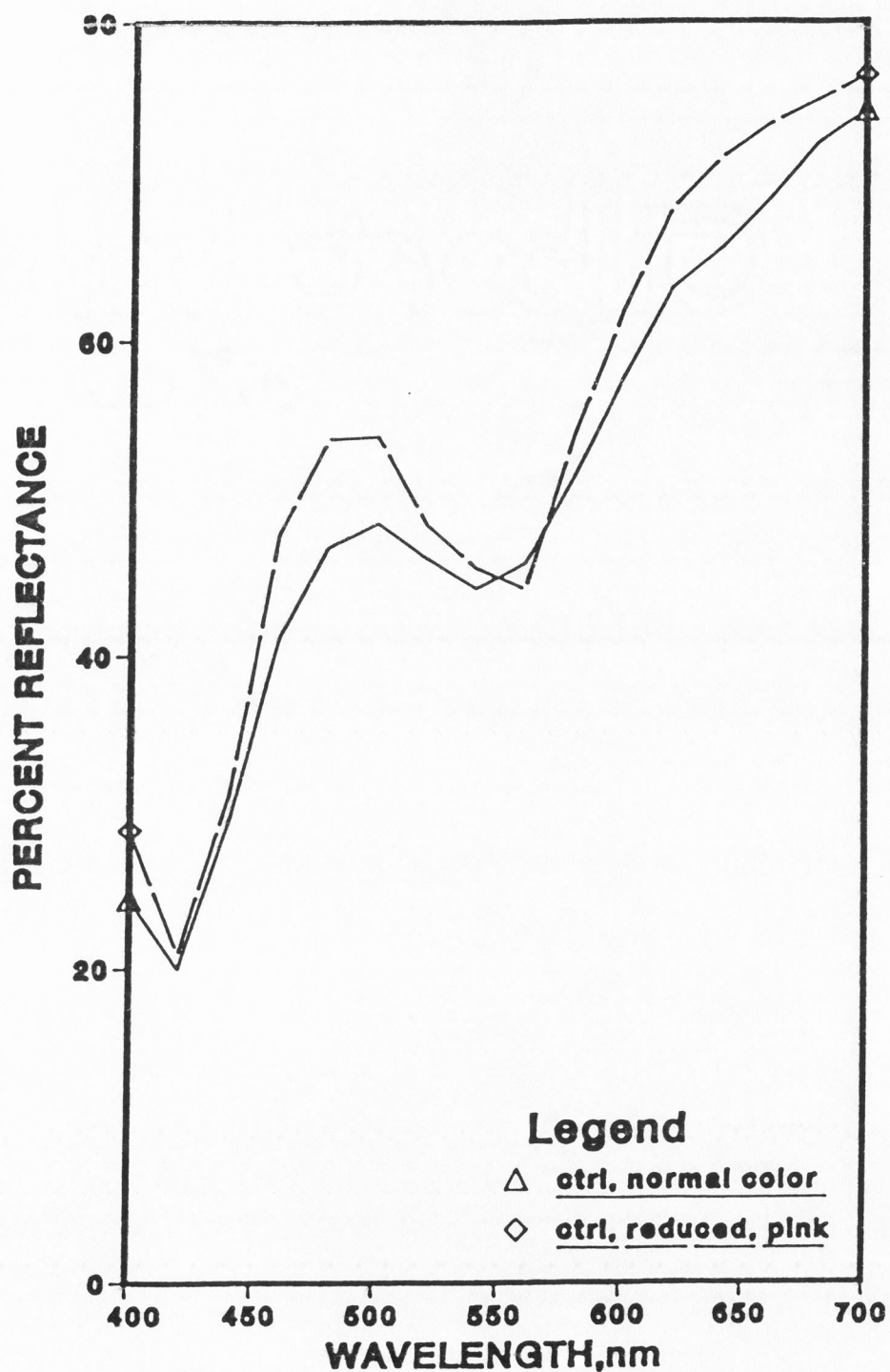


Figure 15. Reflectance spectra of control (ctrl, normal color) and dithionite reduced (ctrl, reduced, pink), turkey slices. Curves are representative of two samples done in duplicate.

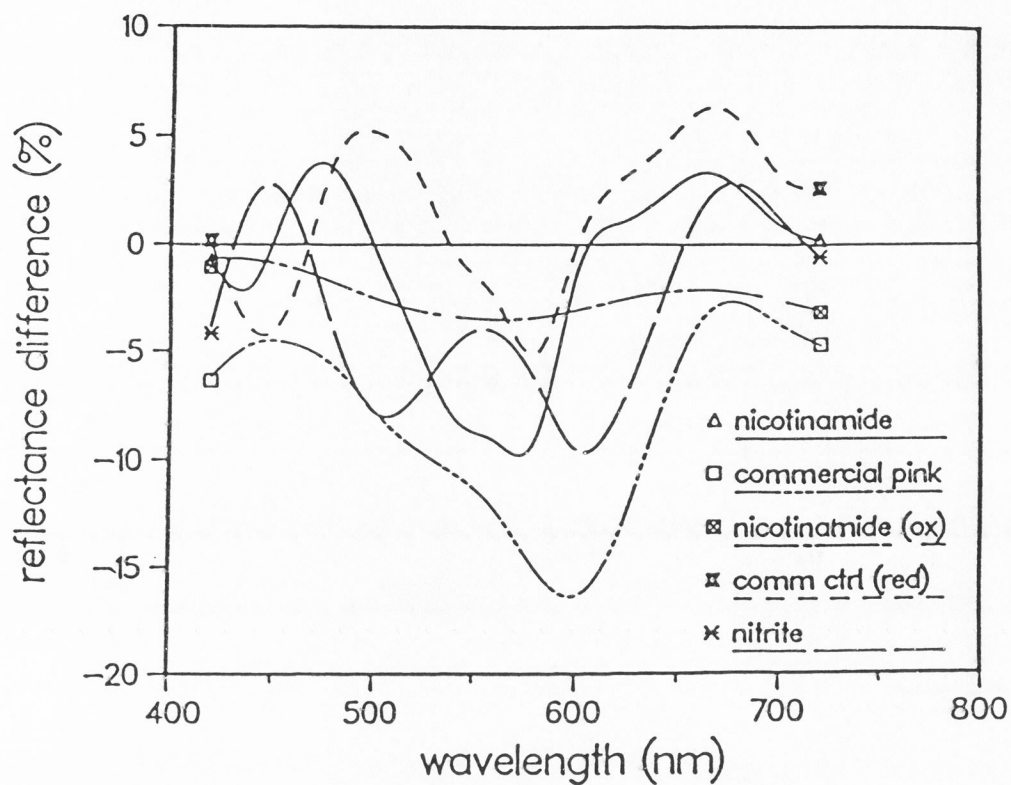


Figure 16. Reflectance difference spectra of commercial and laboratory prepared slices of cooked turkey rolls; (a) 156 ppm sodium nitrite, (b) 2% nicotinamide, (c) commercial sample with pink defect, (d) dithionite reduced commercial sample, (e) oxidized nicotinamide (2%). Curves are representative of two samples done in duplicate.

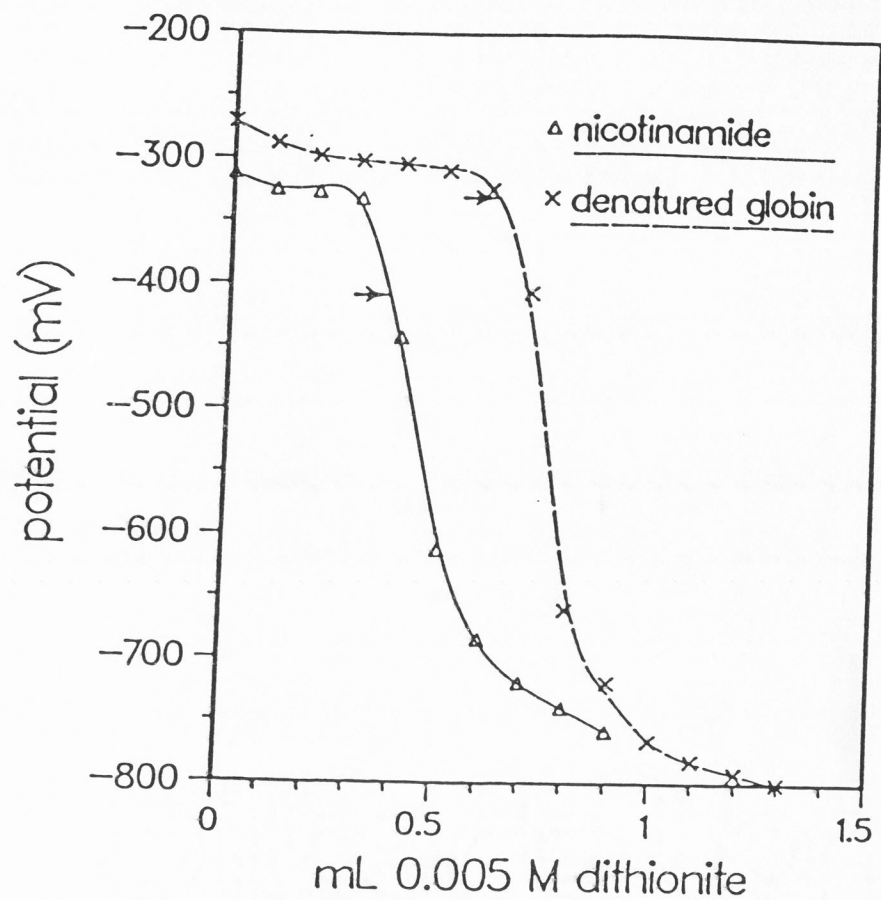


Figure 17. The potentiometric oxidation-reduction titration curves for nicotinamide and denatured-globin-hemochromes. Arrows indicate oxidation-reduction potential at which pink color is first observed. Curves are representative of two samples done in duplicate.

curves are at -550 mV, relative to the hydrogen electrode. The hemichrome/hemochrome are active redox systems and exhibit typical titration curves upon addition of a strong reducing agent such as sodium dithionite. The oxidation-reduction state of the hemochrome /hemichrome is the determining factor in the formation of pink color. Table 4 lists the ORP value at which the first sign of pink could be seen for various hemochromes in solution. At an ORP value of -550 mV or less all of the heme iron is in the ferrous state giving only the pink hemochromes. Titration curves for the other hemochromes listed in Table 4 indicated similar inflection points. Therefore the variable ORP values at which pink appeared for different hemochromes (Table 4) is most likely due to difference in molar absorptivity of each hemochrome and/or its concentration.

Table 5 gives the original color of different meat samples, their ORP values after homogenization, and the potential at which the slurry turned pink during titration with dithionite. The inclusion of an oxidizing agent (KIO_3) in the preparation of the samples with added nicotinamide resulted in a higher redox potential and prevented the formation of the pink color. The normal commercial sample had a higher redox potential than the sample with pink defect but would turn pink upon lowering the ORP value with dithionite. Lowering ORP values promoted hemochrome formation.

In relation to oxidation-reduction potentials of hemoproteins, Williams (1956) states that the most electrophilic (weak base) substituents, such as the nitro groups, give complexes with the lowest oxidation potential. Nitrite and nitric oxide are among the most

Table 4. Oxidation-reduction potential at which pink color is first observed for various hemochromes in solution.

Hemochrome	ORP (mV)
nicotinamide (5.6 mM)	-412 ^a
turkey albumin (400 ppm)	-351
non-fat dry milk (2000 ppm)	-377
denatured globin (100 ppm)	-321
arginine-HCl (160 mM)	-511

^aeach value is the mean of three samples.

Table 5. Color of meat samples and corresponding oxidation-reduction potential.

Sample	Initial		Dithionite addition	
	ORP (mV)	Color	ORP (mV)	Color
Turkey roll + 2% nicotinamide	-274	pink		
Turkey meat + KIO ₃ + 2% nicotinamide	-054	white	-389	pink
Commerical Turkey roll (pink)	-417	pink		
Commercial turkey roll (normal)	-374	white	-529	pink

electrophilic substances (Sykes, 1975) and they develop pink color in cooked meats with even 5 ppm (Ingram, 1974). Nicotinamide is an unsaturated nitrogenous compound with strong basic properties (Tarladgis, 1962a). All indications then are that the constituents of pink color defect are present in turkey meat and that the variable most affecting the appearance of pink defect appears to be the ORP of the meat.

CONCLUSIONS

A nitrite or nitric oxide contamination is the most accepted explanation for pink color defect in cooked turkey rolls. In this study, residual nitrite and nitrosyl hemochrome were not detected in either commercial or laboratory prepared samples exhibiting pink color defect. The pink pigment could not be extracted in acetone and/or water, although a pink colored exudate was obtained in some commercial samples. Turkey rolls made in this study were carefully prepared to exclude any possible nitrite contamination, and yet showed occurrence of pink defect. It is thus concluded that pink defect is usually not due to nitrite contamination.

Another possibility is the formation of various hemochromes, having any of several nitrogenous bases, during cooking. Pink colored pigment of canned tuna has been identified as a hemochrome complex with either nicotinamide or denatured-globin or both (Brown and Tappel, 1957). In this study, the reflectance spectra of the samples with pink defect (commercial or laboratory made turkey rolls) exhibited a pattern of a hemochrome complex. Moreover, the samples with pink defect had similar shaped difference spectra with minima at about 570-590 nm. In contrast samples formulated with sodium nitrite had a definite peak at about 550 nm.

The pink hemochrome in canned tuna is not extractable in several solvents including water and acetone-ether (Brown and Tappel, 1957). Insolubility of the pink defect pigment in the acetone-water solvent of the Hornsey nitroso pigment extraction procedure (1956) as observed in this study, is a characteristic of association of denatured-globin

with heme iron in a hemochrome complex (Tarladgis, 1962b). For the observed pink color in the turkey roll formulated with nicotinamide, the iron must be in reduced state (Livingston and Brown, 1981). Furthermore, white turkey meat has a high concentration of nicotinamide (niacin) as compared to red meats. Niacin is one of the most heat stable vitamins (Fennema, 1976). Niacin is present in white turkey meat at a level of 8.322 mg% while the level of niacin is 4.38, 4.66, and 4.05 mg% in beef, lamb, and pork respectively (Lawrie, 1974; Richardson et al., 1980). Most likely then, the pink defect pigment of cooked turkey samples is a mixed hemochrome in which a denatured-globin and nicotinamide are bonded with the ferrous iron through a weak pi back-bonding allowing for rapid dissociation thus rapid oxidation and fading in air.

The reducing condition of the meat is important in formation of hemochromes, and therefore the development of pink defect. Commercial samples and laboratory prepared turkey rolls with pink color had lower ORP values as compared to non-pink control samples. Control samples had the capability of producing pink pigment when the ORP of the system was lowered, thereby promoting formation of hemochromes. Adding an oxidizing agent was observed to prevent pink color formation by increasing the ORP of the system. These results indicate that the constituents of pink color defect are present in turkey meat and that the variable most affecting the appearance of pink defect appears to be the ORP of the meat.

More research is needed to determine processing conditions or approved additives necessary to maintain mildly oxidizing conditions

in turkey meat, thus preventing formation of pink defect under commercial conditions.

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VITAE

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