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FOOD PROCESSING

The Chemistry of Meat Pigments

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The reactions of the heme pigments of meat, myoglobin and hemoglobin, are important in determining the colors of fresh and cured meats. The effect of the partial pressure of oxygen, the means by which oxidized pigments are re-reduced, and the thermodynamic and electrochemical requirements for the interconversion of the three pigments of fresh meats—myoglobin, metmyoglobin, and oxymyoglobin—are covered. The mechanisms of conversion of the native pigments of meat to the stable red pigments of cured meats are described and the effects of various conditions on the conversion are discussed. The known reactions which produce green heme pigments are related to the development of off-colors and "greening" in both fresh and cured meats.

The chemistry of the color of meat is the chemistry of the heme pigments, myoglobin and hemoglobin, which, in so far as meat color is concerned, are identical in their reactions (Figure 1). The number of reactions are not many, but they are governed or produced by a wide variety of conditions. This paper is concerned with some of the more important of these conditions. The muscle heme pigment myoglobin is the principal but not the whole source of meat color. Even in a well bled piece of meat, hemoglobin, the blood pigment, will comprise 20 to 30% of the total pigment present, sometimes more. Although most of the reactions of the two pigments are identical, several reactions of importance in meat color, such as autoxidation, reaction with nitrite, and denaturation, have different rates for the two pigments. It therefore seems highly improbable that color changes or color intensity in meat with pigment concentration could be correlated without considering the quantities and reactivities of both hemoglobin and myoglobin.

Color Cycle in Fresh Meats

This is a dynamic cycle and in the presence of oxygen the three pigments, oxymyoglobin, myoglobin, and metmyoglobin, are constantly being interconverted. The take-up of oxygen by myoglobin converts the purple reduced pigment to the bright red oxygenated pigment, oxymyoglobin. This process produces the familiar "bloom" of fresh meats; at high oxygen pressures the reaction, as written in Figure 1, is shifted mainly toward the left. The red complex, once formed, is stabilized by the formation of a highly resonant structure; and as long as the oxygen remains complexed to the heme, the pigment will undergo no further color changes. However, the oxygen is continually associating and dissociating from the heme complex, a process which is accelerated by a number of conditions, among them low oxygen pressures. When this occurs the reduced pigment is subject to oxidation by oxygen or other oxidants. It is not exactly known whether the oxidation takes place during association or, as is indicated by the dashed arrow in Figure 1, during dissociation. Regardless of how it is accomplished, there is a slow and continuous oxidation of the heme pigments to the met form. When the meat is fresh, the production of reducing substances endogenous to the tissue will constantly re-reduce the pigment to the purple form, and the cycle continues if oxygen is present. The two areas of

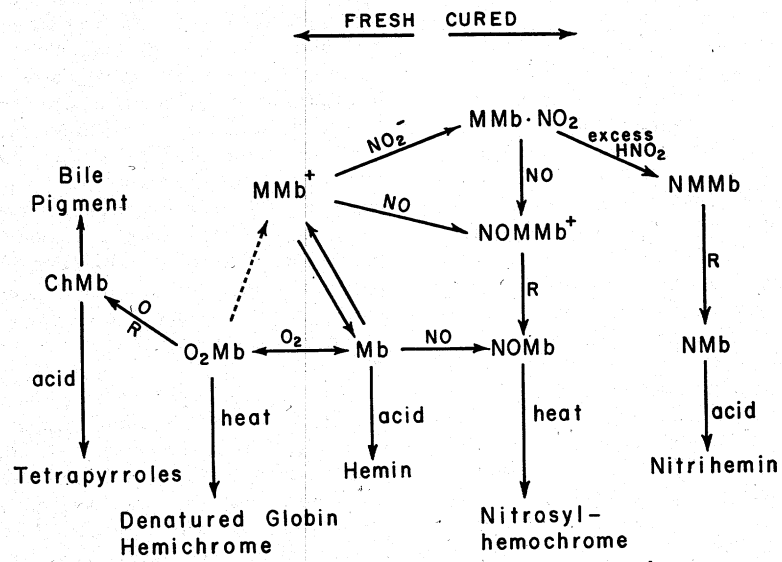

Figure 1. Heme pigment reactions of meat and meat products

ChMb, cholemyoglobin (oxidized porphyrin ring); O_2Mb, oxymyoglobin (Fe^{+2}); MMb, metmyoglobin (Fe^{+3}); Mb, myoglobin (Fe^{+2}); MMb.NO_2, metmyoglobin nitrite; NOMMb, nitrosylmetmyoglobin; NOMb, nitrosylmyoglobin; NMMb, nitrimetmyoglobin; NMb, nitrimyoglobin, the latter two being reaction products of nitrous acid and the heme portion of the molecule; R, reductants; O, strong oxidizing conditions

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interest or importance in this cycle are the oxidation of heme pigments at low oxygen pressures and the determination of the mechanisms whereby the heme pigments are reduced.

The phenomenon of the accelerated rate of oxidation of the heme pigments at low partial pressures of oxygen was first described by Neill and Hastings (*13*) for hemoglobin. Later Brooks (*3*) and George and Stratmann (*8*) further defined the reaction and found that the maximum rate of conversion occurred at partial pressures of oxygen in the range of 1 to 20 mm. of Hg, depending upon the pigment, pH, and temperature. This effect is important when considering packaging films for fresh meats. As the oxygen permeability of the film decreases, a partial pressure is reached where oxygen utilization by the tissues balances oxygen penetration at a pressure level which favors the oxidation reaction. Landrock and Wallace (*12*) determined that a packaging film must have an oxygen penetration rate of 5 liters of O_2/sq. meter/day/atm. to prevent such browning. If the packaging material were to be completely oxygen-impermeable, the metabolic processes would eventually stop for lack of oxygen, with the heme pigments in the fully reduced state. Upon re-exposure to air, the reduced pigments would oxygenate and the meat would "bloom."

Constantly improving sanitation techniques, automated methods of meat handling, pasteurization and sterilization processes, and other advances in the art and practice of fresh meat handling have led to increasing shelf lives for fresh meats. To keep pace, information is needed on the factors which govern color stability. The area of greatest concern and interest is that of mechanisms whereby the oxidized pigments are reduced, both by endogenous and exogenous reductants and/or reducing systems.

Heme pigments can be reduced by a number of reductants, some of which are endogenous to muscle tissues. A question in current research is whether the observed rates and concentrations are sufficient to account for the in vivo reduction of the oxidized pigments. The only extensive work to date on this subject has been done on blood components, the intact erythrocyte and isolated hemoglobin. Endogenous reductants can account for the observed recovery rates of methemoglobin to hemoglobin in vivo, but the reductants that can accomplish the reduction are not usually found in erythrocytes. Kiese (*11*) found evidence for an enzyme in red blood cells, a TPNH oxidase, which can use either oxygen or methemoglobin as an electron acceptor. Further work led to the isolation of an enzyme, but Beutler and Baluda (*2*) have shown that diffusable reductants in the serum can reduce methemoglobin, and it may be that the reduction is partly enzymatic and partly nonenzymatic.

A similar picture appears when we examine work on the muscle pigment and muscle tissue. Although it is assumed that the muscle pigment autoxidizes in vivo like the blood pigment, the rate of autoxidation and quantity of the oxidized pigment have not been established, so that there is no criterion for the requisite reducing capacity in tissue. Walters and Taylor (*19*) and Stewart *et al.* (*16*) found that the reduction of metmyoglobin apparently involves an enzyme system because the addition of iodoacetate and 5% salt, respectively, inhibited the reduction. Work is in progress to define further the mechanisms involved.

Because they have a highly complex resonant structure, centered about an iron atom, heme pigments have special requirements in so far as reduction-oxidation reactions are concerned. The reduction of an oxidized heme pigment is governed by the basic thermodynamic requirements that the free energy change favors the reaction and that the activation energy is not so great that the reaction will take place only at high temperatures, where denaturation of the protein occurs. More specifically, the reductant and the heme pigment must be sterically capable of reacting to form a reactive intermediate. Lastly, the reductant must transfer the same number of electrons as the heme pigment; or one of them must be capable of accepting or giving an intermediate number of electrons, thus forming a radical-type intermediate. From the kinetics of the reduction of methemoglobin using cysteine and ascorbate as reductants, Kiese (*11*) concluded that both cysteine and ascorbate form intermediate complexes with the heme pigment. Both ascorbate and cysteine oxidations involve two-electron steps as opposed to the one-electron reduction of methemoglobin or metmyoglobin, but Fox and Thomson (*6*) and Weil (*20*) have found that both reductants will form a one-electron intermediate. Kiese also found that methylene blue accelerated the cysteine reaction, which is probably a reflection of the ability of methylene blue to form one-electron intermediates readily. We therefore conclude that the reactions which reduce oxidized heme pigments involve the formation of radicals and that the reductants used must be capable of forming one-electron intermediates.

An interesting aspect of the activity of reductants is the oxidation reaction of heme pigments in the presence of reductants. Many reductants, ascorbate in particular, react with the oxygen complexed to heme pigments to form hydrogen peroxide, which then, under conditions not yet thoroughly understood, oxidatively cleaves the prophyrin ring of the heme pigments (Figure 1, left). With hydrogen peroxide alone, the reaction produces bile pigments which are polypyrrolic compounds. In the presence of reductants, the reaction appears to be more or less slowed down and appreciable amounts of the green pigment, choleglobin, are observed. If the reductant is a sulfhydryl compound, the reaction apparently involves the addition of a sulfur atom to the heme ring. These mechanisms, or similar ones, are pathways of heme pigment breakdown in fresh meats and in part in cured meats as well, but the exact mechanisms are not known. Reports in the literature do not help much in this regard, for although most authors have found that the reaction proceeds to an appreciable extent only above pH 7, outside the physiological range, some workers have observed appreciable green pigment formation (cholemyoglobin) and heme pigment oxidation at pH 5 to 7.

Pigments of Curing and Cured Meats

In the presence of nitrite, nitric oxide, and reductants a number of different pathways can lead to the cured meat pigment, nitrosylhemochrome (Figure 1). All these reactions have been observed in vitro, but most of them take place only under fairly strong reducing conditions, since many of the intermediates—for example, nitrosylmetmyoglobin—are unstable in air. When the system in which the cured meat pigment is formed contains nitrite, the heme pigments will be in the oxidized state initially, nitrite being a strong heme pigment oxidant. All these reactions are faster the more acid the conditions, a situation which is also true for the formation of the cured color in meat. For purposes of study, the formation of the cured meat chromophore is generally viewed as two processes, the biochemical reduction reactions which reduce nitrite to nitric oxide and the iron in the heme to the ferrous state, and the thermal denaturation of the protein portion of the molecule. The latter occurs only when the cured meat product is heated to 150° F. or higher and may involve the coprecipitation of the heme pigment with other proteins in the meat. Although the mechanisms involved in the complete reaction have not been determined in meat or meat products, it has been established that the end product is either nitrosylmyoglobin if uncooked, or the denatured globin nitrosylhemochrome if cooked. Some question has been raised recently concerning the identity of the cooked pigment as a denatured globin derivative. Tarladgis (*17*) has presented arguments, based on the correlation of position of the optical absorption maxima to the bond type in iron-porphyrin compounds, to the effect that the cured meat pigment is the dinitrosylhemochrome—that is, two nitric oxide molecules complexed to the heme. Although the evidence is strong, stoichiometric experiments are needed to establish the point.

Perhaps the most important but least understood part of the sequence has been the formation of nitric oxide from nitrite, and recent work has elucidated some of the mechanisms of formation. The endogenous reducing systems in meat have been studied by Walters and Taylor (*18*), who found that nitric oxide forms in the mitochondria and is coupled to the cytochrome *c* oxidation-reduction system. They found evidence for the formation of a nitric oxide–ferricytochrome *c* complex and postulated the reaction to be the nitrite oxidation of ferrocytochrome *c* with formation of the complex. Subsequent reduction of the complex releases nitric oxide, which is then available for formation of the nitrosylhemochrome. Work in this area is continuing to define further the reaction and its relationship to the reduction of nitrite in cured meat products, as well as to investigate other possible endogenous nitrite-reducing systems.

The mechanisms of the reduction of nitrite by exogenous reductants have been investigated by Fox and Thomson (*6*), who found that the formation of nitric oxide could not be accounted for by the termolecular dismutation of nitrous acid, but requires the presence of a reductant. They demonstrated that the reaction proceeds through the formation of an intermediate radical-type complex of nitrite and reductant with subsequent decomposition to form nitric oxide, the complexing of the latter with the oxidized form of the heme pigment, and reduction of the nitrosylmetheme pigment. Continuing these studies, Fox and Ackerman (*5*) investigated the effect of one-electron transfer reducing agents on the formation of nitrosylmyoglobin from nitrite and heme pigments. A number of quinoid-type structures were investigated, but none were so effective as ascorbate. Several combinations of reductants were tried, and it was found that ascorbate plus cysteine increased the initial velocity of the reaction, introducing a curvature in the rate curve (Figure 2). The reaction is normally linear with respect to time if only one reductant is used. However, the conversion of metmyoglobin to nitrosylmyoglobin by ascorbate was completed in the same time, whether or not cysteine was present. As shown in Figure 3, the first part of the reaction, above the broken line, involves the production of nitric oxide from nitrous acid and a reductant, in this case ascorbate. When ascorbate is used as the reductant, the intermediate complex will react in two ways: decompose to yield the initial reagents, nitrous acid and ascorbic acid; or, decompose to yield nitric oxide and oxidized ascorbic acid. Ascorbate is a much more effective reductant than cysteine for this reaction and, in a mixture of the two with nitrite, the production of nitric oxide is dependent upon the concentration of ascorbate. However, cysteine was almost as effective as ascorbate for the reduction of the reaction intermediate, nitrosylmetmyoglobin. If only ascorbate is used, the rate of the reaction is constant with time—that is, the reaction is of zero order with respect to the heme pigment. This lack of rate dependence upon the concentration of the heme pigment indicates that the reaction intermediate, nitrosylmetmyoglobin, remains at constant concentration during the reaction. Thus, the formation of nitrosylmyoglobin by ascorbate involves an equilibrium process wherein the formation of nitric oxide and its complexing to metmyoglobin are balanced against the reduction of the nitrosylmetmyoglobin. If cysteine is added, this balance is upset by the increased rate of reduction of nitrosylmetmyoglobin by both ascorbate and cysteine, with no concomitant increase in the rate of nitric oxide formation. This results in an initial surge in the production of nitrosylmyoglobin. However, since the time for conversion of metmyoglobin to nitrosylmyoglobin is dependent upon the rate of production of nitric oxide, governed by the rate of the ascorbic acid–nitrous acid reaction sequence, both of the ascorbate reaction sequences, with and without cysteine, go to completion at the same time.

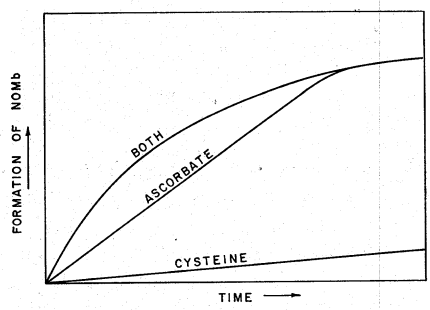

Figure 2. Formation of nitrosylmyoglobin

Generalized reaction for system containing heme pigment, nitrite, and reductant(s)

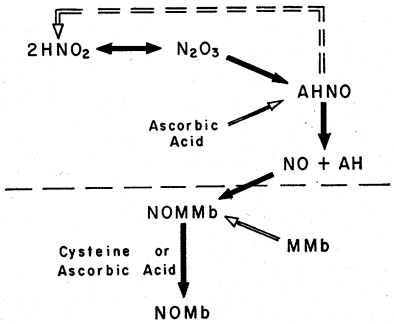

Figure 3. Formation of nitrosylmyoglobin

AHNO. Ascorbic acid–nitrous acid intermediate
AH. Ascorbic acid radical

The pH dependence of the rate of reduction of nitrosylmetmyoglobin was also investigated. The reaction with either ascorbate or cysteine was independent of pH. By comparison, the rate of the reaction of reductants with nitrite is faster under acid conditions, whereas emulsification properties and water retention are improved at neutral or alkaline pH values. Nitric oxide gas introduced directly into meat emulsions will immediately form the nitrosyl derivatives of the two oxidation forms of the heme pigment, metmyoglobin and myoglobin, regardless of pH. The nitrosylmetmyoglobin pigment will then be reduced to the fully reduced form, again regardless of pH. Thus, the use of nitric oxide directly may simultaneously achieve good color production and good emulsions.

The cured meat pigment, once formed, is more stable at higher pH values. Bailey, Frame, and Naumann (*1*) found that the nitrosylhemochrome was more stable to light fading at pH 6.8 than at pH 6.2. Light fading is a two-step reaction, the first being the light-accelerated dissociation of the nitric oxide from the heme, and the second the oxidation of nitric oxide by oxygen. If oxygen is excluded from the meat by the use of an oxygen-impermeable packaging material, the second step does not occur and the color is stable (*15*). Nitric oxide may be regenerated by the action of residual or added reductants and/or nitrite, as demonstrated by Kelley and Watts (*9*). This reaction is slow at neutral pH values. Thus, if fading is slower, so is regeneration; and the question remains to be resolved as to which reaction is the more important to the maintenance of the cured meat pigment.

Little information has been accumulated concerning the chemistry of discoloration of cured meats because of the inherent difficulties in studying color changes in cooked meats and finished products. Most discolorations are commonly referred to as "greening" and usually consist of greenish brown spots on the surface, and of green centers in fermented sausages. Deibel and Evans (*4*) found that these discolorations were always associated with high nitrite levels and/or low pH values, and the problem was almost always cured by avoiding these conditions. The chemical reactions involved in cured meat color discoloration probably follow this sequence: First, nitrosyl heme pigment dissociates, a reaction which may be accelerated by light. The free reduced pigment is then oxidized by excess nitrite to form metmyoglobin, which is probably the pigment of brownish surface discolorations. Recent studies by Fox and Thomson (*7*) showed that, in the presence of high concentrations of nitrite, the heme of the pigment may react further to produce a green nitrited porphyrin ring compound (Figure 1). This may be the pigment of green centers in fermented

sausage. The reaction is greatly accelerated at low pH values, and the compound formed cannot be reconverted to the pink cured meat pigment; it only becomes brighter green on reduction. Upon continued exposure to nitrite, these green intermediates may be even further degraded to yellowish and colorless compounds. These reactions were studied using the protein-heme complex. Because of the difficulties involved, the reaction of nitrite with the corresponding hemochromes has not been so thoroughly investigated. The latter reaction is of interest with respect to discoloration in cooked cured meats. Fox and Thomson (*7*) isolated a nitrite-green hemin, but the reaction has not been observed as the result of direct action of nitrite on a hemochrome.

Conclusions

Much of our knowledge of heme chemistry has come from physiological and technological studies. One of the pressing needs of the field today is to determine to what extent known chemical and biochemical mechanisms are applicable to meat physiology and technology. In many cases this means development of new techniques or improvement of old in order to determine the course of color changes in fresh and cured meats and meat products.

It is clear that progress in all phases of meat processing lies in automation of processes and standardization of the raw material, the live animal, and the products made therefrom. To do this, increasing sophistication is required in chemical and technological studies on all aspects of meat processing, including color chemistry. Perhaps some of the more exciting and pertinent studies in solving meat color problems are being made today in the field of molecular biology, specifically in relating of the structure of a molecule to its function. Such studies in simpler organic molecules have always been the basis for the improvement of desirable properties by structural alteration. With the heme pigments, little work along classical chemical lines has been possible, for the structure of the protein part of the molecule has only very recently been defined. Thanks principally to the work of Perutz and coworkers (*14*) on horse hemoglobin and Kendrew and coworkers (*10*) on whale myoglobin, the structures of the heme pigments are probably the most thoroughly documented of any protein. For heme pigment chemists in general, and the meat color chemist in particular, this means it is now possible to relate color changes, which reflect the changing function of the molecule, to changes in structural features of the molecule, opening up the possibility of controlling these changes at the molecular level.

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