Effect of packaging conditions, nitrite concentration, sodium erythrobate concentration and length of storage on color and rancidity development of sliced bologna

by

Hwei-Shen Lin

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Meat Science

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1978

TABLE OF CONTENTS

INTRODUCTION

The curing of meat is based in part upon the art as practiced through aeons of time and perhaps to a far greater extent upon sound scientific principles developed over the past years. The origin of the use of nitrate in curing meat is lost in history, but it is certain that the preservation of meat with salt preceded the intentional use of nitrate by many centuries. The technology of sea salt production was known at least by 1200 B.C. by the Chinese, who apparently preserved meat by salting.

Sodium nitrite is permitted to be used as an additive due to the very beneficial results obtained. However, the increasing concern regarding the use of nitrite is due to the problem of nitrosamines which possess carcinogenic activity. The possible health hazard due to the reaction of nitrite and nitrosatable amines in foods to form nitrosamines have made it necessary to reevaluate the functions of nitrate and nitrite in cured meats in order to justify levels of addition and to search for possible substitutes. These functions and levels must be established for a wide variety of processed meats due to variations in additives, processing conditions, and subsequent time and temperature of storage of different types of products.

There has been great interest in the use of nitrite for curing with current suggestions for reducing the amounts used. Reduction of nitrite will probably require more specific packaging conditions, but limited information is available on cured meat color under specific packaging conditions, sodium erythrobate concentrations, and reduced nitrite levels.

In this study three experiments were conducted to evaluate the effects of:

(1) eight different packaging films

(2) three sodium nitrite levels, and five different packaging films

(3) three sodium erythrobate levels, and five different packaging films.

In addition, the effect of three initial degrees of vacuum and six storage times on color and rancidity of sliced bologna was examined.

LITERATURE REVIEW

Chemical-Physical Characteristics of Meat Pigment

Hemoglobin, the blood heme pigment, transports oxygen from the lungs via the blood stream into the capillaries where it diffuses into the muscle tissue and is bound by myoglobin for subsequent use in aerobic metabolism. Oxygen is transferred to mitochondria where it is used to oxidize carbohydrates. During the process, a certain amount of myoglobin is oxidized to the met or ferric form which can no longer store oxygen. However, the oxidation of carbohydrates supplies electrons to reduce the iron to the ferrous state, and the pigment again becomes functional for oxygen binding. In general, the conjugated protein myoglobin is primarily responsible for the red color of muscle. The amount of myoglobin present in various muscles is due to different levels of activity of the tissue, the amount of blood supply, the general oxygen availability, and the age of the animal.

Kagen and Gurevich (1967) found that myoglobin is located next to the cell wall to facilitate transport of oxygen across the cell wall and subsequent storage of oxygen. Liu and Watts (1970) pointed out that myoglobin is in solution in the cytoplasm and thus is separated from phospholipids, which are in the membranes. However, it may be that, as cooking breaks down the membranes, the lipid constituents and heme components are brought into contact for accelerated development of rancidity.

Myoglobin is a complex protein composed of both a peptide (globin) and nonpeptide portion (heme). The heme consists of an iron atom and an

organic part, protoporphyrin. This porphyrin 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 tetrapyrrole ring. Stryer (1975) indicated that the iron atom of the heme is directly bonded to one of the histidines, namely residue F8 which occupies the fifth coordination position of the iron. The oxygen-binding site is on the other side of the heme plane, at the sixth coordination position. A second histidine residue is nearby and not bonded to the heme. The conformations of deoxymyoglobin, oxymyoglobin, and ferrimyoglobin appear to be very similar except at the sixth coordination position. Lee and Cassens (1976) found that the heated samples of myoglobin contained twice the amount of ^{15}N as unheated samples. Therefore, Lee and Cassens (1976) and Tarladgis (1962) pointed out that the globin portion was likely detached from the myoglobin by heating, and that cooked, cured meat pigment presents two binding sites for nitric oxide.

There is about 75% of the main chain of the globin folded in a α-helical conformation and four prolines which cannot be accommodated in an α-helix. The interior helix consists almost entirely of nonpolar residues such as leucine, valine, methionine, and phenylalanine. Residues that have both a polar and nonpolar part, such as threonine, tyrosine, and tryptophan, are oriented so that their nonpolar portion points inward. The only polar residues inside of the myoglobin structure are two histidines which have a critical function at the active site. The outside of the molecule contains both polar and nonpolar residues. Satterlee and Zachariah (1972) reported that bovine and ovine myoglobin

have identical amino acid compositions, except for a small difference in alanine and leucine. Only a few amino acids of porcine myoglobin were found to be present in quantities similar to those of ovine and bovine myoglobin; however, glycine and methionine are found in equal amounts in all three types of myoglobin. The molecular weight of porcine myoglobin (17,142) is only slightly less than the molecular weight given for bovine (17,693) and ovine (17,735). Satterlee and Zachariah (1972) found that porcine myoglobin is a very unstable molecule which is very susceptible to acid denaturation and there were no significant heat denaturation differences among these three metmyoglobins at a pH of 5.5. Bembers and Satterlee (1975) reported that the instability of PSE porcine muscle myoglobin and oxymyoglobin to both heat and conversion to MetMb could result in a rapid loss of the normal red muscle color and aid in the development of the pale color of PSE muscle compared to that of normal porcine muscle.

Nitrite

Sodium nitrite, the salt of a relatively weak acid and a strong base, is a pale yellow crystalline substance which is very soluble in water. Aqueous solutions are highly ionized, slightly alkaline, and pale yellow. The nitrite ion is considered to be a highly reactive ion which can serve as both a reducing agent and an oxidizing agent. It can be oxidized to nitrate ion by bromine, permanganate, chromate, and similar oxidizing agents. In turn, it is able to oxidize iodide.

Nitrite performs four main functions in cured meats. It is responsible for the development of cured meat color through its reduction to nitric oxide and reaction with the meat pigment myoglobin (Fox, 1966;

Eakes et al., 1975). Secondly, nitrites have been shown to contribute to the development of cured flavor in some processed products, such as frankfurters (Wasserman and Talley, 1972; Simon et al., 1973), cured ham (Brown et al., 1974; Kemp et al., 1974), pork loins (Cho and Bratzler, 1970), canned comminuted pork (Ockerman et al., 1973), and Lebanon bologna (Zaika et al., 1976). Thirdly, nitrite provides an important degree of protection against toxin production by Clostridium botulinum (Greenberg, 1972; Hustad et al., 1973; Christiansen et al., 1973) and the growth of other putrefactive organisms (Bulman and Ayres, 1952). Finally, nitrite has been shown to inhibit development of off-flavors by oxidation of fatty acids (Sato and Hegarty, 1971).

Sources of Nitrite

Nitrates are formed from nitrogen and atmospheric oxygen in the atmosphere. They are, indirectly, by-products of photosynthesis that are used by green plants as precursors of ammonia and hence of amino acids. Nitrate, when used in meat processing, is not reactive or dangerous in any way other than the pathway of reduction to nitrite.

White (1975) indicated that nitrite may also arise in the environment, primarily in food, either through addition as a food additive or by reduction of nitrate by bacteria or enzymes. Tannenbaum et al. (1974) have demonstrated that microorganisms in the human mouth convert salivary nitrate (the bulk of which comes from vegetables and fruits) into nitrite.

The four vegetables, lettuce, celery, spinach, and beets, account for about one-half of the supply of nitrates of vegetable origin per

capita in the United States and about 42% of the nitrates from all food sources. However, it is interesting to note that the average American gets two-thirds of his nitrite from his own saliva (Greenberg, 1975). When vegetables and vegetable juices that are high in nitrates are consumed, the salivary nitrite concentration may increase. Tannenbaum (1976) reported levels of more than 400 ppm in the saliva of volunteers who had consumed one portion of organic celery juice containing 240 mg of nitrates. This is several times as high as the maximum level, 120 ppm, for bacon.

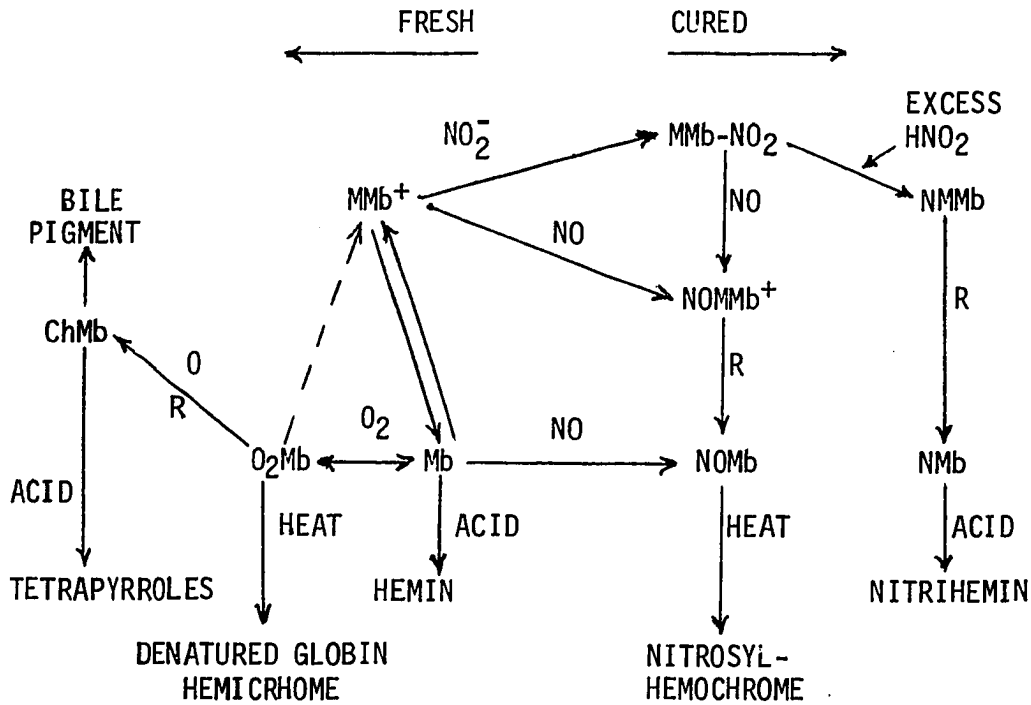
In addition, Correa et al. (1975) proposed a hypothesis that drinking water containing high levels of nitrate may give rise to stomach cancer, mostly because acid conditions promote formation of nitrite and subsequent production of nitrosocompounds. A level of less than 45 ppm in drinking water is recommended. Therefore, limiting the formation of nitrite from nitrates in the digestive tract or saliva and the reduction of nitrate content of well water or vegetables merit further study.

Functions of Nitrite

Color development

Myoglobin and other meat pigments can undergo various reactions under both fresh or cured conditions (Hornsey, 1964; Fox, 1966; Fox and Ackerman, 1968) which are influenced by variables such as pH, enzyme activity, additives, oxidation-reduction conditions, light, and heat (Figure 1).

Upon exposure of cut surfaces of fresh red muscle to the atmosphere, the three pigments oxymyoglobin, myoglobin, and metmyoglobin



ChMb, cholemyoglobin (oxidized porphyrin ring); O_2Mb, oxymyoglobin (Fe^{+2}); MMb, metmyoglobin (Fe^{+3}); Mb, myoglobin (Fe^{+2}); $MMb{\cdot}NO_2$, 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.

Figure 1. Heme pigment reactions of meat and meat products

are constantly being interconverted in the presence of oxygen. At high oxygen pressures, gaseous oxygen enters and diffuses through the aqueous environment surrounding each myoglobin molecule and then into the hydrophobic heme cleft to occupy the vacant sixth coordination site of the iron (Giddings, 1977; Chang and Taylor, 1975). This process produces the familiar "bloom" of fresh meat resulting from the bright red oxygenated pigment, oxymyoglobin, converted from the purple-red pigment, myoglobin. However, the oxygen is continually associating and dissociating from the heme complex and this is accelerated by a number of conditions, among them low oxygen pressure. When this low oxygen pressure occurs, the myoglobin or oxymyoglobin heme iron undergoes a univalent oxidation to the ferric oxidation state; the fresh red muscle loses its attractive bright cherry-red surface appearance and becomes brown. 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 (Fox, 1966). Ferrimyoglobin is unable to form an oxygen adduct, probably because the ferric heme iron is a poorer donor to the sixth ligands than is its ferrous counterpart (Rifkind, 1973). This is compensated for in ferrimyoglobin complexes with the sixth ligands (CN^-, NO, N_3^-) that are superior donors. As such they form low spin complexes with electronic properties and therefore optical spectra and appearance similar to those of low spin ferrous complexes, such as oxymyoglobin.

Neill and Hastings (1925) first described the phenomenon of the accelerated rate of oxidation of the hemoglobin. George and Stratmann (1952) 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 pH, pigment, and temperature. This effect is important when considering packaging films for fresh meat.

In addition, it is recognized that incident light can result in red meat surface discolorations. The extent of the effect is dependent upon wavelength and intensity of the light, temperature and oxygen pressure, meat pH, and length of holding (Rifkind, 1974; Hunt et al., 1975). Based upon their experimental results and cited earlier reports, Solberg and Franke (1971) hypothesized that a photoenergized molecule such as riboflavin may interact with and oxidize oxygenated heme pigments. More recently, Lynch et al. (1976) suggested the same for the semiquinone of riboflavin generated by photoreduction. Constantly improving sanitation techniques, meat handling, pasteurization, and sterilization processes have led to increasing shelf-life and color stability for fresh meat. 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. These problems will be discussed in detail in a later section.

The color reaction of cured meat is shown by several different pathways to be the reaction among nitrite, nitric oxide reductants, and myoglobin resulting in formation of the cured meat pigment, nitrosyl-hemochrome (Figure 1). The meat pigment is largely oxygenated to oxymyoglobin at the time of contact with the curing mixture. The first reaction then appears to be oxidation of the meat pigment to metmyoglobin, which is evident by the color of the sausage emulsion immediately after the "cure" is added. There are several alternate pathways by which

metmyoglobin can be converted to the cured meat pigment (Fox and Ackerman, 1968). The metmyoglobin must be reduced and combined with nitric oxide to yield the final desired pigment.

An equilibrium is established between the ionized salt of nitrite and the unionized nitrous acid in an acid environment, depending upon the pH of the solution (pK_a = 3.4). From the Henderson-Hasselbach equation, pH = pK_a + log salt/acid, the amount of undissociated nitrous acid at various pH levels can be calculated. Theoretically, only a small quantity of added nitrite exists as nitrous acid at the usual pH values of meat.

In slightly acid solutions, nitrous acid appears to decompose into nitric oxide (Treinin and Hayon, 1970; Bard and Townsend, 1971):

$$3HONO \rightleftarrows HNO_3 + 2NO + H_2O$$

The reaction is reversible and the nitric oxide is a colorless, difficultly condensable gas which is slightly soluble in water. Nitric oxide can form complexes with such porphyrin-containing compounds as catalase, the peroxidases, cytochromes, and cytochrome oxidase, as well as with hemoglobin and myoglobin. However, myoglobin, the major meat pigment in meat (Fox, 1966), is of greatest interest in the curing reaction.

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 which 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. The end product of curing is nitrosylmyoglobin if uncooked and the denatured globin nitrosylhemochrome if cooked.

It is well-established that the sixth position ligand of cured meat myoglobin is nitric oxide which binds to the heme iron via the nitrogen atom to form an extremely stable reddish color complex. It has long been known that NO combines with both ferric and ferrous iron of unhindered heme proteins. Ferric myoglobin and hemoglobin complexes of nitric oxide are gradually autoreduced and assume a partial ferrous nitrosyl (Fe^{+2} - NO^{+}) configuration. This results from partial transfer of the odd nitrogen electron to iron (Chien, 1969; Yonetani et al., 1972). Antonini and Brunori (1971) indicated that the affinity of Mb or Hb complexes with NO is extremely high. The affinity is 100-fold greater than for CO which, in turn, has a much greater affinity than O_2. Once formed the Mb and Hb complexes of NO are very stable in the absence of oxygen and rapidly oxidize free NO to NO_2 in the presence of O_2. The stability of the complexes is limited by the rate of NO dissociation since O_2 does not react directly with the bound NO (Brunori et al., 1973). This dissociation rate is very low; however, nitric oxide complexes are photodissociable. Moore and Gibson (1976) reported that due largely to the unpaired electron on the nitrogen atom, NO heme complexes possess certain properties not exhibited by O_2 and CO complexes in addition to exceptional stability.

Kearns (1971) pointed out the fact that NO is a much better quencher than O_2 and has a higher electronic affinity. This may explain why cured meat color is more susceptible to light-induced discoloration than is fresh meat color. Tarladgis (1962) was the first to

conclude that cooked cured meat pigment, which is a heme compound, contains NO groups at both axial coordination sites. 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 converted to the fully reduced form, again regardless of pH. The cured meat pigment, once formed, is more stable at higher pH values. Bailey et al. (1964) found that the nitrosylhemochrome was more stable to light fading at pH 6.8 than at pH 6.2. Tarladgis (1962) indicated that the fading of cured meat color by lipid oxidation or photocatalyzed dissociation of NO from heme involves withdrawal of electron density from iron to porphyrin. This weakens the Fe-NO bond. He viewed green discoloration as being caused by electrophilic and nucleophilic attacks on areas of high and low electron density in the porphyrin ring π electron cloud which causes ring opening.

Light fading is a two-step reaction, the first being the light-accelerated dissociation of the nitric oxide from the heme, and the second being the oxidation of nitric oxide by oxygen. In addition, the formation of sulfmyoglobin and cholemyoglobin also can produce green chromophores. Deibel and Evans (1957) and Fox and Thomson (1963) showed that the greenish brown spots on the surface and the green centers in fermented sausage result from a green porphyrin ring compound in the presence of excess concentrations of nitrite. Tarladgis (1962) reported that reducing conditions in storage and absence of light exposure can prevent color fading. As demonstrated earlier by Ramsbottom

et al. (1951) and explained by Fox (1966), elimination of oxygen essentially eliminates photoinduced color fading as a problem by preventing oxidation of dissociating NO by O_2.

Flavor development

Meat flavor is of great academic as well as practical importance; however, it is very difficult to evaluate and describe. Thus, we are still unable to completely understand this important sensory quality. There are many factors, both antemortem and postmortem, affecting meat flavor. Examples are age, breed, sex, feeding, carcass condition, slaughtering and aging conditions, freezing, length of storage, food additivies, and various cooking methods such as braising, broiling, frying, or roasting. In general, the flavor of raw meat is weak, salty, and mildly serum-like. The true meaty flavor develops during cooking.

Meat flavor research has been characterized by a dual approach: a study of flavor precursor systems, and an analysis of the meat flavor volatiles and the distinctive contributions of the lean and lipid portions of meat. Meat flavor components identified in earlier research (Bender and Ballance, 1961; Yueh and Strong, 1960; Sanderson et al., 1966) included a few simple sulfides, mercaptans, carbonyl compounds, alcohols, carboxylic acids, ammonia, hydrocarbons, esters, and aromatic compounds. Thus, a hypothesis was formulated that if the appropriate relative concentrations were known, one could duplicate the characteristic flavor of different meats using the various identified compounds in the proper proportions. However, it seems that meat flavor could not be reconstituted without heterocyclic sulfur and nitrogen containing

components (Chang and Peterson, 1977). Hornstein and Crowe (1960) reported that meat flavor contributions are produced by the lipids during cooking. Indeed, the fatty tissue may serve as the solvent for the precursors of meat flavor but the lipids themselves cannot produce the sulfur and nitrogen containing heterocyclic compounds. Chang and Peterson (1977) pointed out that artificial flavors such as beef, pork, or chicken cannot yet be completely synthesized because the key compounds from lipids which play the major role in a specific flavor have not yet been identified. Many workers are studying cured meat flavor which is still a wide open area because it is not completely understood.

The relationship of nitrite to flavor was first described by Brooks et al. (1940). These authors stated that a panel showed preference for nitrite cured bacon and hams, although no taste panel data were presented. It is well-established that the concentrations of nitrite used for curing meat are important for the development of cured flavor in processed products such as frankfurters (Wasserman and Talley, 1972; Simon et al., 1973; Hadden et al., 1975; Sebranek et al., 1977), cured ham (Brown et al., 1974; Eakes et al., 1975; Kemp et al., 1975), pork loins (Cho and Bratzler, 1970), Lebanon bologna (Zaika et al., 1976), and sausage (Hustad et al., 1973; Dethmers et al., 1975). Bailey and Swain (1973) reported that in view of existing data, no individual compound among those identified can be said to be unique in cured meat relative to noncured meat. Thus, they concluded that a major function of nitrite is its ability to retard oxidation of lipids in cooked meats.

An important flavor difference is due to accumulation of oxidative end products in uncured cooked meat. The minor differences in flavor between nitrite-cured and nonnitrite-cured meats soon after cooking are probably due to the so-called "warmed-over" flavor caused by rapid oxidation of unsaturated fatty acids catalyzed by iron or its complexes. Swain (1972) stated that lipid oxidation is considerably delayed in cured meats. Cross and Ziegler (1965) concluded from their comparison of the volatile fractions of cured and uncured hams that nitrite interferes with the oxidation of unsaturated lipids. The key components of fresh meat and cured meat flavor still need more extensive study before food scientists will be able to synthesize desirable meat flavors simply by adding proportional concentrations of various compounds to a mixture.

Antioxidation

Nitrite has been shown to eliminate warmed-over flavor (WOF) at a level of 220 ppm and to inhibit development of WOF at 50 ppm (Sato and Hegarty, 1971). Cross and Ziegler (1965) found that certain aldehydes were present to a much greater extent in uncured ham than in cured ham, especially hexanal and valeraldehyde which are derived by oxidative cleavage of unsaturated fatty acid residues such as linoleate.

A similar comprehensive study of the volatiles from cured and uncured ham was performed by Swain (1972) who found the uncured ham had more concentrated isobutanal, n-pentanal, and higher molecular weight compounds than those of cured ham. Westerberg (1973) indicated that cured frankfurter had lower TBA values than uncured samples. Bailey and Swain (1973)

have also confirmed the effectiveness of nitrite in preventing oxidation of meat when stored under refrigeration. They further confirmed the effectiveness of nitrite in preventing WOF by subjective panel scores, thus verifying the inhibition of oxidation as shown by low TBA values. Such results offer an explanation for the "better" flavor of nitrite-cured pork as reported by Cho and Bratzler (1970).

Since the muscle membranes appear to be the site of oxidation during the development of WOF (Sato and Hegarty, 1971), the nitrite must either stabilize the lipid components of the membranes or else inhibit the natural prooxidants present in muscle. Liu and Watts (1970) concluded that both heme and nonheme iron function as catalysts of lipid oxidation in cooked meat. Nitrite functions as an antioxidant by removing the catalytically active ferric hemochromogen by the formation of inactive ferrous nitric oxide hemochromogen (Zipsen et al., 1964).

In contrast, Sato and Hegarty (1971) and Love and Pearson (1974) indicated that nonheme iron is the major lipid prooxidant in meat systems; it seems more probable that nitrite complexes and stabilizes the lipids in the membranes. Liu and Watts (1970) pointed out that myoglobin is in solution in the cytoplasm and separated from the phospholipids which are in membranes. The lipid constituents and heme components are brought into contact when cooking breaks down the membranes. In this case, the stabilization of myoglobin by nitrite would have the same effects as stabilization of the membranes per se. The presence of nitrite in the meat product inhibits oxidation of the lipids while the formation of off-flavor compounds by oxidation results in uncured products resulting in less organoleptic acceptability.

Antimicrobial effects

Curing meat provides an important degree of protection against botulism. The inhibitory effects of nitrite are very complex because the necessary concentration of nitrite depends on several factors such as pH, salt, number of bacteria, and storage temperature. Nitrite may provide similar protection against other food poisoning bacteria, e.g., Clostridium welchii or Staphylococci (Ingram, 1973). Staphylococcus aureus (Buchanan and Solberg, 1972), Bacillus sp. and Clostridium sporogenes (Perigo et al., 1967; Duncan and Foster, 1968), Clostridium perfringens (Labbe and Duncan, 1970), Strep. faecalis (Stoychev and Djejeva, 1971), Pseudomonae, Enterobacter, and Micrococcus (Spencer, 1971), and Salmonella sp. have all been shown to be inhibited by nitrite. Much of the work has been directed at Clostridium botulinum due to the fact that an aspirin-sized tablet of toxin would be capable of killing the entire population of New York City (Anonymous, 1972; Greenberg, 1972).

Shank et al. (1962) indicated that the antimicrobial effect increases with falling pH to a maximum effectiveness near pH 5.0, and that nitrous acid is the compound responsible for clostridia inhibition. Christiansen et al. (1975) reported that growth and toxin production of Clostridium botulinum did not occur in fermented sausage due to the rapid acid production. The concentration of nitrous acid is very pH dependent and it would be present in very low concentrations at the normal pH of cured meat. Thus, the other factors, heating (Grever, 1973), anaerobic or in the presence of reducing agent (Ingram, 1973), probably work synergistically with nitrite to produce the

inhibitory effect on clostridia. Kueper and Trelease (1974) reported that no toxin developed in fermented sausage even in the absence of nitrite. In unfermented sausage, if there is no nitrite present there is little protection against botulism toxin development; however, if dextrose was present in the formula, protection from the development of botulism toxin resulted with 50 ppm or more added nitrite. If there was no dextrose in the formula, some reduction in toxin development occurred when 100 ppm or 150 ppm nitrite was used (Eddy and Ingram, 1956).

Bowen and Deibel (1974) concluded that ascorbate in frankfurters has no effect on the ability of nitrite to inhibit the formation of botulinal toxin. However, in a bacon study, they found that ascorbate decreases the efficiency of nitrite to inhibit botulinal growth, especially at levels above 500 ppm of ascorbate. They pointed out that a significant result for cures of bacon is the finding that the lower the level of ascorbate used, the more effective nitrite is in inhibiting botulinal growth. In addition, they reported that the initial nitrite level, rather than the residual amount, is the important factor in determining inhibition of botulinal toxin formation. Several authors have also indicated that increased nitrite levels decreased the probability of botulinal toxin formation in canned perishable cured meat (Christiansen et al., 1973), summer style sausage (Christiansen et al., 1975), wieners (Hustad et al., 1973), thuringer (Kueper and Trelease, 1974), and bacon (Christiansen et al., 1974).

The value of nitrite as an inhibitor of Clostridium botulinum in canned meat has been recognized for several decades, but it was believed that nitrite per se caused the inhibition. Perigo et al. (1967) and

Perigo and Roberts (1968) described the inhibition of vegetative cells of several clostridia by very low amounts of nitrite present in the growth medium during sterilization. They suggested that an unknown antimicrobial inhibitor (PF) might be important in the safety of canned cured meat. However, Johnston and Loynes (1971) and Roberts (1971) studied this Perigo effect in sterilized meat products and could not find such an effect. Growth inhibition in samples inoculated after sterilization occurred with rather high amounts of ascorbic acid or cysteine and small amounts of nitrite. The inhibiting effect which Perigo discovered in his medium does not seem probable in meat under normal curing and sterilizing conditions. Johnston and Loynes (1971) were not able to find growth inhibition with Perigo's medium after addition of coagulated meat protein. These findings do not rule out the formation of an inhibiting agent in sterilized cured meat but tend to indicate that the activity of such a substance is perhaps inhibited by adsorption onto meat protein. Van Roon (1973) found that the black Roussian salt and nitrosyl-cysteyl-ferrate can be formed in canned cured meat products during heating. Both complexes inhibit the growth of clostridia spores. In order to restrict the use of nitrite in food, further study is necessary to understand the minimum nitrite concentration needed in meat products to prevent growth of clostridia.

Residual Nitrite

Studies on residual nitrite or so-called "free nitrite" in cured meat indicated that most or all of the remaining nitrite is involved in the formation of nitroso-reductant intermediates or products.

Greenberg (1975) indicated current nitrite regulatory limits for the typical pickle cured, chopped, and dry cured meat products at respective rates of 211 ppm, 156 ppm, and 624 ppm sodium nitrite at the time of formulation. A further condition specifies that the finished product may not contain more than 200 ppm sodium nitrite. A survey in 1970 (American Meat Institute, 1978) showed that less than 2% of samples contained more than 100 ppm of residual nitrite levels in cured meat at the plant level. Thus, when properly used in regular industry practice the cured meats conform rather well to nitrite restrictions. After several meetings and the careful consideration of much data, the expert panel (a six member panel appointed by the Secretary of Agriculture to the advise the Department on the safety and continued use of nitrate and nitrites) agreed on some broad recommendations:

1. That nitrate use should be prohibited wherever possible.
2. Nitrite salt be standardized at 156 ppm for curing all processed products except bacon and dry-cured products.
3. The current permitted 200 ppm residual nitrite level should be reduced dependent on specific product category:
   a. 50 ppm in sterile products
   b. 100 ppm in cooked sausage products
   c. 125 ppm in canned and pickle-cured products.

In September, 1977, the expert panel recommended that: "As an anti-botulism agent, nitrite has been the most acceptable ingredient yet found and is responsible for the excellent safety record of commercially produced meats." The panel approved a table of nitrite, nitrate, and ascorbate amounts for various classes of products. For instance, bacon

data submitted by industry indicated that 120 ppm nitrite and 550 ppm ascorbate (or erythrobate) should not result in the formation of confirmable nitrosamines at 10 ppb (O'Brien, 1978).

A substantial portion of the nitrite disappears and is unaccounted for. Most results indicate that immediately after formulation, approximately 50 to 75% of the originally added nitrite can be measured by analytical procedures (Greenwood, 1940). Additional nitrite loss of 20 to 80% occurs with thermal processing and nitrite also declines during storage (Nordin, 1969; Greenberg, 1972). AMIF (1971) also cited that the nitrite content is greatly reduced and sometimes completely eliminated during curing, cooking, and aging. Most cured meats at retail, therefore, contain a residual nitrite level between 10 and 50 ppm. Fox and Nicholas (1974) indicated in a kinetic study that loss of nitrite is related to the reduction reaction which produces nitric oxide. Production of the nitric oxide accounts for a large part of total nitrite loss and evidence indicates that most or all of the remaining nitrite not accounted for is involved in the formation of nitroso-reductant intermediates or products. In addition, other gas production and evolution of N_2O and N_2 by reaction of nitrite with a meat system may also account for some of the nitrite loss (Sebranek et al., 1973; Sebranek, 1974; Cassens et al., 1974).

Woolford et al. (1976) showed that bovine serum albumin and myosin have the ability to bind appreciable amounts of nitrite with resulting modifications of the protein. The sulfhydryldisulfide group of myosin was responsible for only a small proportion of the total nitrite lost in the curing process. The rate of this reaction is affected by nitrite

concentration, pH, and temperature (Kubberd et al., 1974). Woolford et al. (1976) demonstrated that nitrite can combine with sulfhydryl groups in meat to form nitrosothiols. Water insoluble cured meat fractions showed that up to 25% of the added nitrite had reacted to form nitrosothiols, while the same fraction from a heat-treated sample showed that only about 8% of the added nitrite formed nitrosothiols. The amount of nitrosothiols formed was linear with respect to increasing added nitrite concentration in the uncooked sample.

Sebranek et al. (1973) and Cassens et al. (1974) indicated the meat myoglobin binds about 15 ppm of nitrite which may also react with other porphyrin-containing pigments such as cytochromes and hemoglobin. They assumed that between 10 to 20% of nitrite added for curing reacts with porphyrin-containing compounds in the meat. Lee et al. (1976) found that the slightly lower residual nitrite in products made from red muscle than that of white muscle probably is due to the greater content of myoglobin in red muscle. Autoxidation of nitrite resulted in the formation of nitrate and nitric oxide which should also account for disappearance of nitrite (Smith, 1921).

Nitrosamines

Nitrite itself acts as a vasodilator and hypotensive agent (Rubin et al., 1963). It can reduce the storage of vitamin A in the liver, and it may also disturb thyroid function (Emerick et al., 1963). The main acute or subchronic toxic effect of ingested nitrites and nitrates (which are easily reduced to nitrites mainly by bacteria) is the formation of methemoglobin. In this form the pigment is incapable of

carrying oxygen in the bloodstream. Prominent symptoms are vomiting, cyanosis, shock, and unconsciousness in man (Greenberg et al., 1945). This is particularly serious for infants who have a high liquid intake per body weight and also lack methemoglobin reductase. Nitrite formation in infants under six months of age, due to low acid secretion in the stomach and the subsequently easy bacterial nitrate reduction, has led to serious intoxication and even death, especially after ingestion of nitrate-rich drinking water or spinach (Phillips, 1971).

In addition, Magee and Barnes (1967) have shown that large doses of the dialkyl nitrosamines produce severe liver necrosis and extensive hemorrhages both in the liver and at other sites. They indicated that the required dose (LD_{50} for rats) increases with the length of the chain in the alkyl group. Thus, the LD_{50} value for N-nitrosodimethylamine is 27 to 40 mg/kg, for N-nitrosodiethylamine 216 mg/kg, and for N-nitroso-dibutylamine 1200 mg/kg. Acute poisoning by N-nitrosodimethylamine in humans has been reported following an industrial accident (Barnes and Magee, 1954) and in animals, particularly mink. At levels below the toxic dose, nitroso derivatives of dimethylamine and diethylamine are known to cause a carcinogenic response in a wide range of animal species. No known animal has been shown to be totally resistant to this class of compound. These compounds produce tumors in almost all important organs in animals (liver, kidney, esophagus, stomach, brain, bladder, lung, and alimentary system) and are active at low dosage levels (Magee and Barnes, 1967). The organ specificity of the action depends mainly on the chemical structure of the compound and to a minor degree on the animal species, rate application, and the dose. Other compounds in this series are also

known to be active, although the relationship between compound, dose, animal diet, and so on are very complex.

Although no direct evidence of the carcinogenicity of nitrosamines in human is available, it has been shown as a result of an industrial accident that similar toxic symptoms arise in the human liver as with experimental studies on animals. In addition, due to many factors that may contribute to the observed results, it is usually very difficult to establish evidence for a single factor influencing tumor incidence. Nevertheless, many relationships can be found from epidemiological studies. Examples are the relation between lung cancer and smoking, bladder cancer and the rubber industry, lung cancer and uranium, mesothelioma and asbestos, lung cancer and mustard gas, and liver cancer and aflatoxin (Epstein, 1972).

As mentioned previously, nitrosamines can be formed by reaction of secondary amines with nitrous acid or nitrite under acidic conditions. Nitrite is used for curing meat and fish. Nitrates are also widespread in the environment in vegetables such as spinach, beets, celery, and lettuce as well as in some well water supplies (Magee and Barnes, 1967). Amines or amine precursors such as proteins, amino acids, phospholipids, or other compounds are present in most foods and may be available for reaction with nitrite. In fact, high levels of secondary amines have been reported in fish, vegetables, and fruit juices (Phillips, 1968). In addition, nitrosamines have also been found in mushrooms, smoked fish, and smoked meats (Ender and Ceh, 1968) and in wheat plant, wheat grain, and wheat flour (Hedler and Marquardt, 1968).

Bacon is currently the most critical product because, unlike other meat products, fried bacon has consistently been found to contain detectable quantities of nitrosamine, particularly nitroso-pyrrolidine (N-Pyr). Herring (1973) indicated the concentration of the N-nitrosamine increased with increasing nitrite levels. Pan fried samples had the most and microwave cooked samples the least N-Pyr. All uncooked samples contained no N-Pyr. Pensabene et al. (1974) found the maximal formation of N-Pyr at 185°C frying temperature.

Lijinsky and Epstein (1970) have proposed that the formation of N-Pyr may arise either through N-nitrosoproline from proline and sodium nitrite with subsequent decarboxylation to N-Pyr or by direct interaction of pyrrolidine which arises from proline or putrescine and nitrite. Bills et al. (1973) have reported that N-Pyr was produced from N-nitrosoproline (N-Pro), pyrrolidine (Pyr), spermidine, proline (Pro), and putrescine in yields of 2.6, 1.0, 1.0, 0.4, and 0.04% of their respective theoretical values.

Barnes and Magee (1954) showed that nitrosodimethylamine was uniformly distributed in the body tissues of the rat following oral administration, there being no selective concentration in the liver. Tannenbaum et al. (1978) demonstrated that nitrite and nitrate are formed by endogenous synthesis in the human intestine. These findings significantly alter previous conceptions of human exposure to nitrite and suggest an even wider role for nitrite in the etiology of human cancer.

The metabolism of nitrosamines *in vivo* takes place very rapidly and none of the original dose was recovered after 4 hours in mice

or after 24 hours in rats. Only a very small percentage of the dose was excreted in the urine. The lack of accumulation of dimethylnitrosamine in any organ of the body following injection and the fact that the liver, the center of metabolic activity in the animal, was the organ most frequently affected, suggested that a metabolite of nitrosamines was the true active species and not the compounds themselves (Magee and Barnes, 1967).

Dutton and Heath (1956) used nitrosodimethylamine labeled with ^{14}C and found that the principal radioactive product was expired as CO_2 during the first few hours following injection. Using ^{14}C and ^{15}N as a label indicated that dimethylnitrosamine was oxidized to one carbon atom intermediate and that the nitroso group is partly reduced to ammonia. Heath (1962) extended this work to other nitroso compounds. He suggested the toxic metabolite for the dialkylnitrosamines was a diazoalkane, or a monoalkylnitrosamine or carbonium ions formed from it.

Sander (1974) reported that the nitrosation reaction may be catalyzed as well as it may be inhibited by several agents. Catalyzing agents, changing the reaction rates of the nitrosation reaction, are known to be halide ions and the pseudo halide thiocyanate (SCN^-). The latter arises from several goitrogenic substances which are contained in certain vegetables, and also are formed in vivo from cyanide which is inhaled with cigarette smoke. Some aldehydes may also be important as catalysts, e.g., formaldehyde. However, inhibition can manifest itself in a reduction of the tumor yield and/or a longer latency period.

Suppression of carcinogenic action may be caused by nutritional factors, inhibition or stimulation of enzyme systems, and hormonal and

immunological status of the host. It is known that the nutritional factors are the caloric restrictions inhibiting tumor development, as do dietary fat, protein, and histamine deficiency. However, excess of some constituents also seems to reduce tumor growth (Magee, 1969). Inhibitory effects of the stimulation or suppression of enzyme systems may occur through increased metabolism to inactive derivatives (Miller and Miller, 1971). Hormonal status may affect the tumor induction by carcinogens. An interesting finding was that one carcinogen may inhibit the effect of another (Likhachev, 1968).

Ascorbates at present are the best means to suppress or reduce the formation of nitrosamines in cured meat products. It is critically important to establish the optimal balance of nitrite and ascorbates, particularly in bacon. In vitro and in vivo addition of ascorbic acid inhibited nitrosamine formation from dimethylamine, morpholine, piperazine, N-methylamiline, and amidopyrin (Kamm et al., 1973; Greenblatt, 1973). With high doses of ascorbic acid the inhibition was complete. One mole of ascorbic acid destroys two moles of nitrite (Sander, 1974). The reaction between nitrite and ascorbic acid is fast enough to inhibit the nitrosation of most secondary amines in vitro. In animals, the inhibition is almost complete if equimolar concentrations of the amine, of ascorbic acid, and of nitrite are applied. Very often the amount of ascorbic acid in the food exceeds the nitrite content. In these cases the nitrosamine formation should be very low.

Potential Substitutes

Nitrates and nitrites may represent a potential public health hazard. If their use is prohibited, a new substitute to produce the cured meat characteristic would be in great demand. Tarladgis (1962) recommended replacing NO-based curing salts with strong electron-donor compounds such as purines, pyrimidines, and other nitrogenous bases having desired ligand-electronic properties for curing meat. Howard et al. (1973) studied color formation by nicotinic acid esters and amides in a model system and in a ground meat mixture. This study confirmed that ascorbic acid or glucono-Δ-lactone improved color. Dymicky et al. (1975) reported that the best pink color was formed by 3-acylpyridines. Isoquinoline, pyrazine, and imidazole also formed stable pigment. Von Elbe et al. (1974) found that cured meat color can be simulated to a high degree with some levels of betalains pigment. However, these products are water soluble and tend to leach out if the meat is prepared in water.

There are several patents for nitrite substitutes but no acceptable replacement has been found. In general, these patents disclose the use of compounds such as nicotinic acid, other pyridine derivatives, and other heterocyclic compounds such as tetrazole, purines, pyrimidines, imidazole, pyrazine, triazine, and similar ring systems (Brown, 1973). These substitutes appear to have two common problems: (1) they are substantially less stable to oxidation than is the nitrite derivative, and (2) many of the compounds are vasodilators, thus posing a problem for approval in human food.

Brown (1973) reported the derivatives of pentaerythritol-tetranicotinate gave good results in a sausage model system. This compound was stable to oxidation in the model system but has not yet been evaluated in aerobic systems. In general, all researchers agree that it is highly unlikely that any substitute will match the all around effectiveness of nitrite.

Regulation and Impact if Nitrite is Banned

The use of nitrite in food is of concern because the potential exists for reaction of nitrite with amine or amides to form N-nitroso compounds which are carcinogenic. The residual nitrite content of cured meats represents one source of nitrite consumed by humans.

One of the earliest public calls for the reduction or removal of nitrite from the diet was cited by Lijinsky and Epstein in 1970. Nitrite was singled out as the most potentially dangerous food additive capable of directly causing mutations and indirectly causing cancer in man. Jacobsen (1973) issued a pamphlet entitled "How Sodium Nitrite Can Affect Your Health" and subtitled "Don't Bring Home the Bacon". In addition to the problems of methaemoglobinemia, mutation, and cancer, the pamphlet added the hazards of headaches, changes in brain waves, and death, and called for nitrite to be outlawed. However, Birdsall (1976) showed that more than 90% of the bacon samples had no nitrosopyrrolidine after frying. A few samples were confirmed to have less than 10 ppb of nitrosopyrrolidine, and all but one of these had only 5-7 ppb. All available information indicates that minute amounts of

nitrosopyrrolidine are not harmful to humans in the amounts present in the diet and in the environment. However, the Community Nutrition Institute (1977) petitioned the USDA to ban nitrite from all uses in the processing of meat and food products intended for human consumption (cited by American Meat Institute, 1978).

Madsen (1976) reported on some of the economic consequences of a ban on nitrite. If nitrite would no longer be allowed in bacon, for example, hog producers would have an annual income loss of at least 500 million dollars. Much greater losses would occur if nitrite were to be banned in all cured meats. It would all but destroy the U.S. hog industry since nearly 70% of pork ends up in processed meat products. Only about 30% is sold fresh. Banning the use of nitrite could lead to possible botulism outbreaks and affect the entire chain of production including loss of farm income; loss of employment in farming, meat packing, distribution, and retailing; loss of export market for pork; depressed trimmings market; losses from closing of facilities; less choice for consumers at the meat counter; and loss of cash and future markets. All of these costs must be measured against an assumed benefit of a reduction in the risk of cancer.

Oxidation-Reduction System

The endogenous reducing systems and/or added reductants to meat which play an important role in nitric oxide myoglobin formation include not only enzymatic systems but also nonenzymatic ones. These systems are factors limiting the rate of formation of nitric oxide myoglobin in meat. Koizumi and Brown (1971) studied the formation of nitric oxide

myoglobin under anaerobic conditions. NADH (reduced nicotinamide adenine dinucleotide) and NADPH (reduced nicotinamide adenine dinucleotide phosphate) as reducing agents alone did not produce nitric oxide myoglobin from metmyoglobin and nitrite. However, in the presence of FMN (flavin mononucleotide), FAD (flavin adenine dinucleotide), and riboflavin, either NADH or NADPH readily produced nitric oxide myoglobin. Mb is necessary to reduce nitrite to NO similarly to methylene blue-diaphorase system and itself oxidized to MetMb. NO is immediately bonded to excess Mb, while MetMb returns to the circulatory system as shown in Figure 2.

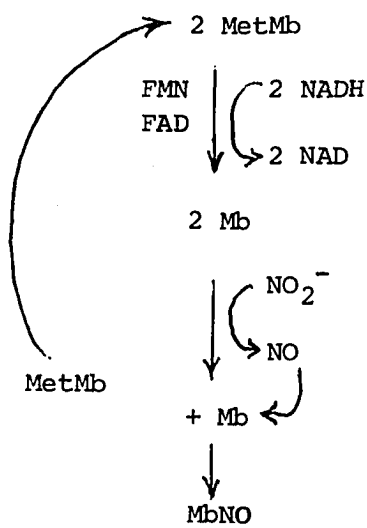


Figure 2. Nonenzymatic formation of NO-Mb

Watts et al. (1966) pointed out that the intermediates of glycolytic pathway such as glyceraldehyde-3 phosphate and fructose-1-6 diphosphate and other substrates such as α-glycerophosphate, malate, and glutamate increased metmyoglobin reduction when added to meat. Fox and Ackerman

(1968) also reported that ascorbic acid, cysteine, hydroquinone, and NADH involves the production of a nitroso-reductant intermediate which breaks down to release nitric oxide. Added ascorbic acid has a stabilizing effect on the nitric oxide pigments, particularly when packaged in film impermeable to oxygen. It also has a function prior to its stabilizing value during storage, distribution, and display, in that during processing it speeds the curing action by reacting with the nitrite to give a controlled positive release of nitric oxide for ready combination with myoglobin (Hollenback and Monaham, 1953). Competing reactions of ascorbic acid in meat are shown in Figure 3 (Bauernfeind and Pinkert, 1970).

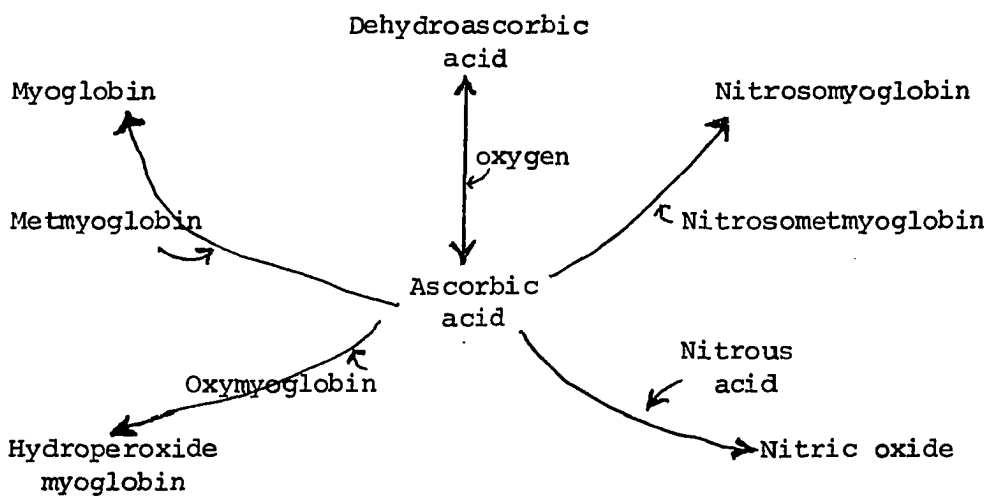


Figure 3. Competing reactions of ascorbic acid in meat

Sodium ascorbate added to curing pickles of salt, sugar, and phosphate produced superior results in hams. Bailey et al. (1964) pointed out that cured meat pigments of ham were more stable to light irradiation in the presence of nicotinamide and sodium ascorbate than those treated

with niacin and ascorbate or ascorbate alone; a pH environment of 6.8 was favored over 6.2. Fox and Ackerman (1968) found that ascorbate plus cysteine increased the initial velocity of the reaction. The reaction is normally linear with respect to time if only one reductant is used. The ascorbic acid together with its isomer, erythorbic acid, and their salts constitute probably the most effective added reductants in cured meat systems, apparently counteracting photo induced discoloration as well (Cassens et al., 1974; Walters et al., 1975). Both ferrous and ferric myoglobin will combine with NO to yield the same pigment. The latter is believed to autoreduce with time via internal electronic rearrangement. Further, ferrous myoglobin can reduce NO_2 to generate NO which will combine with either oxidation state of the pigment. Endogenous or added reductants such as ascorbate, sulfhydryl compounds, and NADH-flavins accelerate the process by either reducing ferrimyoglobin which can then reduce NO_2, or reducing NO_2 to NO, or both, in addition to perhaps accelerating reduction of ferri Mb-NO. Thus, the use of ascorbic acid or its salts results in substantial reduction of curing time, a more uniform product color, and a better flavor and color during storage (Bauernfeind and Pinkert, 1970).

During the storage of intact beef muscle for 2 to 4 weeks, most of the glycolytic enzymes were found to be active (Andrews et al., 1952). Similar studies have been reported in fish muscle (McLeod et al., 1963). Walters and Taylor (1965) and Walters et al. (1975) demonstrated the enzymatic formation of NO-Mb by skeletal muscle mitochondria containing cytochrome c and cytochrome oxidase reducing systems. However, the mechanisms of formation of nitric oxide myoglobin in meat are not fully

established. They found functional mitochondria appear to survive the bacon curing process and may be able to mediate the transfer of NO from nitrosylferricytochrome c to ferrimyoglobin to form MbNO. They noted that ascorbate and some sulfhydryl compounds as well as NADPH flavin can also accomplish this. Cheah (1976) stated that nitrite is an inhibitor of mitochondrial respiration, acting as an inefficient electron acceptor from cytochrome oxidase. Then the oxidations caused here are repaired by the NADH-dehydrogenase system. Figure 4 shows that ferrocytochrome c has been oxidized by nitrite to ferricytochrome, and that NO, as a product of reduction, has been bound to the cytochrome (Walters et al., 1968). Nitrosylferricytochrome c is now reduced by NADH with the aid of the dehydrogenase system. Ferrocytochrome c can return into circulation. Since nitrosylferrocytochrome is unstable, NO separates and is transferred to MetMb. Thus, hypothetic NO-MetMb develops. The NADH-dehydrogenase system reduces the MetMb, the desired cured pigment is produced.

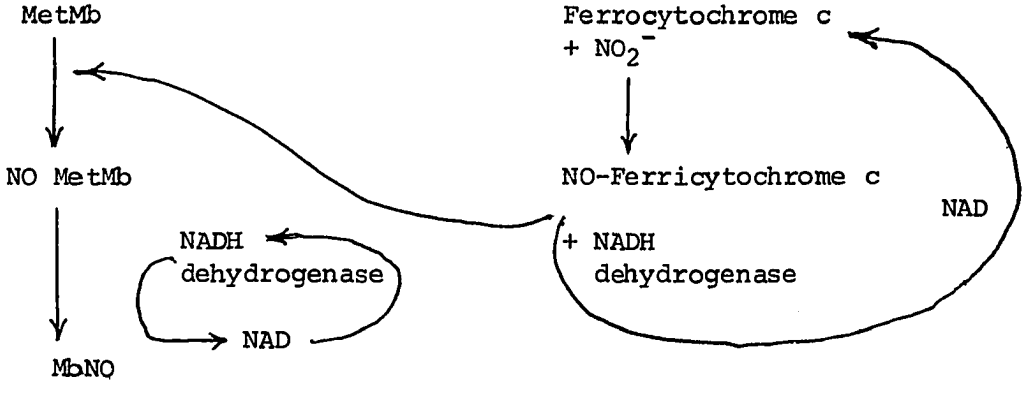


Figure 4. Enzymatic formation of NO-Mb

Oxidation of Meat and Meat Products

Oxygen uptake by intact post-rigor muscle has been measured by Bendall and Taylor (1972) and DeVore and Solberg (1974). Mitochondrial respiration was the main element determining post-rigor oxygen consumption, and the decline of oxygen consumption rate occurred as a result of a deterioration of mitochondrial structure (Bendall and Taylor, 1972), enzyme degradation (Grant, 1955), and substrate depletions (Atkinson et al., 1969). DeVore and Solberg (1974, 1975) reported that the respiratory oxygen consumption rate decline appeared to be related to a reduction in respiratory enzyme activity and substrate depletions.

Lipid oxidation is a major cause of quality deterioration in meat and meat products involved in the oxidation of lipid and ferrous myoglobin. Lundberg (1962) has reviewed the free radical chain mechanisms involved in autocatalytic autoxidation. Dugan (1961) indicated that hydroperoxides are the primary products of the oxidation of unsaturated lipids; the products resulting from hydroperoxide degradation are responsible for the occurrence of off-flavors in oxidized lipids. Phospholipids have been shown to be the lipid component most rapidly oxidized in cooked meat (Younathan and Watts, 1960) due to their high content of unsaturated fatty acid (Lea, 1957). It is the traditional view that hemoproteins are the major catalysts of lipid oxidation in meat and meat products (Younathan and Watts, 1959; Tappel, 1962). In contrast, Sato and Hegarty (1971) and Love and Pearson (1974) proposed that nonheme iron plays a major role in accelerating lipid oxidation

in cooked meat. The latter authors found that metmyoglobin did not influence TBA values in cooked meat which had been water extracted to remove prooxidants prior to cooking. Rhee (1978) found that the rate of MetMb (a heme iron catalyst) catalysis was not proportional to MetMb concentration whereas the rate of Fe^{+2} EDTA catalysis increased linearly with the concentration of Fe^{+2}-EDTA complex. Liu and Watts (1970), however, indicated that both heme and nonheme iron function as catalysts of oxidative rancidity in meats, although heme was reported to be the dominant one.

In addition, Ingold (1962) reported that the heavy metals, such as iron, cobalt, and copper, are important catalysts of oxidative rancidity due to increase in the rate of formation of free radicals. Sato and Hegarty (1971) and Love (1972) have reported that low levels of ascorbic acid increase the efficiency of iron as a catalyst for lipid oxidation. Ascorbic acid can function in the release of nonheme iron from iron containing protein. This may partially explain why low levels of ascorbic acid enhance autoxidation (Sato and Hegarty, 1971), probably by upsetting the balance between ferrous and ferric iron or by acting as an oxygen scavenger. They reported that ascorbic acid and phosphates in combinations have an important synergistic action in preventing the development of oxidation in cured meat. They also pointed out that any process causing disruption of the muscle membrane system, such as grinding, emulsifying, or cooking, resulting in exposure of the lipid components to oxygen, thus accelerates development of oxidative rancidity.

Ellis et al. (1968) reported that an increase in NaCl concentration accelerated autoxidation but did not affect the decomposition of hydroperoxides to monocarbonyls. Zipsen et al. (1964) indicated that addition of NaCl resulted in acceleration of oxidation in freezer-stored, cooked, cured meat.

Several investigators (Wasserman and Talley, 1972; Simon et al., 1973; Bailey and Swain, 1973) have shown that nitrite improves the flavor of frankfurters, apparently by inhibiting oxidation. It seems that nitrite either stabilizes the lipid components of the membranes or it inhibits the nature prooxidants present in muscle (Sato and Hegarty, 1971; Love and Pearson, 1974).

A number of researchers have noted the accelerating effect of heating on the development of oxidative rancidity in meat and meat products (Sato and Hegarty, 1971; Keller and Kinsella, 1973). Cooked meat exposed to oxygen can develop off-flavors in a few hours. The rapid oxidation of lipids in cooked meat has been attributed to the irreversible conversion of iron in the porphyrin ring of myoglobin pigments to the ferric form during heating (Younathan and Watts, 1959). Sato et al. (1973), however, found overcooking, as is common in canned meat, protects against warmed-over flavor, apparently by producing compounds that possess antioxidant activity. Lower levels of lipid oxidation have been observed in cooked, cured meat than in uncured samples. The pink, ferrous form of the cured meat pigment apparently does not cause rapid lipid oxidation.

On storage of cured meat, conversion of the pigment to the brown ferric form can result in increased TBA values for stored product

(Younathan and Watts, 1959). Greene (1969) reported that wrapping meat in oxygen impermeable film prevented metmyoglobin formation and lipid oxidation during storage, if sufficient reducing activity was present in the meat.

Watts (1962) demonstrated that extracts from a number of plant sources (green onions, green peppers, potato peelings, and green pepper seeds) are effective antioxidants for meat. Sweet (1973) pointed out the most potent inhibitors of oxidation were combinations of either butylated hydroxyanisole (BHA) or butyl-hydroquinone (BTHQ) with EDTA or citric acid.

In addition, the natural level of tocopherols present in the tissues may be sufficient to retard oxidation (Pearson et al., 1977). Thus, unexplained differences in the prevalence of rancidity may be due to differences in the amount of tocopherols in the meat itself and may be worthy of further study.

Vacuum Packaging

Some factors which influence the rate of oxidative rancidity include amount of oxygen present, the degree of unsaturation of the lipid component, amount of antioxidants present, metals, organic catalysts such as hematin compounds and lipoxidase, certain salts, processing treatment, storage temperature, exposure to light, and packaging.

The primary functions of a package for meat and meat products are to protect against physical damage, chemical changes, and microbial contamination, and to present the product to the consumer in the most attractive manner. It can only maintain, not improve, the quality of

the product. Packaging requirements are influenced by the type of product involved, the nature of any processing that may be employed, and the method of merchandising that is anticipated. The way to maintain the bright red color of fresh meat is to have a high partial pressure of oxygen in the surrounding atmosphere. Thus, in wrapping fresh meat, a packaging material should be chosen that will allow for the passage of oxygen.

Westerberg (1971) pointed out that there are eight film requirements for packaging processed meats used in vacuum and gas flushing procedures as follows: (1) barrier to oxygen, (2) barrier to moisture, (3) form hermetic seals, (4) tough puncture-resistant packages, (5) good optical properties, (6) thermoformable, (7) can be printed, and (8) can be used on packaging machines. He recommended that films with oxygen transmission rates less than 1 cc/100 in^2/24 hr ATM were required to package sliced bologna while transmission rates of about 2.5 cc/100 in^2/24 hr ATM were required for frankfurters. He also indicated the properties of six general types of films such as cellophane, polyethylene, polyvinyl chloride, polyvinylidene chloride, copolymer, polyester, and nylon.

According to Baltzer (1969), vacuum packaged meat has certain advantages over aerobically packaged meat because: (1) total bacterial counts increase more slowly, (2) putrefaction and slime formation are reduced, and (3) the final bacterial counts after storage usually are lower than in comparable samples packaged in oxygen permeable films. The principle involved in using oxygen impermeable films is to prevent reentry of oxygen into the package after residual oxygen is converted to carbon

dioxide. Johnson (1974) and Ingram (1962) reported that the oxygen remaining in a vacuum package after closure is converted to carbon dioxide by respiration of meat tissue and bacterial activity. Pierson et al. (1970) reported that vacuum packaging creates an anaerobic condition that prohibits the growth of common spoilage bacteria such as Pseudomonas and Achromobacter species and allow the development of facultative anaerobes such as Lactobacilli, Leuconostoc species, and Clostridium botulinum. However, the Clostridium botulinum is unlikely to grow in cured meats at normal refrigeration temperatures. Also, the concentration of nitrite in the meat tends to inhibit its growth.

Ramsbottom (1971) reported that vacuumized packages extend the shelf-life of processed meats up to three times over that of nonvacuum packages because the vacuum helps to prevent the light-catalyzed oxidation of the cured meat pigments under display lighting as well as aerobic microbial growth. Thus, the level of vacuum is very important.

In commercial practice, processors insist on vacuum levels of between 25 and 29 inches for vacuum packages and oxygen levels of 1% or less in controlled atmosphere packages. Kraft and Ayres (1954) recommended the 29 inch Hg vacuum pressures for bologna and 27 inch Hg for frankfurters (Ertle, 1969). These corresponded to initial residual oxygen concentrations of 1% and 2% in the package. The slightly higher oxygen content in packages of frankfurters is permissible due to pigments on the dehydrated surface which are usually subjected to the deposition of smoke ingredients. Apparently they are less sensitive to oxygen-caused and light-catalyzed discolorations than are pigments in the inner core of bologna. However, the higher vacuum pressures such

as 29 inch Hg used for packaging frankfurters showed more juices and more squaring or distortion of the products than packaging at lower vacuum pressures, such as 27 inch Hg. Seideman et al. (1976a,b) reported that high vacuum packaged wholesale cuts of beef tended to be lower in Lactobacilli and anaerobic counts and have better fat appearance, color retention, and total desirability than those of low and intermediate vacuum.

Meat Color as Influenced by Light

The concentration and chemical state of myoglobin (Mb) and hemoglobin (Hb) in the muscle are responsible for the color of fresh meat. The effect of display conditions is important since this affects autoxidation of the MbO_2 of fresh meat. Ramsbottom et al. (1951) indicated that fading and subsequent flavor losses in fresh meat were dependent upon the display lamps used, the intensity of the lamps, and the exposure time.

Kraft and Ayres (1954) showed that ultraviolet light caused the rapid autoxidation of MbO_2 in fresh meat, resulting in dark brown color very rapidly. To obtain similar degrees of discoloration under fluorescent light requires a much longer time than ultraviolet light. Lentz (1971) indicated that frozen beef would discolor in 1 to 3 days when held in a lighted display case, whereas storage at the same temperature in the absence of light caused no discoloration during the same period of storage. Satterlee and Hansmeyer (1974) and Zachariah and Satterlee (1973) indicated that soft white fluorescent light did affect the autoxidation of purified bovine, ovine, and porcine MbO_2.

There were few reports of work directly related to the specific wavelength of radiant energy affecting muscle pigment. On the basis of work with colored filters, Townsend and Bratzler (1958) suggested that 560 to 630μ light was the region of the spectrum most damaging to frozen meat pigments.

Solberg and Franke (1971) studied the effect of visible light (from 420 to 632.8 nm) on the autoxidation of beef MbO_2 in intact meat held at either 1.1 or 5°C. They concluded that visible light had a small, but significant, effect on the autoxidation of the MbO_2 in intact beef.

The cured meat pigment, once formed, is more stable at higher pH values. Bailey et al. (1964) found that the nitrosylhemochrome was more stable to light fading at pH 6.8 than at pH 6.2. Ramsbottom et al. (1951) indicated that 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.

Tarladgis (1962) advanced the view that fading mechanism of cured meat color by lipid oxidation or photocatalyzed dissociation of NO from heme involves withdrawal of electron density from the iron to the porphyrin, thus weakening the Fe-NO bond. The NO dissociates, leaving the iron susceptible to oxidation by electronegative groups present in the medium. He viewed green discoloration as being caused by electrophilic and nucleophilic attacks on areas of high and low electron density in the porphyrin ring π electron cloud causing ring opening. The fact that NO is a much better quencher than O_2 and has a higher electronic

affinity (Kearns, 1971) may explain why cured meat color is apparently more susceptible to light induced discoloration than is fresh meat color.

MATERIALS AND METHODS

Large diameter bologna was made at the Meat Lab of Iowa State University with a mixture composed of 3% salt, 0.5% Heller's number 531 seasoning, 0.05% sodium erythrobate, 10% ice, and with three different nitrite levels of 50 ppm, 100 ppm, and 156 ppm. Fresh beef and pork were trimmed to desired fat levels, approximately 10 and 50%. All trim was ground through the 9.5 mm plate of a grinder. Samples weighing 5.9 kg were taken from each batch of ground meat and placed in an Anyl-Ray (Anyl-Ray Corp.) machine for rapid fat analysis. The weights of analyzed meat were prepared in the proper proportions to give a 25±1% fat mixture. The beef trim was first placed in Kramer Grebe Silent Cutter, followed immediately by ice and salt. After 3 minutes of chopping, the pork trim and other ingredients were added. The final temperature was about 50 to 52°F. The emulsion was stuffed (Vemag Robot 1000S Type 116 model) into large diameter fibrous casing and smokehouse processed to 65°C internal temperature. After processing, samples were sliced (10 mm) and randomly packaged in eight different types of film characterized in Table 1.

Table 1. Characteristics of packaging films[a]

Film code no.	Oxygen permeability $ml/m^2/24$ hr
1	0.1
2	7.2
3	6.5
4	57
5	120
6	0.3
7	0.2
8	1592

[a]All films and permeability data provided by American Can Company.

Three studies were conducted as follows:

Study I, sodium nitrite: 156 ppm

8 packaging films: 1, 2, 3, 4, 5, 6, 7, 8
3 initial vacuum levels: maximum, 90%, 70%
5 storage times: 1 day, 4 days, 10 days, 21 days, 35 days

Study II

5 packaging films: 2, 3, 4, 6, 7
3 initial vacuum levels: same as study I
3 nitrite levels: 156 ppm, 100 ppm, 50 ppm
6 storage times: 1 day, 4 days, 10 days, 21 days, 35 days, 56 days

Study III, sodium nitrite: 156 ppm

5 packaging films: same as study II
3 sodium erythrobate levels: 0 ppm, 500 ppm, 940 ppm
3 initial vacuum levels: same as study I
6 storage times: same as study II

Three different initial vacuum levels of maximum attainable, 90% of maximum, and 70% of maximum were used in a Multivac Pouch machine MG 2 equipped with heat sealing bar. Measuring the vacuum levels inside packages immediately showed the three vacuum levels to be 27 to 29 in. Hg, 23 to 25 in. Hg, and 18 to 21 in. Hg, respectively. All samples were stored under 200 foot candles of cool white fluorescent light at 2 to 5°C in a display case for 1, 4, 10, 21, 35, and 56 days. At the time of packaging, the sliced bologna was very fresh and very bright pink in color, free of surface discoloration, and very desirable in general appearance.

Residual nitrite was determined according to the method of AOAC (1970).

Randicity measurements were made by the TBA (2-Thiobarbituric Acid) number according to the method of Tarladgis et al. (1960). An objective vacuum level inside of packages was determined by placing each package

in a plastic bell jar. The chamber was evacuated by an electric vacuum pump and the equilibrium vacuum pressure between package and chamber was determined. As the film separated from the meat surface, a measurement in millimeters was recorded as the equilibrium vacuum level.

Quantitation of nitrosopigment was performed by acetone water extraction according to techniques described by Hornsey (1956). Pigment conversion was the percentage of total heme pigments converted to nitric oxide haemochrome.

Reflectance spectroscopy also was used to determine the degree of pigment nitrosation. A ratio of K/S values for percent reflectance readings at 570 and 650 nm was used with a Beckman DK-2A spectro-reflectometer (Wodicka, 1956; Judd and Wyszecki, 1963; Giddy, 1966).

A photovolt with a green filter also was used to measure color change as total reflectance values.

Subjective evaluation of surface discoloration was included by use of an 8-point scale scored from 8 dark reddish-pink to 1 brown-green abnormal.

RESULTS AND DISCUSSION

Effect of Packaging Conditions and Length of Storage on Color and Rancidity Development

Table 2 shows that film 1 had the greatest cured meat color retention. Film 1 was a good oxygen barrier and also included aluminum foil, making it nontransparent. This was followed by films 2 and 3 which showed quite similar results. These were in turn followed by films 6, 7, and 4. Films 5 and 8 showed the worst color retention as measured by nitric oxide heme pigment and pigment conversion. The terminal vacuum degree was only slightly different between films except for films 6 and 7 which were significantly lower ($P<0.05$). This might be due to the basic material of films 6 and 7 which was of a more rigid type which did not adhere as closely to the product as did the others. In addition, rancidity development was not significantly different between films except for films 5 and 8 which had high values, probably resulting from higher oxygen permeability (120 and 1592 $ml/m^2/24$ hr, respectively). The principle involved in using oxygen impermeable films was to prevent reentry of oxygen into the package after residual oxygen was converted to carbon dioxide.

Reflectance ratios (K/S 570/K/S 650) for sliced bologna treated with different packaging films and storage are shown in Table 3. A higher value in this ratio was indicative of greater color development. Spectral reflectance analysis revealed that a ratio of 3.5 or greater for sliced bologna samples indicated acceptable color. Comparisons for different films indicated that films 4 and 5 appeared brownish-gray at 10 days and 4 days storage, respectively. Film 8 appeared abnormally greenish in

Table 2. Effect of packaging films on development of cured meat color and rancidity in sliced bologna[1]

Variables	Films							
	1	2	3	4	5	6	7	8
Nitric oxide heme pigment (ppm)	101.65^{a} ±10.79	76.33^{b} ±6.11	75.62^{b} ±14.76	59.20^{d} ±25.74	38.51^{e} ±26.92	66.42^{c} ±26.79	60.91^{d} ±28.99	9.80^{f} ±9.33
Pigment conversion (%)	72.62^{a} ±10.03	64.11^{b} ±10.55	62.14^{b} ±9.49	48.41^{d} ±20.08	31.85^{e} ±18.34	54.40^{c} ±18.88	51.38^{cd} ±21.27	12.35^{f} ±5.77
Terminal vacuum (in)	23.10^{ab} ±5.25	24.55^{a} ±3.73	23.60^{ab} ±4.08	23.23^{ab} ±5.46	23.97^{ab} ±4.24	19.36^{c} ±10.24	17.04^{d} ±10.29	22.16^{b} ±7.73
TBA no.[2]	0.67^{c} ±0.17	0.52^{c} ±0.06	0.59^{c} ±0.11	0.98^{c} ±0.79	3.77^{b} ±4.47	0.60^{c} ±0.16	0.78^{c} ±0.47	8.23^{a} ±5.75

[1]All means in a row with the same superscripts are not significantly different ($P<0.05$); X±SD = mean ± standard deviation.

[2]TBA is expressed as mg malonaldehyde/1000 g sample.

Table 3. Effect of packaging films and storage interval on the development of cured meat color in sliced bologna as measured by reflectance Spectra ratios (K/S 570/K/S 650)[a]

Storage times (days)	Films							
	1	2	3	4	5	6	7	8
1	6.25 ±0.82	5.60 ±1.02	4.69 ±1.07	4.58 ±0.61	4.24 ±0.88	4.18 ±1.36	3.54 ±1.47	1.60 ±0.08
4	7.08 ±0.48	5.30 ±1.34	4.65 ±1.54	4.03 ±1.06	2.20 ±0.61	3.80 ±1.77	4.03 ±1.77	1.57 ±0.04
10	7.23 ±0.53	4.85 ±1.24	4.63 ±0.85	2.77 ±1.40	1.70 ±0.38	4.85 ±1.43	4.12 ±1.81	1.62 ±0.30
21	6.32 ±0.43	4.17 ±1.10	4.26 ±1.07	2.00 ±0.59	1.32 ±0.09	4.24 ±1.52	4.50 ±1.93	1.33 ±0.03
35	6.46 ±0.96	3.96 ±0.93	3.86 ±0.32	1.67 0.53	1.41 0.25	3.92 ±1.61	3.91 ±1.52	1.23 ±0.01

[a]X ± SD = mean ± standard deviation.

color after only one day storage. Film 1 retained pink color even at 35 days storage. Films 2, 3, 6, and 7 were found to be quite similar in that color decreased with storage time but was still acceptable at 35 days. This indicated, as expected, that the better barrier film will provide longer shelf-life and better quality product with the storage period than will film with inferior oxygen barrier. All values were confirmed by extracted pigment concentrations (Table 2) and subjective color scores although all values are not shown.

Figures 5 and 6 show the significant interaction effect of packaging films and initial vacuum degree on cured meat color retention as measured by reflectance ratios. In general, all films had a higher value for maximum vacuum followed by 90% and 70% of maximum vacuum. The films 4, 5, and 8 all showed very significant discoloration corresponding with initial vacuum level. This suggests that the films with oxygen permeability higher than 57 $ml/m^2/24$ hr cannot accomplish good color retention even with high initial vacuum. Films 6 and 7 had very good cured meat color development as long as maximum vacuum was used; otherwise, they showed complete fading of color. This might be due to loss of vacuum between initial and terminal evaluations, particularly for packages of lower initial vacuum.

These values were also confirmed by examination of initial vacuum degree as affecting the development of cured meat color and rancidity as indicated in Table 4. The maximum initial vacuum samples showed no significant differences on residual sodium nitrite or TBA numbers with 90% vacuum level; however, they had significantly higher residual nitrite and lower TBA numbers than those of the 70% initial vacuum group.

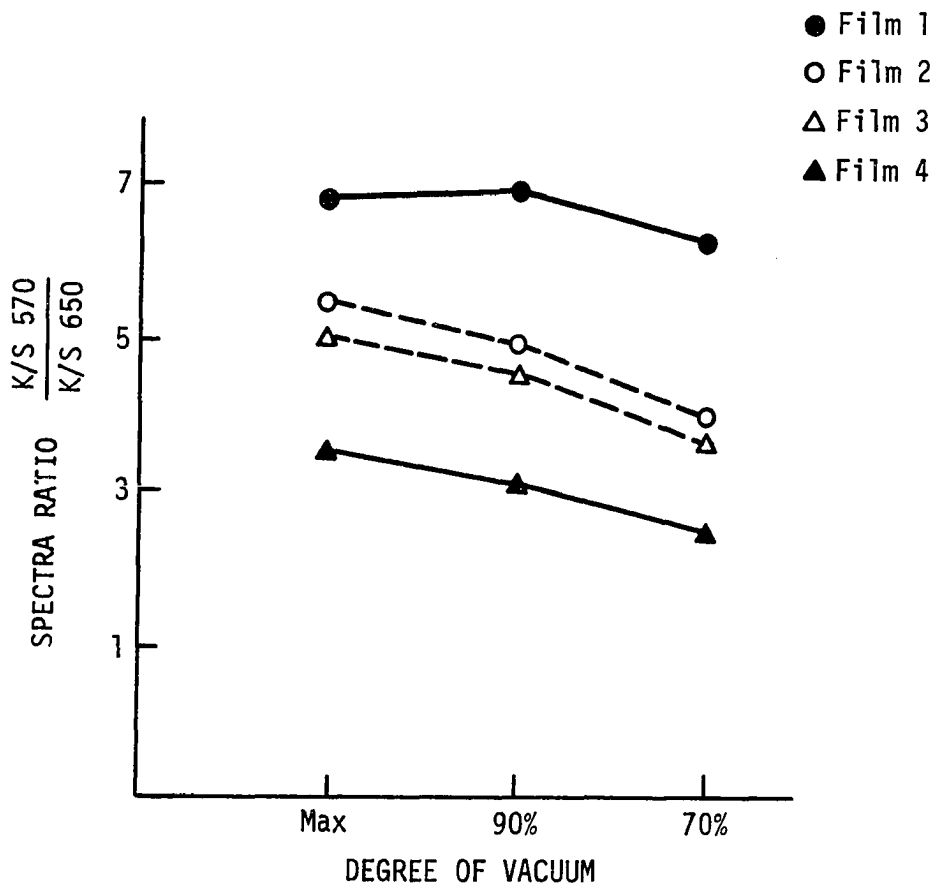


Figure 5. Effect of packaging films and degree of vacuum on spectra ratio

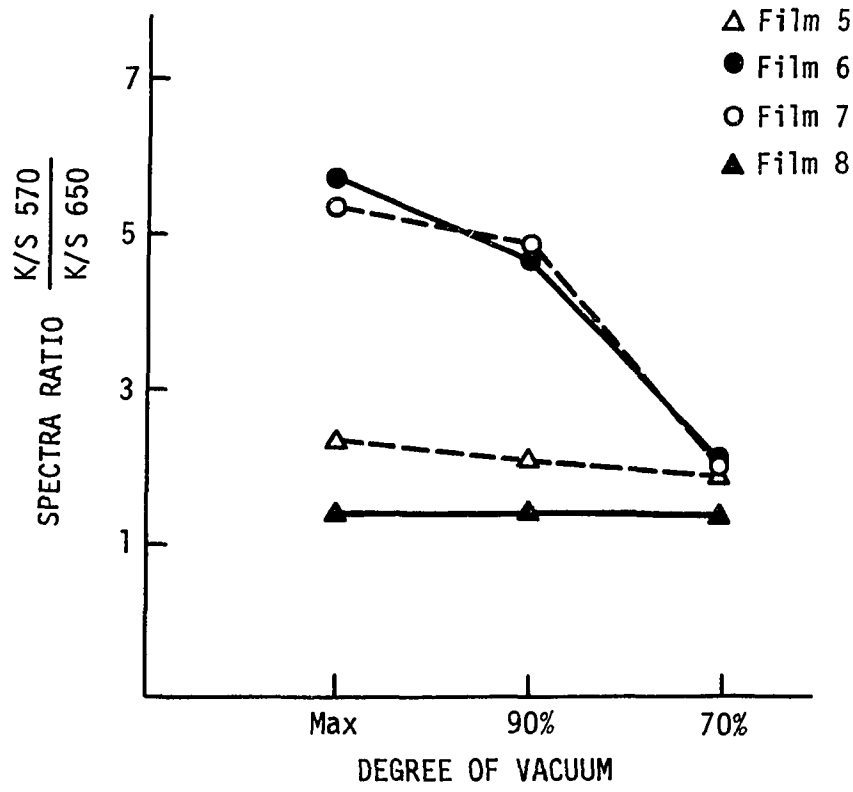


Figure 6. Effect of packaging films and degree of vacuum on spectra ratio

Table 4. Effect of initial vacuum degree on development of cured meat color and rancidity in sliced bologna[1]

Variable	Degree of vacuum		
	Maximum	90%	70%
Color score	5.17[a] ±1.70	3.93[b] ±1.53	2.50[c] ±1.40
Nitric oxide heme pigment (ppm)	75.99[a] ±26.08	71.14[b] ±24.94	47.14[c] ±26.56
Pigment conversion (%)	60.78[a] ±13.94	57.52[b] ±13.43	41.39[c] ±19.77
$NaNO_2$ residual (ppm)	8.71[a] ±3.77	8.34[a] ±3.69	7.12[b] ±3.37
TBA	1.74[b] ±3.50	1.93[b] ±3.55	2.39[a] ±3.80

[1]All means in a row with the same letter are not significantly different ($P<0.05$); X ± SD = mean ± standard deviation.

Packages with higher initial vacuum levels resulted in greater film adhesion to the product surface. Generally, cured meat products with good film to product contact had less surface discoloration. Bowling et al. (1977) pointed out that the higher initial vacuum levels were associated with more complete removal of residual air, tighter package adhesion, less purge flow over fat surfaces, less browning of lean and fat surfaces, and possibly less metmyoglobin formation. Discoloration was usually started from the edge of product surface. It might be due to lack of package adhesion and more residual air spaces. Seideman et al. (1976a) agreed with this finding. Greater bacterial activity in 70% initial vacuum packages also might have caused surface discoloration. Seideman et al. (1976b) reported that wholesale cuts showed lower bacterial count

at high initial vacuum levels than at lower initial vacuum levels in packaged samples.

Figure 7 shows that maximum and 90% initial vacuum packages experienced only slight changes in terminal vacuum within packages. However, the 70% initial vacuum packages showed more loss of vacuum with storage time which may have resulted from more production of gas inside the bag through greater bacterial activity or from more atmospheric air passing through the film.

The interaction between initial vacuum and packaging film is shown in Table 5. Generally, the TBA number increased as vacuum levels decreased. Film 1, 2, or 3 showed only slight differences for TBA values among the three vacuum levels. This indicated that vacuum level had little influence on TBA value for any of these three films. Film 6 and film 7 showed slightly different values between the maximum and 90% vacuum level. However, the 70% vacuum was found to be a great influence on TBA number. Film 4 showed higher rancidity development under 90% and 70% vacuum levels compared to films 1, 2, 3, 6, and 7. Due to high oxygen permeability of films 5 (120 $ml/m^2/24$ hr) and 8 (1592 $ml/m^2/24$ hr), these films showed extremely high TBA numbers even for maximum vacuum level.

Cured color development was evaluated by determining percentage of pigment conversion. Maximum and 90% vacuum were noted to have better color development for films 1, 2, 3, 6, and 7. Only films 1, 2, and 3 showed good color retention for all three vacuum levels. Film 1 showed the best color retention among films and was slightly different at the three vacuum levels. Films 5 and 8 showed unacceptable color even at the maximum vacuum level. However, oxygen permeability as high as

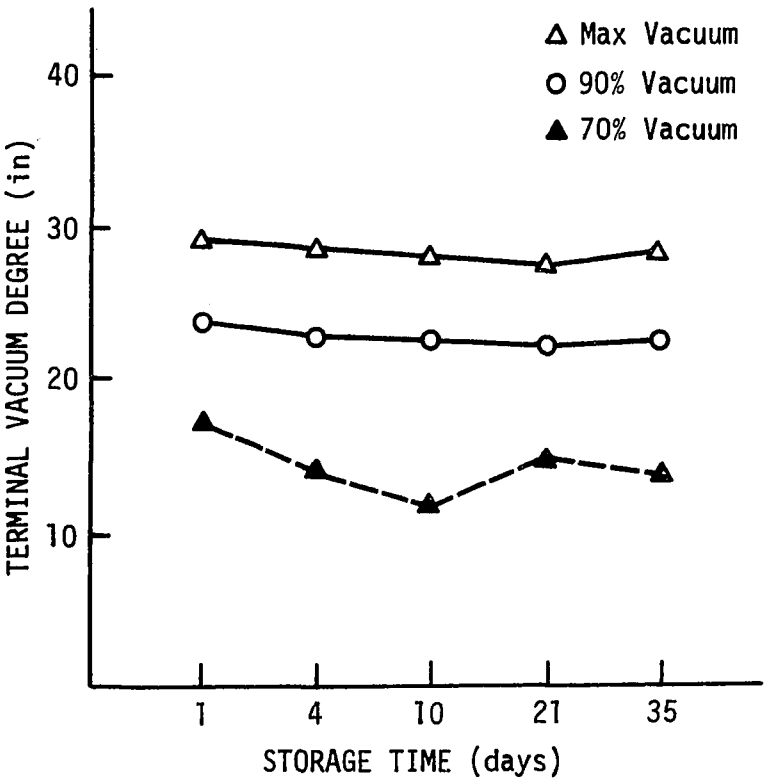


Figure 7. Effect of initial vacuum degree and storage time on terminal vacuum level

Table 5. Effect of packaging films and initial vacuum degree on TBA number and cured color development[a]

Films	Initial vacuum degree					
	Maximum		90%		70%	
	TBA no.	Pigment conversion (%)	TBA no.	Pigment conversion (%)	TBA no.	Pigment conversion (%)
1	0.66±0.19	73.86±10.70	0.67±0.16	72.86±7.35	0.69±0.21	72.20±10.89
2	0.50±0.05	65.82±11.52	0.52±0.08	63.02±10.59	0.53±0.06	59.16±9.39
3	0.58±0.07	64.18±9.51	0.60±0.16	64.33±7.73	0.60±0.12	55.70±11.33
4	0.51±0.07	58.52±10.17	0.77±0.41	56.92±14.07	1.68±1.04	30.02±18.37
5	1.88±2.79	32.17±24.16	3.92±5.04	28.27±17.20	5.51±5.32	23.67±17.05
6	0.55±0.13	62.52±6.09	0.53±0.07	58.93±6.34	0.70±0.24	30.92±9.96
7	0.54±0.09	59.00±5.19	0.59±0.13	57.39±6.74	1.21±0.66	27.63±16.08
8	8.66±6.43	12.84±5.78	7.87±5.92	13.01±6.26	8.18±6.25	14.99±7.92

[a]X ± SD = mean ± standard deviation.

57 ml/m^2/24 hr such as in film 4 still can retain desirable color as long as maximum vacuum is used. This indicated that maximum vacuum packaging and good barrier films can eliminate the oxidation of fat, thus decreasing rancidity, oxidation of heme pigments, and discoloration. These results tend to agree with Kraft and Ayres (1954) who recommended 29 in. of Hg vacuum pressure and Westerberg (1971) who recommended that films with oxygen transmission rates less than 10 ml/m^2/24 hr be used for packaging sliced bologna.

This study made no consideration for microbial growth under the various conditions discussed. The microbial question needs to be addressed before any application of this approach is made in commercial practice.

Due to the nontransparent nature of film 1, which is very effective in retarding fading, consumers would not be able to see the product when they purchased it. Therefore, the use of film 1 might create some sales resistance. Films 5 and 8 were unacceptable after one day storage. Thus, the other five films were considered to be more important for the study of reduced nitrite levels and were utilized in study II.

Conclusions

A maximum (27 to 29 inches of Hg) initial vacuum level combined with films of low oxygen permeability (7.2 ml/m^2/24 hr or less) resulted in higher color score, greater cured pigment conversion, and lower TBA numbers.

Effect of Sodium Nitrite Concentration, Packaging Conditions, and Length of Storage on Color and Rancidity Development of Sliced Bologna

The effects of packaging films on color and rancidity development are given in Table 6. There are no differences in pH value or total pigments among all films. Films 2 and 3 showed quite similar and better color retention than the other three films, possibly due to a lower oxygen permeability (7.2 and 6.5 $ml/m^2/24$ hr, respectively). These are followed by films 6 and 7 which have oxygen permeability of 0.3 and 0.2 $ml/m^2/24$ hr, respectively. Film 4 showed significantly lower color retention as measured by color score, nitric oxide heme pigment, and pigment conversion. This may be due to the higher oxygen permeability (57 $ml/m^2/24$ hr) resulting in high oxidation of the heme pigment. Although films 6 and 7 have lower oxygen permeability than films 2 and 3, they still showed significantly less cured color retention and significantly higher TBA numbers compared to films 2 and 3. The rigid material in these films may make it difficult to produce or hold a good vacuum package. This effect was also indicated by significantly lower terminal vacuum levels. Films 6 and 7 had values of 19.51 and 16.68 inches of Hg, respectively.

Table 7 indicates that the initial vacuum has no significant influence on pH value. However, the samples in maximum initial vacuum showed significantly better color retention as indicated by lower surface reflectance, higher reflectance ratio, higher pigment extraction and conversion. This was followed by 90% and 70% initial vacuum group samples. The maximum vacuum group also showed significantly lower TBA

Table 6. Effect of packaging films on color and rancidity development[1]

Items	Films				
	2	3	4	6	7
pH	6.00±0.11^{a}	6.00±0.11^{a}	6.01±0.11^{a}	6.00±0.13^{a}	6.03±0.10^{a}
Color score	4.12±1.37^{a}	4.26±1.35^{a}	2.85±1.74^{c}	3.78±1.77^{b}	3.65±1.80^{b}
K/S 570/K/S 650	4.61±1.26^{a}	4.32±1.09^{ab}	2.74±1.44^{d}	4.15±1.62^{bc}	3.95±1.62^{c}
Nitric oxide heme pigment (ppm)	73.61±17.32^{a}	73.60±15.78^{a}	52.14±29.48^{d}	65.67±26.90^{b}	58.98±28.74^{c}
Total pigment (ppm)	117.06±17.37^{a}	120.06±20.52^{a}	113.44±25.70^{b}	117.49±22.94^{a}	111.69±26.51^{b}
Pigment conversion (%)	62.61±10.93^{a}	61.32±9.43^{a}	43.10±22.82^{d}	54.66±18.02^{b}	51.09±20.87^{c}
Terminal vacuum (in.)	24.42±3.93^{a}	23.64±4.07^{a}	23.42±5.22^{a}	19.51±10.11^{b}	16.68±10.54^{c}
$NaNO_2$ residual (ppm)	7.31±4.03^{ab}	7.63±3.55^{a}	6.83±3.53^{b}	7.70±3.73^{a}	7.08±3.94^{b}
TBA no.	0.56±0.34^{c}	0.53±0.33^{c}	0.80±0.71^{b}	0.80±1.08^{b}	1.18±1.36^{a}

[1]All means in a row with the same superscripts are not significantly different ($P<0.05$); X ± SD = mean ± standard deviation.

Table 7. Effect of initial vacuum degree on color and rancidity development[1]

Items	Degree of vacuum		
	Maximum	90%	70%
pH	6.01±0.12[a]	6.02±0.11[a]	5.00±0.12[a]
Color score	5.22±1.31[a]	3.79±1.17[b]	2.21±0.94[c]
Reflectance	33.29±3.08[c]	34.09±3.19[b]	36.13±3.21[a]
K/S 570/ K/S 650	4.89±1.39[a]	4.24±1.14[b]	2.73±1.26[c]
Nitric oxide heme pigment (ppm)	76.96±20.60[a]	72.72±19.98[b]	44.72±23.14[c]
Total pigment (ppm)	121.61±19.91[a]	121.76±21.30[a]	104.47±23.09[b]
Pigment conversion (%)	62.99±14.82[a]	59.54±14.70[b]	41.15±18.07[c]
Terminal vacuum (in.)	27.76±1.07[a]	23.17±3.31[b]	13.68±8.53[c]
$NaNO_2$ residual (ppm)	8.04±3.73[a]	7.62±3.61[b]	6.26±3.70[c]
TBA no.	0.55±0.56[b]	0.65±0.75[b]	1.13±1.15[a]

[1]All means in a row with the same superscripts are not significantly different ($P<0.05$); X ± SD = mean ± standard deviation.

values. These differences probably resulted from the maximum vacuum resulting in no visible residual air spaces between the meat surface and the film, with the film adhering tightly to the meat surface. Samples in the 70% vacuum group had numerous visible air spaces between the meat surface and film, particularly in films 6 and 7. Mean values for terminal vacuum measurements revealed significant differences among levels of vacuum used for packaging. Objective vacuum measurements averaged over storage time, nitrite concentration, and films in maximum, 90%, and 70% vacuum groups were 27.76, 23.17, and 13.68 in. of Hg, respectively. Surface discoloration was usually evident from the edge of the package in the

70% vacuum group. These were the areas of incomplete contact of product surface to the film, allowing residual air spaces. Seideman et al. (1976a) indicated the same findings on vacuum packaged wholesale and subprimal beef cuts. Ledward (1970) claimed that 1 to 2% oxygen inside the package could theoretically result in the formation of substantial amounts of surface discoloration via metmyoglobin formation. Seideman et al. (1976b) also pointed out the increasing surface discoloration of wholesale beef cuts in the low vacuum treatment might have resulted from greater bacterial activity, differences in bacterial types due to availability of oxygen and other gases, or the presence of more residual oxygen which allowed increased formation of metmyoglobin.

Figure 8 shows highly significant effects of the interaction of films and initial vacuum degree on cured meat color development of sliced bologna as measured by reflectance ratios (K/S 570/K/S 650). A higher value in this ratio is indicative of greater color development. Reflectance spectra analyses revealed that a ratio of 3.5 or greater for sliced bologna samples indicated acceptable color. In general, the maximum vacuum showed better color retention than 90% or 70% initial vacuum. Films 2 and 3 retained a pink color even in the 70% vacuum group up to 21 and 10 days, respectively (Table 8). Figure 8 also indicates that film 4 has poor color retention traits even under maximum vacuum. Films 6 and 7 allow the greatest color development, particularly at maximum vacuum. The 90% vacuum group showed acceptable color; however, the 70% vacuum group showed extremely poor color due to the extremely low terminal vacuum.

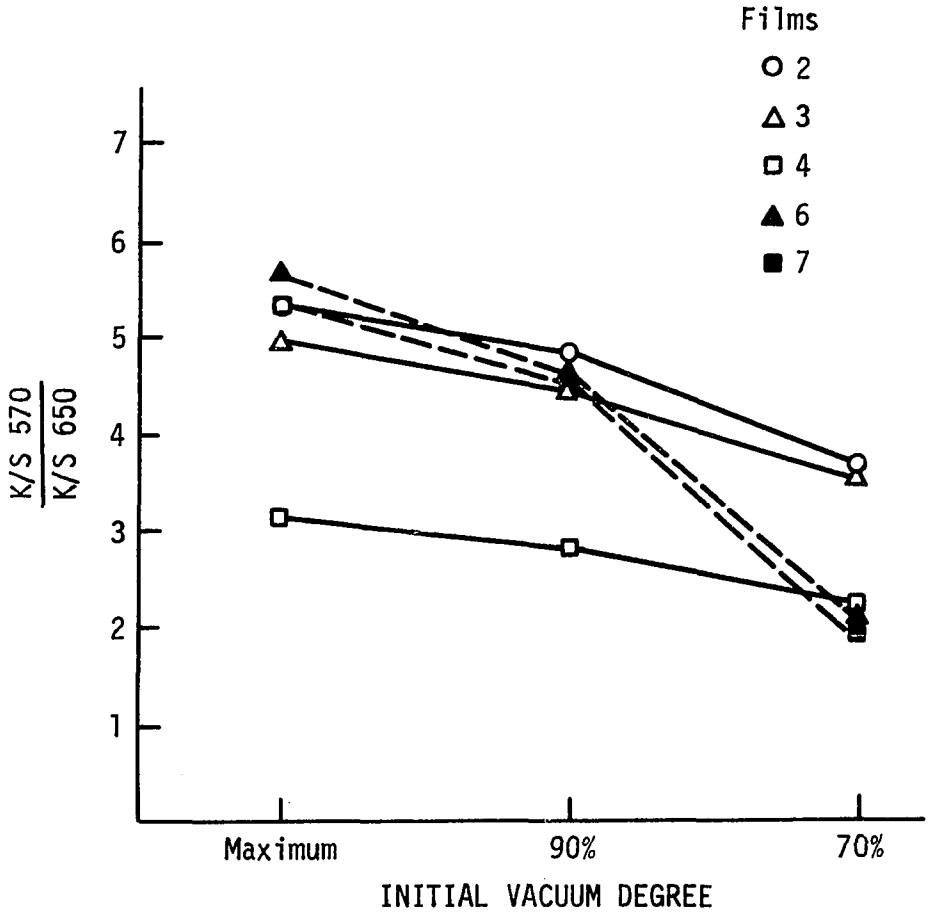


Figure 8. Effect of packaging films and initial vacuum degree on cured color development

Table 8. Effect of packaging films, initial vacuum degree, and storage period on cured color development as measured by percentage of pigment conversion[a]

Storage time (days)	Initial vacuum degree	Films 2	3	4	6	7
1	Maximum	66.38±11.28	66.11±10.06	65.18±3.83	69.28±6.93	65.32±10.73
	90%	63.36±10.22	67.59±5.25	63.90±4.94	63.65±7.61	54.54±11.17
	70%	58.41±1.92	57.27±10.27	53.60±10.06	26.44±3.58	24.27±13.42
4	Maximum	72.73±16.26	69.71±11.55	65.57±16.76	67.94±17.57	70.31±22.19
	90%	69.08±17.13	65.92±9.07	64.89±14.07	64.28±19.40	64.56±16.81
	70%	67.27±14.64	54.04±3.30	56.13±14.38	17.42±3.21	27.25±17.06
10	Maximum	74.54±10.67	74.31±7.48	61.08±16.67	70.28±2.22	69.15±3.51
	90%	69.37±7.15	68.14±9.86	58.01±22.27	62.34±3.03	66.24±9.48
	70%	57.40±6.55	59.09±13.90	44.90±7.73	33.57±11.32	21.29±5.52
21	Maximum	66.27±4.12	66.29±5.70	40.72±8.50	66.05±11.00	67.79±11.27
	90%	66.96±5.11	61.82±4.90	40.97±12.47	58.97±11.21	54.90±19.84
	70%	59.90±7.36	53.04±9.28	35.28±13.84	49.26±9.27	38.37±8.98
35	Maximum	60.77±8.48	59.11±1.02	34.79±23.82	65.84±8.37	60.42±6.82
	90%	61.98±7.56	59.16±1.87	33.64±22.62	65.14±7.00	64.20±3.79
	70%	47.15±3.34	50.56±6.97	7.46±2.29	35.54±5.02	22.01±9.45
56	Maximum	60.39±12.42	59.44±5.39	23.85±23.99	67.03±7.77	63.22±8.45
	90%	53.57±10.16	24.29±2.45	25.56±2.02	60.25±11.53	59.78±12.13
	70%	51.48±9.73	52.32±9.41	6.97±1.95	40.67±1.59	26.04±9.50

[a]X ± SD = mean ± standard deviation.

Table 8 shows the interaction among films, initial vacuum, and storage time on pigment conversion. Pigment conversion of 55 percent or higher indicated an acceptable color. Films 2, 3, 6, and 7 were found to increase the pigment conversion up to 10 days storage and then showed a decrease gradually for the maximum and 90% group. These groups remained pink up to 56 days. The reason for this is probably due to the reducing activity of the cured product. The nitrite takes the place of oxygen in part of the respiratory chain and the oxidation caused hereby is repaired by the NADH-dehydrogenase system (Walters et al., 1968). Hornsey (1956) pointed out that free SH compounds, such as cysteine and reduced glutathione, are the most likely compounds capable of reducing the ferric heme pigments to the corresponding ferrous compounds in cooked meat. This increase in color is also due to the decrease in moisture over this period, resulting in a corresponding increase in pigment concentration and the elevation of pH due to denaturation of protein (Eakes et al., 1975). For the 70% vacuum group, films 6 and 7 showed unacceptable color even in the one day samples. However, films 2 and 3 maintained good appearance up to 21 days. This may be due to the lower terminal vacuum. Watts et al. (1966) reported that reduction of metmyoglobin to myoglobin will not occur until residual oxygen is substantially reduced. Film 4 showed an increase in color up to 4 days storage and still retained a good color appearance up to 10 days storage for maximum and 90% initial vacuum groups. From all these findings, it is suggested that the reducing activity of cured cooked meat product can exist longer if a good barrier film and high initial vacuum are used.

The reason for poor color in the 70% initial vacuum group is indicated in Figure 9. Films 2 and 3 have relatively slight changes in terminal vacuum with storage time. However, films 6 and 7 showed significantly lower terminal vacuum over all the storage times. They even seemed different at the first day compared to the other films which may be caused by the 70% initial vacuum not being high enough to make these rigid material films adhere tightly to the meat surface. Therefore, these films contained more residual air spaces and resulted in higher TBA numbers for films 6 and 7 as compared to the other films (Figure 10).

Table 9 indicates that there were no significant differences between 156 ppm and 100 ppm nitrite levels on nitric oxide heme pigment and TBA number. However, the 156 ppm nitrite level was found to have significantly lower pigment conversion (54.27%) than 100 ppm nitrite level (60.23%), and both of these results were significantly higher than that of 50 ppm nitrite level (49.17%). The product was prepared with 25±1% fat in a combination of beef with about 10% fat and pork with about 50% fat for all studies. However, it was still very difficult to control the myoglobin content of the raw material in making the product. Different amounts of myoglobin or different environmental conditions within the meat source, such as reducing activity, may be the reasons for the lower pigment conversion of 156 ppm nitrite level compared to 100 ppm nitrite level. It is also possible that the O. D. value was not read immediately after pigment extraction. Hornsey (1956) indicated that the bright red color of extracted solution was converted to yellow-brown if the solution was exposed for one hour. The change was indicated by a decreasing O. D., meaning lower nitric oxide heme pigment concentration. In regard to the

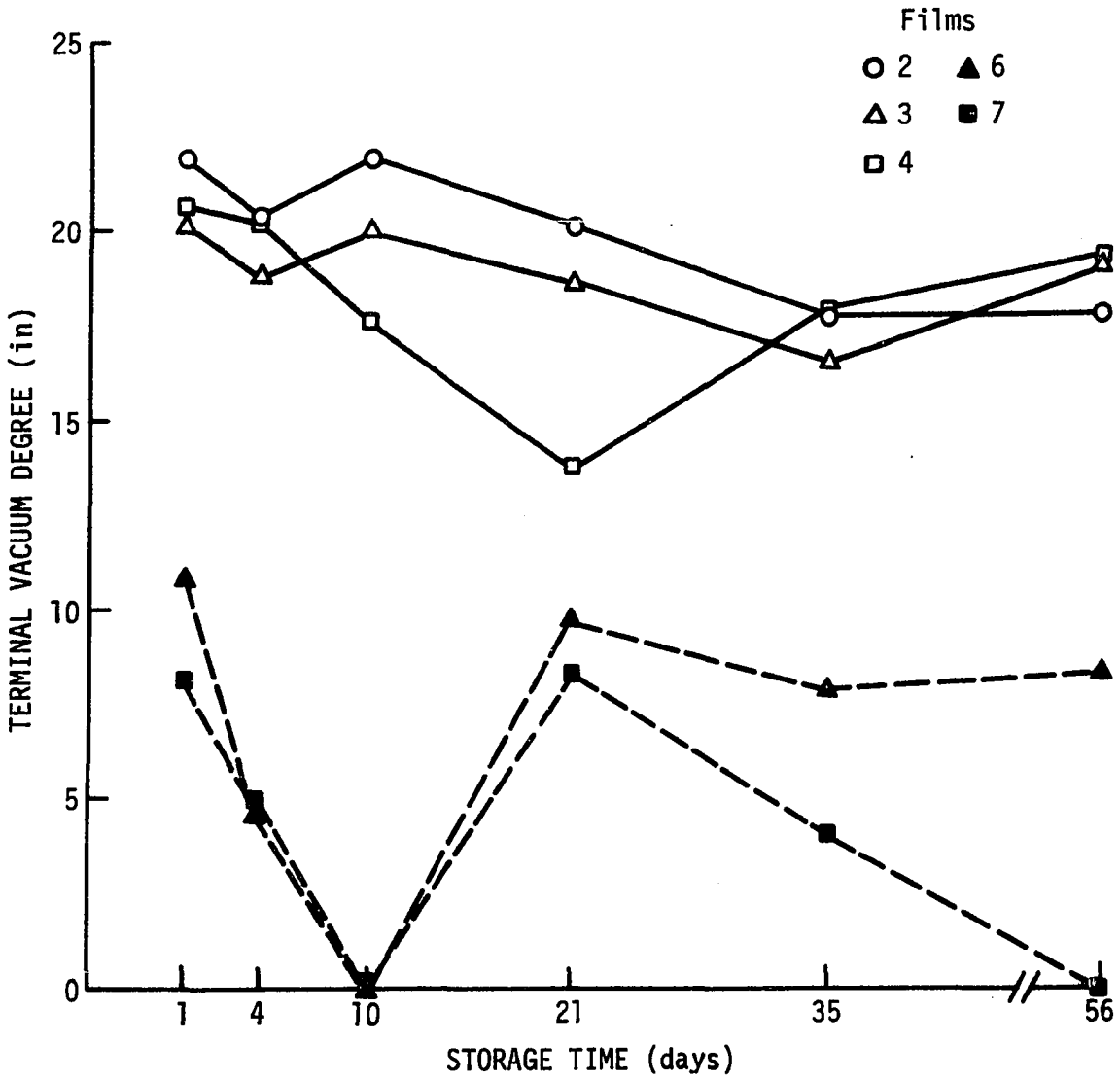


Figure 9. Effect of packaging films and storage period on terminal vacuum degree under 70% initial vacuum

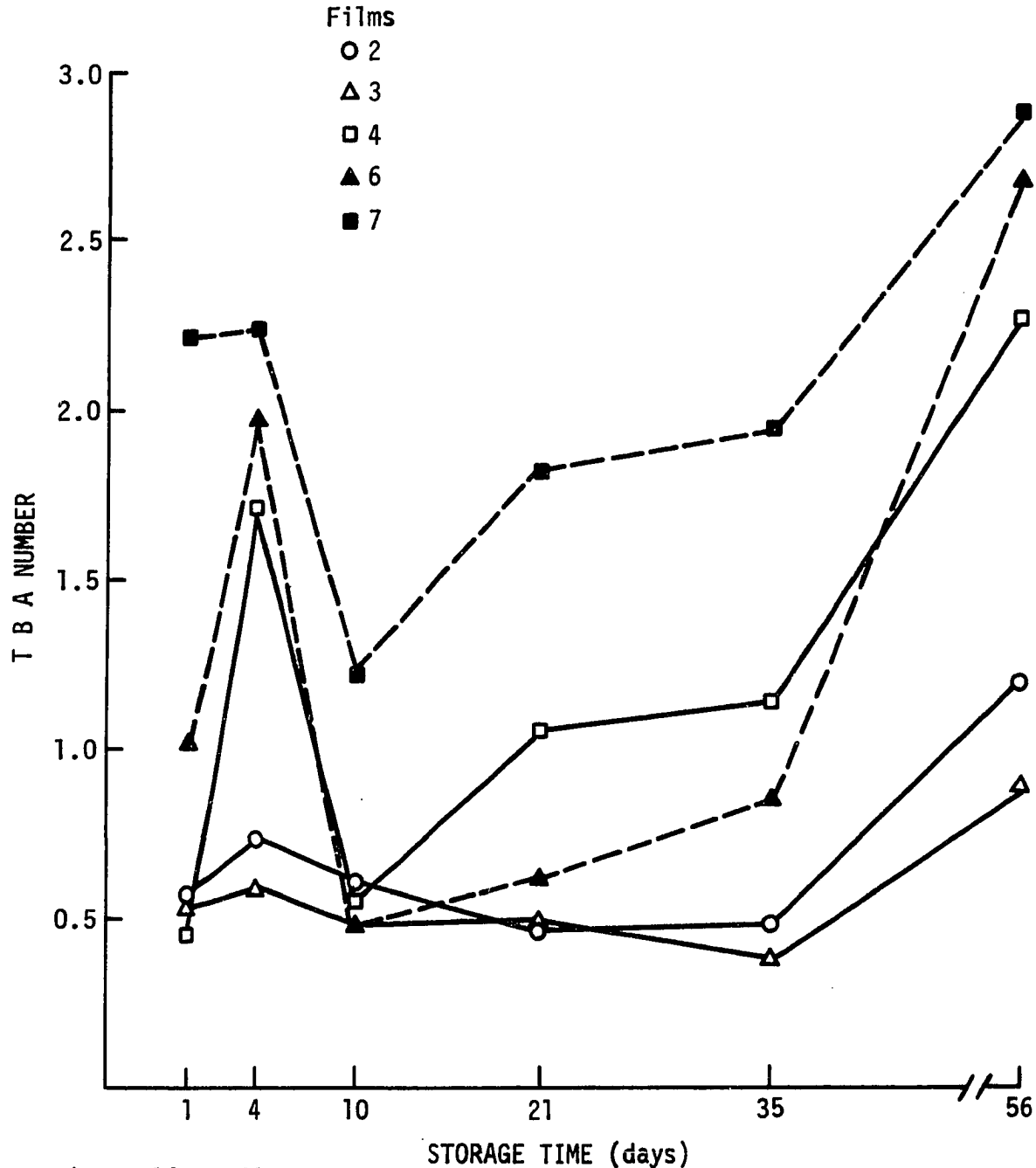


Figure 10. Effect of packaging films and storage period on TBA number under 70% initial vacuum

Table 9. Effect of nitrite concentration on color and rancidity development[1]

Items	Levels of nitrite		
	156 ppm	100 ppm	50 ppm
Color score	4.20±1.81[a]	3.51±1.48[b]	3.51±1.66[b]
Reflectance	31.74±2.61[c]	34.62±2.52[b]	36.67±3.03[a]
Nitric oxide heme pigment (ppm)	71.13±24.38[a]	70.66±26.59[a]	52.60±21.26[b]
Total pigment (ppm)	128.30±24.33[a]	114.78±17.70[b]	104.76±19.87[c]
Pigment conversion (%)	54.27±16.19[b]	60.23±19.75[a]	49.17±18.07[c]
$NaNO_2$ residual (ppm)	9.25±3.75[a]	7.41±3.89[b]	5.26±2.27[c]
TBA no.	0.69±0.45[b]	0.58±0.63[b]	1.03±1.26[a]

[1]All means in a row with the same superscripts are not significantly different ($P<0.05$); X ± SD = mean ± standard deviation.

residual nitrite, the 156 ppm nitrite level showed a significantly higher (9.25 ppm) value, followed by the 100 ppm nitrite level (7.41 ppm), and the 50 ppm nitrite level (5.26 ppm).

The interaction of nitrite concentration and initial vacuum on nitric oxide heme pigment is given in Figure 11. Both the 156 ppm and 100 ppm nitrite groups showed similar results with significantly higher values for maximum and 90% initial vacuum groups compared to 50 ppm nitrite samples. At 70% initial vacuum, very low nitric oxide heme pigment concentrations resulted for all three nitrite groups.

Comparison of nitrite concentrations with storage time for pigment conversion is shown in Figure 12. The color development in 156 ppm and 100 ppm nitrite groups showed better cured color than the 50 ppm group over the entire storage period. An increase in color with up to 4 days

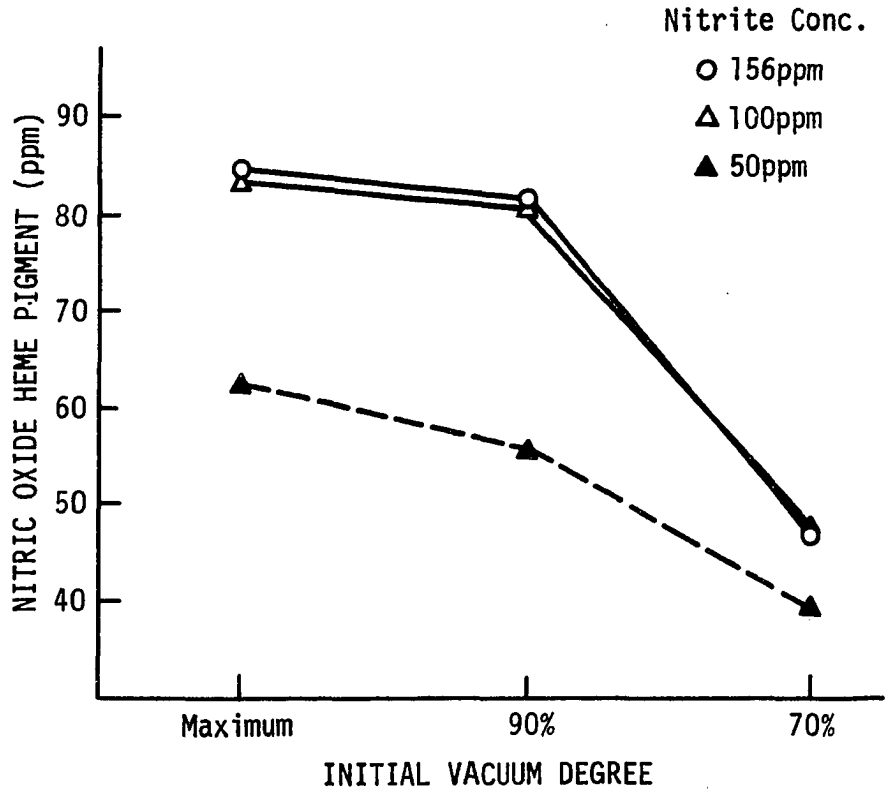


Figure 11. Effect of nitrite concentration and initial vacuum degree on nitric oxide heme pigment

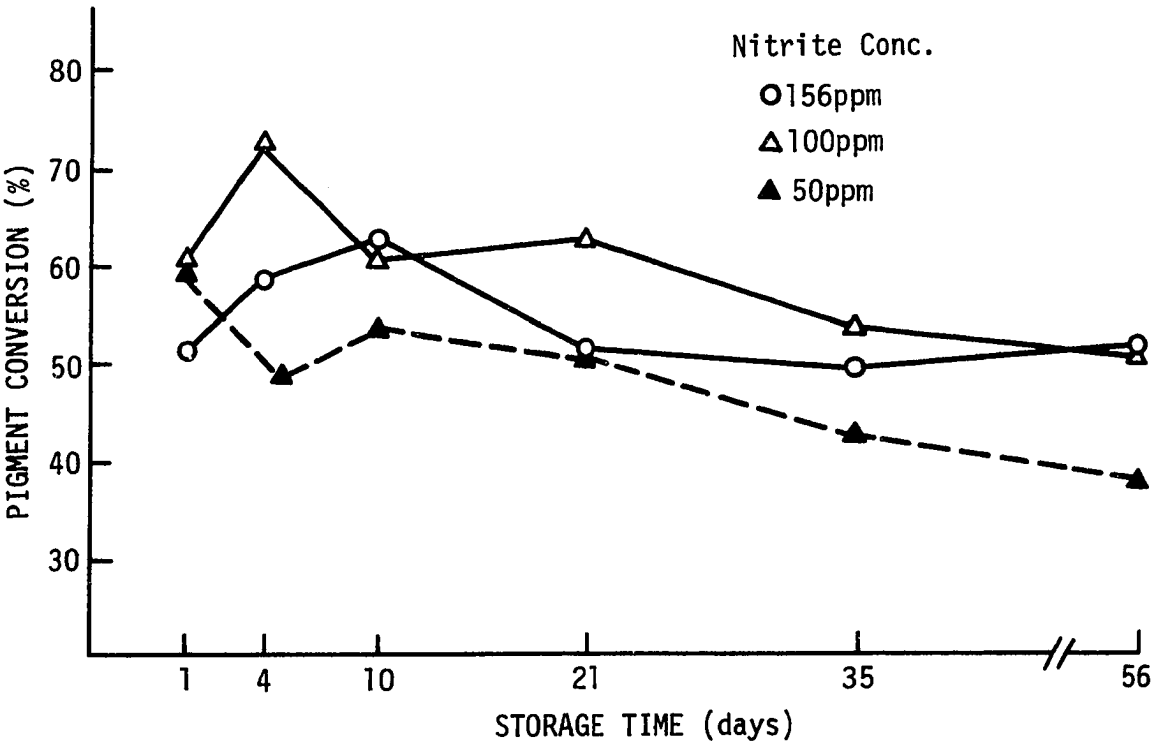


Figure 12. Effect of nitrite concentration and storage period on pigment conversion

storage for the 100 ppm and 21 days storage for the 156 ppm nitrite group was detected, which then gradually decreased. The 50 ppm nitrite group did not show the increased color from the first day of storage and color decreased with storage time. It is obvious that increased levels of nitrite produced higher concentrations of nitrosopigment and also retarded rancidity development.

The interaction influence of films, nitrite concentration, and initial vacuum on pigment conversion is reported in Table 10. The 156 ppm nitrite groups showed acceptable color at all three initial vacuum levels for films 2 and 3. Acceptable color was indicated at maximum and 90% vacuum levels for films 4, 6, and 7, and totally unacceptable color at 70% vacuum for films 4, 6, and 7. At the 100 ppm nitrite level, film 4 showed discoloration at all three vacuum levels, while films 6 and 7 showed discoloration at 70% vacuum. At the lower 50 ppm nitrite level, pink color still appeared for films 2, 3, and 6 at maximum and 90% vacuum levels, but only at maximum vacuum level for film 7 which showed slight color fading at 90% vacuum.

The rancidity development from an interaction between films and nitrite levels (Figure 13) was quite closely related to the color development reported in Table 10. A higher TBA number was found for the 50 ppm nitrite samples and for films 6, 7, and 4. This indicated that the higher level of nitrite retarded more rancidity development in cured meat.

Residual nitrite was determined for three nitrite levels with storage time as shown in Figure 14. Generally, the depletion of nitrite was noted with storage time for all nitrite concentrations. It appeared to be affected by initial level added. The 156 ppm nitrite group was higher

Table 10. Effect of packaging films, initial vacuum degree, and nitrite concentration on pigment conversion[a]

Nitrite levels (ppm)	Initial vacuum degree	Films				
		2	3	4	6	7
156	Maximum	65.82±11.52	64.18±9.51	58.52±10.17	62.52±6.09	59.00±5.19
	90%	63.02±10.59	64.33±7.73	56.92±14.07	58.94±6.34	57.39±6.74
	70%	59.16±9.39	55.70±11.33	30.02±18.07	30.92±9.96	27.63±16.08
100	Maximum	74.47±7.44	70.72±8.75	51.72±25.54	75.93±6.19	76.25±9.99
	90%	71.57±7.57	67.94±5.60	50.65±24.87	71.13±8.48	69.49±12.80
	70%	61.47±9.06	54.93±5.15	41.55±27.77	36.16±13.80	29.57±9.00
50	Maximum	60.26±9.59	62.59±5.78	35.36±23.87	64.76±7.45	62.87±8.08
	90%	57.59±8.46	59.01±6.73	32.60±22.81	57.25±8.26	55.23±12.17
	70%	50.18±7.68	52.54±9.09	30.61±21.80	34.37±12.90	22.42±6.98

[a]X ± SD = mean ± standard deviation.

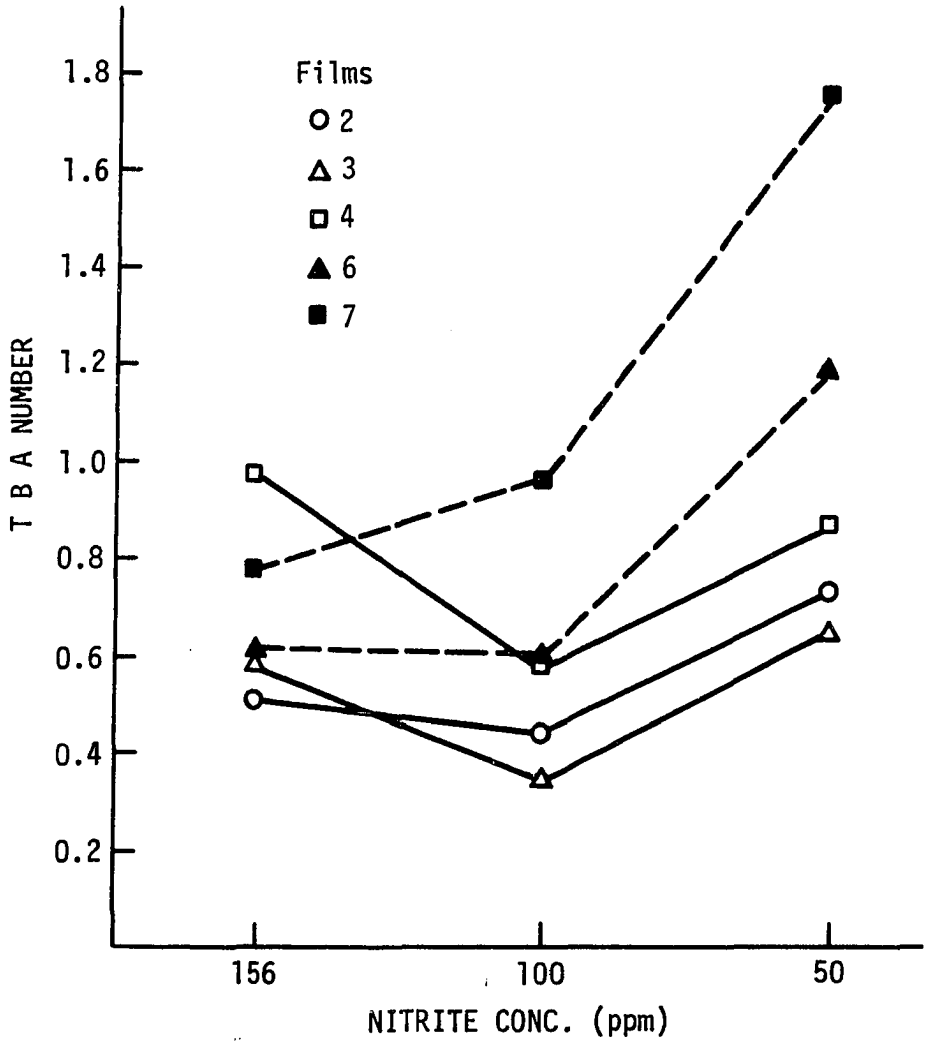


Figure 13. Effect of packaging films and nitrite concentration on TBA number

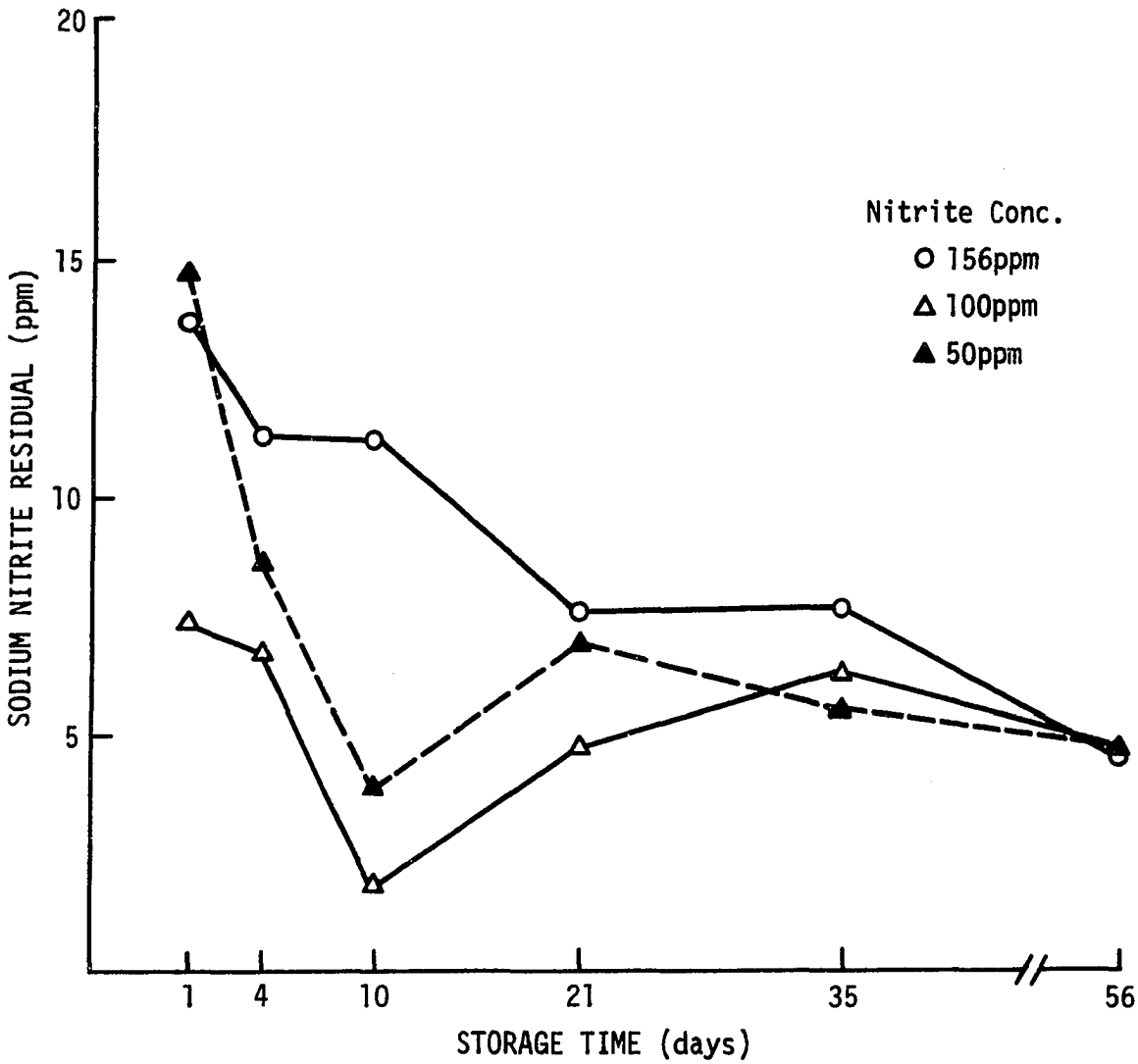


Figure 14. Effect of nitrite concentration and storage period on sodium nitrite residual

in residual nitrite, followed by the 100 ppm and the 50 ppm nitrite groups.

Several significant correlations among various traits are noted in Table 11. Significant correlations were noted between pigment conversion and color score ($P<0.05$), reflectance ratio ($P<0.01$), nitric oxide heme pigment ($P<0.01$), terminal vacuum degree ($P<0.01$), and TBA number ($P<0.01$). The bright pink cured color development associated with sliced bologna had higher terminal vacuum levels and lower TBA numbers. Terminal vacuum was negatively related ($P<0.01$) to TBA number. In general, higher terminal vacuum and lower TBA numbers all were associated with better cured meat color development. The pH value, reflectance value, and residual nitrite did not show correlation with the other traits.

Conclusions

Results of study II suggested the following:

(1) Good barrier films should be used for packaging cured meat products; a minimum oxygen barrier of between 7.2 to 57 $ml/m^2/24$ hr is suggested for films.

(2) As much initial vacuum as possible for all packaging films should be used, particularly for films 6 and 7. The type of material in films 6 and 7 requires maximum initial vacuum for preservation of good color and less rancidity development.

(3) Overall evaluation showed that 100 ppm nitrite was just as effective as 156 ppm nitrite for cured meat development.

(4) Color development can be accomplished with the reduced amount of sodium nitrite (50 ppm) if it is combined with maximum

Table 11. Correlations among variables studied

Variable	pH	Color score	Reflectance	K/S 570/K/S 650	Nitric oxide heme pigment (ppm)	Total pigment (ppm)	Pigment conversion (%)	Terminal vacuum (in.)	$NaNO_2$ residual (ppm)	TBA no.
pH	1.00									
Color score	-0.01	1.00								
Reflectance	-0.20**	-0.01	1.00							
K/S 570/K/S 650	-0.08	0.28**	-0.05	1.00						
Nitric oxide heme pigment (ppm)	0.05	0.28**	-0.11	0.34**	1.00					
Total pigment (ppm)	0.06	0.07	-0.13	0.09	0.39**	1.00				
Pigment conversion (%)	0.07	0.16*	-0.04	0.30**	0.82**	-0.08	1.00			
Terminal vacuum (in.)	0.07	0.27**	-0.07	0.14	0.34**	0.17*	0.29**	1.00		
$NaNO_2$ residual (ppm)	-0.04	0.01	-0.07	0.00	0.07	0.10	0.00	-0.05	1.00	
TBA no.	0.00	-0.07	0.00	-0.06	-0.17*	0.08	-0.29**	-0.22**	0.05	1.00

*Significant at $P<0.05$ level.

**Significant at $P<0.01$ level.

initial vacuum and good barrier packaging films (less than 7.2 $ml/m^2/24$ hr oxygen permeability).

(5) Most of the interactions among packaging films, initial vacuum levels, nitrite concentration, and storage period significantly affected the color and rancidity developments.

(6) Increasing color was found for 156 ppm and 100 ppm nitrite with higher initial vacuum and good barrier films.

(7) Cured meat color development was found to be highly related to the terminal vacuum and TBA number. However, cured meat color did not show any correlation with pH, residual nitrite, and reflectance value.

Effect of Sodium Erythrobate, Packaging Conditions, and Length of Storage on Color and Rancidity Development of Sliced Bologna

Effect of packaging films on color and rancidity is given in Table 12. The findings of study I and study II were confirmed. Superior cured meat color development was indicated by color score, K/S 570/K/S 650 ratio, nitric oxide heme pigment, and pigment conversion in the following order: films 2, 3, 6, 7, and 4. Overall means for film 4 indicated that this film was not acceptable as measured by a color score of 2.37, spectral ratio of 2.92, and pigment conversion of 40.87%. There were no significant differences for terminal vacuum among films 2, 3, 6, and 4; however, film 4 was found to be slightly lower than that of the other three films. Film 7 had the worst vacuum condition, particularly at the 70% initial vacuum level which resulted from the rigid type of material used. This was also noted in study I and may mean more residual air

Table 12. Effect of packaging films on color and rancidity development[1]

Items	Films				
	2	3	4	6	7
Color score	4.13±1.52^{a}	4.06±1.63^{a}	2.37±1.75^{c}	4.06±1.72^{a}	3.39±2.05^{b}
Reflectance	34.12±3.22^{bc}	34.55±2.97^{b}	35.59±3.38^{a}	33.87±3.64^{c}	34.60±3.83^{b}
K/S 570/K/S 650	4.67±1.56^{a}	4.39±1.44^{a}	2.92±1.39^{c}	4.61±1.82^{a}	3.91±1.83^{b}
Nitric oxide heme pigment (ppm)	81.97±15.77^{a}	79.72±19.35^{a}	51.09±25.78^{c}	78.82±22.29^{a}	68.01±30.29^{b}
Total pigment (ppm)	129.26±19.67^{a}	133.67±25.08^{a}	122.87±23.52^{b}	133.47±22.74^{a}	125.39±29.52^{b}
Pigment conversion (%)	63.71±9.69^{a}	60.14±11.69^{a}	40.87±18.70^{c}	59.00±14.15^{a}	52.45±18.98^{b}
Terminal vacuum (in.)	24.98±3.39^{a}	24.15±4.56^{a}	23.98±5.03^{a}	24.28±5.42^{a}	17.86±9.04^{b}
TBA no.	0.72±0.23^{b}	0.71±0.18^{b}	1.05±0.62^{a}	0.79±0.25^{b}	1.12±0.69^{a}

[1]All means in a row with the same superscripts are not significantly different ($P<0.05$); X ± SD = mean ± standard deviation.

existed inside the package. This caused more rancidity development as indicated by higher TBA numbers for the samples of films 4 and 7 compared to the others.

The significant effect of films and initial vacuum level on pigment conversion is shown in Figure 15. Film 2 in all three vacuum groups and films 3, 6, and 7 in maximum and 90% initial vacuum levels exhibited above 55% pigment conversion, which was acceptable. Film 4 showed extremely low values (under 50% pigment conversion) with all three vacuum groups due to oxidation of pigment which resulted from higher oxygen permeability (57 $ml/m^2/24$ hr). Film 7 was found to have serious discoloration at the 70% vacuum level due to the low terminal vacuum previously discussed. The color increased with storage up to 21 days for films 2, 6, and 7, and for film 3 up to 10 days, followed by a decrease is shown in Figure 16. These results suggested that there was reducing activity existing for several days if oxygen was completely excluded. Because film 4 had higher oxygen permeability, the pigment conversion decreased after the first day of storage.

Initial vacuum level had a significant influence on color and rancidity development (Table 13). The package with the maximum initial vacuum level appeared to have higher color retention as indicated by higher color score, 5.28; lower reflectance, 33.4; higher spectral ratio, 5.56; higher nitric oxide heme pigment, 85 ppm; and higher pigment conversion, 62.80%. The maximum vacuum level also showed higher terminal vacuum, 27.95 in. of Hg; higher residual nitrite, 15.07 ppm; and lower TBA number, 0.75; compared to the other two initial vacuum levels. The 70% initial vacuum level did not show acceptable color; the color score was less than 3.5,

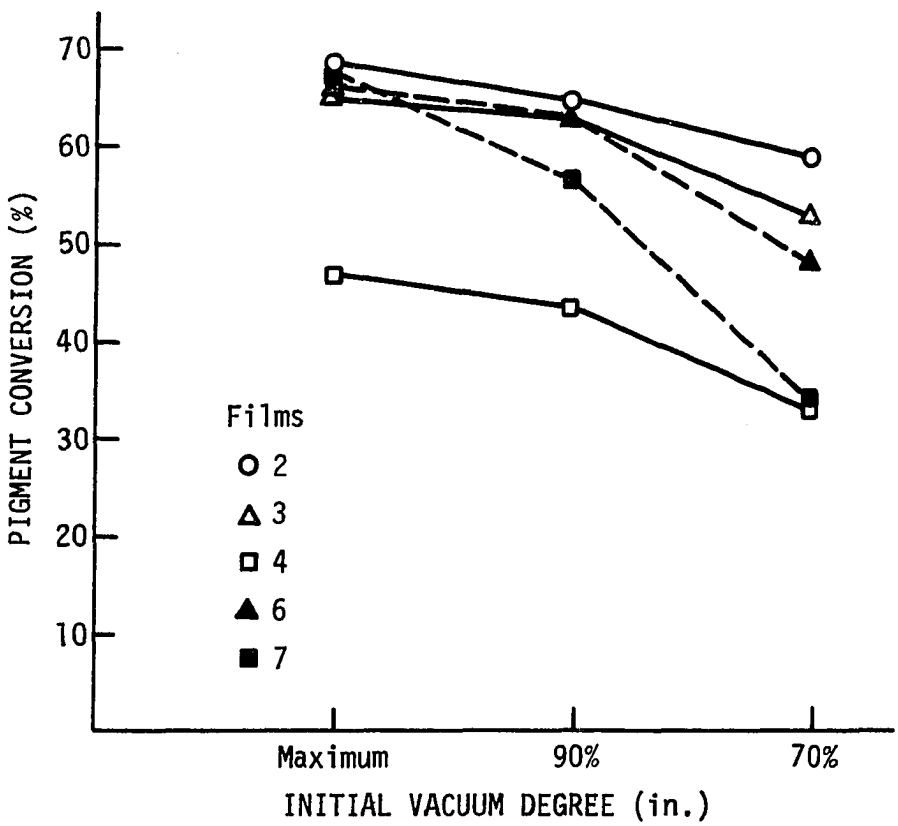


Figure 15. Effect of films and initial vacuum degree on pigment conversion

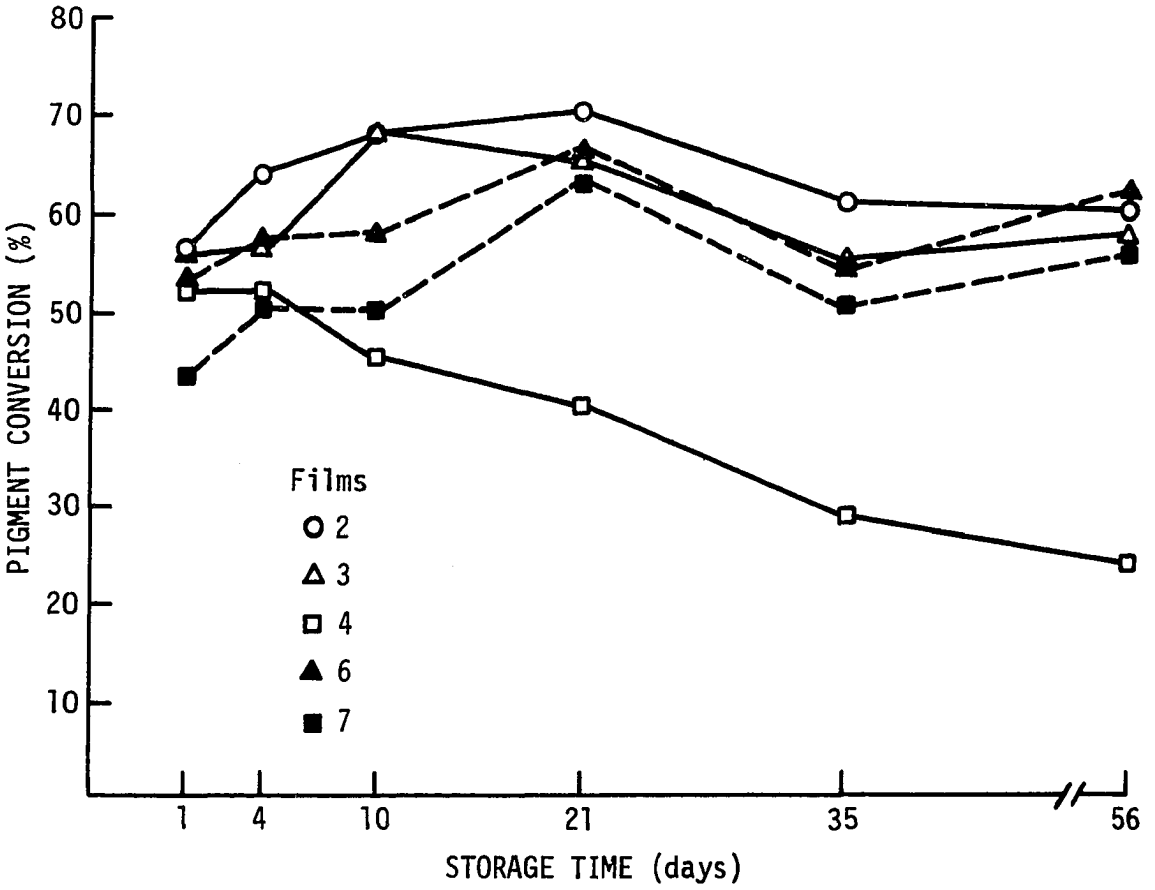


Figure 16. Effect of films and storage time on pigment conversion

Table 13. Effect of initial vacuum on color and rancidity development[1]

Items	Degree of vacuum		
	Maximum	90%	70%
Color score	5.28±1.51[a]	3.54±1.33[b]	1.98±0.92[c]
Reflectance	33.40±3.17[c]	34.35±3.35[b]	35.88±3.38[a]
K/S 570/K/S 650	5.56±1.52[a]	4.11±1.29[b]	2.63±0.87[c]
Nitric oxide heme pigment (ppm)	85.00±21.64[a]	76.33±20.04[b]	54.43±25.23[c]
Total pigment (ppm)	135.46±22.33[a]	132.63±22.78[a]	118.34±25.02[b]
Pigment conversion (%)	62.80±13.51[a]	57.89±14.07[b]	45.01±18.02[c]
Terminal vacuum (in.)	27.95±1.14[a]	23.59±4.07[b]	17.61±6.99[c]
Residual nitrite (ppm)	15.07±9.37[a]	14.12±9.14[b]	13.47±10.41[b]
TBA no.	0.75±0.31[b]	0.81±0.32[b]	1.08±0.66[a]

[1]All means in a row with the same superscripts are not significantly different ($P<0.05$); X ± SD = mean ± standard deviation.

the spectral ratio was less than 3.5, pigment conversion was less than 50%, and the TBA number was more than 1.

Influence of films, initial vacuum, and storage time on pigment conversion is given in Table 14. This effect was similar to the previous study. Good barrier films interacted with maximum initial vacuum to retain good color development. The maximum and 90% initial vacuum for films 2, 3, 6, and 7 still retained acceptable color up to 56 days of storage. The 70% initial vacuum level remained acceptable for 56 days with film 2, 21 days with film 3, and was not acceptable after only one day of storage for films 6 and 7. In addition, the poor barrier film 4 remained acceptable after 4 days with maximum initial vacuum and for 1

Table 14. Effect of films, initial vacuum degree, and storage time on pigment conversion[a]

Storage time (days)	Initial vacuum degree	Films				
		2	3	4	6	7
1	Maximum	62.82±9.89	61.19±9.35	61.44±6.14	67.76±5.56	63.11±9.06
	90%	54.94±10.43	57.11±15.13	54.11±13.00	54.55±11.91	50.15±10.67
	70%	52.70±14.26	51.45±14.70	43.24±11.17	40.29±21.29	26.09±1.69
4	Maximum	68.36±3.61	65.74±1.63	58.54±2.47	64.61±5.52	66.38±6.98
	90%	63.03±6.03	58.84±6.77	52.07±18.13	63.50±10.23	55.92±8.96
	70%	62.72±13.68	45.38±13.69	47.57±11.31	43.92±26.40	28.55±15.98
10	Maximum	75.58±8.85	73.51±7.96	52.65±26.13	67.16±2.99	68.70±2.80
	90%	70.52±6.32	68.47±10.46	50.28±28.69	62.34±3.04	57.58±10.72
	70%	58.52±6.98	64.30±10.28	33.62±11.91	43.60±16.86	24.23±21.41
21	Maximum	72.58±7.80	69.62±7.42	43.45±22.27	69.09±13.59	71.80±14.49
	90%	74.65±6.07	66.59±10.13	41.80±12.09	69.95±14.42	63.47±13.50
	70%	64.27±12.08	59.01±13.55	35.72±15.88	60.32±16.24	25.61±17.36
35	Maximum	66.49±9.33	62.89±2.64	35.69±18.36	63.51±2.21	63.78±8.77
	90%	62.16±4.30	61.81±4.31	31.59±21.91	59.16±4.55	62.48±3.90
	70%	54.84±8.07	42.49±12.98	20.83±15.66	43.72±13.20	25.59±12.32
56	Maximum	64.03±5.25	62.53±0.90	27.62±22.06	67.64±1.70	65.76±2.75
	90%	60.85±8.97	61.38±6.41	29.29±8.91	66.69±4.83	61.35±3.19
	70%	57.74±0.81	50.12±13.88	16.09±6.51	44.19±13.34	43.63±22.53

[a]X ± SD = mean ± standard deviation.

day with 90% initial vacuum. Increasing color occurred, particularly in the maximum and 90% initial vacuum levels, for films 2, 3, 6, and 7. This was also true in the 70% initial vacuum level but only for film 2. As previously stated, anaerobic conditions are extremely important with regard to color development and how long it will remain acceptable.

Sodium ascorbate, or its isomer erythrobate, is used in cure mixtures and has been shown to inhibit nitrosation reactions, thus helping to reduce the potential for nitrosamines in cured meat (Mirvish et al., 1972; Fiddler et al., 1973). It appears that the use of a reductant such as ascorbate or erythrobate in concentrations greater than that now permitted offers potential for the preparation of nitrosamine-free cured meat products. The amount allowable in comminuted meat product is 7 to 8 ounces per 100 pounds of meat.

Sodium ascorbate and erythrobate act by reducing NO_2^- to nitric oxide which reacts with the meat pigment myoglobin and forms the stable pink nitric oxide hemochrome upon heating. The higher nitric oxide heme pigment found in groups without erythrobate or with 500 ppm erythrobate may be due to different raw meat sources resulting in higher total pigment content in these groups compared to the 940 ppm erythrobate samples. However, the 940 ppm erythrobate group was noted to have significantly higher pigment conversion compared to the 500 ppm and zero erythrobate groups. This suggested that erythrobate was accelerating the development of cured color (Table 15). Similar findings have been reported by other authors (Fox et al., 1967; Counsell, 1971).

Residual nitrite levels were higher in bologna cured without erythrobate than in bologna cured with erythrobate (Table 15).

Table 15. Effect of sodium erythrobate levels on color and rancidity development[1]

Items	Levels of sodium erythrobate		
	0 ppm	500 ppm	940 ppm
Nitric oxide heme pigment (ppm)	80.20±3.03[a]	71.13±24.38[b]	64.44±19.23[c]
Total pigment (ppm)	149.20±15.59[a]	128.30±24.34[b]	108.94±12.62[c]
Pigment conversion (%)	52.97±19.01[c]	54.27±16.19[b]	58.46±15.35[a]
Residual nitrite (ppm)	22.27±11.11[a]	9.25±3.75[c]	11.13±6.58[b]
TBA no.	0.94±0.48[a]	0.69±0.45[b]	0.98±0.47[a]

[1]All means in a row with the same superscripts are not significantly different ($P<0.05$); X ± SD = mean ± standard deviation.

This suggests that erythrobate decreased the residual nitrite content. This concept is also supported by the work of Brown et al. (1974). It seems that the use of erythrobate altered the distribution of nitrite and related compounds in cured meat. The higher rancidity development of samples without erythrobate or with 940 ppm of erythrobate as compared to that of 500 ppm erythrobate might be due to less reducing activity and excess gas production.

The 500 ppm level or somewhere between 500 ppm and 940 ppm is a critically important amount of erythrobate for cure mixtures from the standpoint of TBA numbers. The influence of sodium erythrobate and initial vacuum level indicated that the higher initial vacuum packages resulted in lower TBA numbers for all three erythrobate levels (Figure 17). The TBA number was similar between zero and 940 ppm erythrobate groups. The 70% initial vacuum samples showed TBA values above 1.0, which means they were unacceptable. The TBA numbers increased with

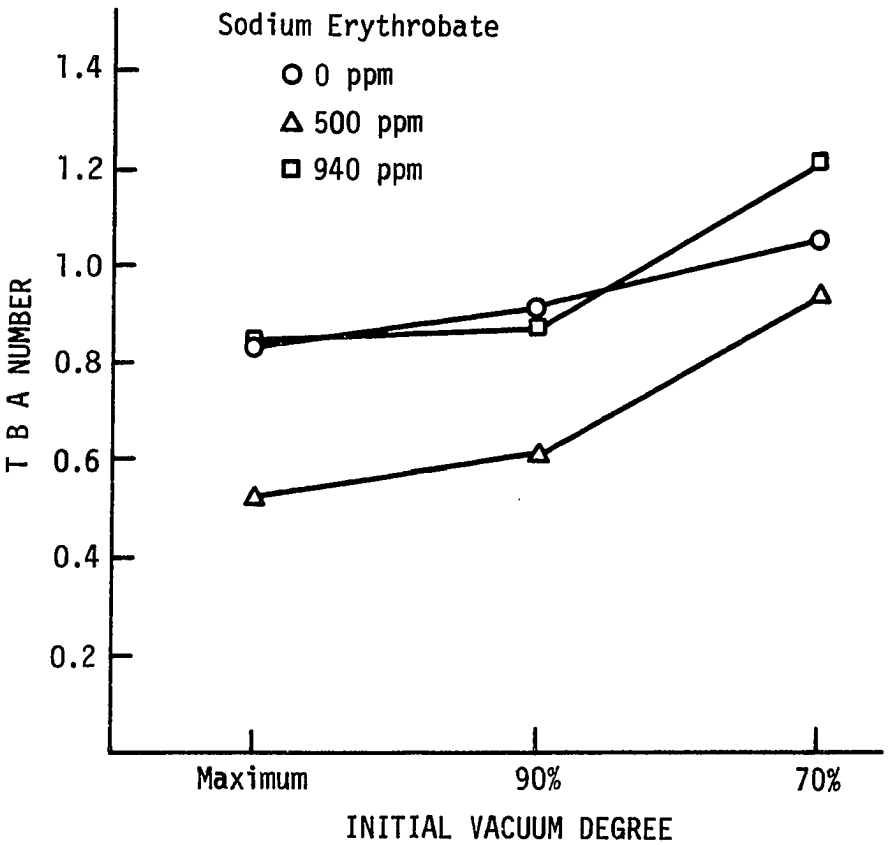


Figure 17. Effect of sodium erythrobate and initial vacuum degree on TBA number

storage time and also showed higher values in the samples with 940 ppm and zero ppm erythrobate levels compared to the 500 ppm erythrobate group (Figure 18).

Erythrobate accelerated the nitrite depletion rate as shown in Figure 19. Bologna with erythrobate had lower residual nitrite from the first day of storage as compared to the zero erythrobate level. Residual nitrite was also found to decrease with storage time and the depletion rate was much faster during the first few days of storage.

Cured meat product with 500 ppm and 940 ppm sodium erythrobate and packaged with maximum initial vacuum was found to have the best color retention as described by pigment conversion (Figure 20). The color development of the 940 ppm erythrobate samples was better than the 500 ppm or zero erythrobate groups when compared at both maximum and 90% initial vacuum levels, respectively. The samples of three erythrobate levels at maximum or 90% initial vacuum level were acceptable due to the pigment conversion value of above 55%. However, all the 70% initial vacuum samples had conversion values of less than 50%, even the 940 ppm erythrobate group. Thus, even erythrobate cannot help the packages which had certain quantities of residual air inside. These results are confirmed by spectral ratio as indicated in Table 16. In addition, it seems that the initial vacuum level was more important for zero erythrobate samples than for groups with erythrobate. Because the difference in spectral ratio between the maximum and 90% vacuum levels in the zero erythrobate group was more than in 500 ppm or 940 ppm erythrobate groups, the improvement of cured color by maximum vacuum was more important in the zero erythrobate group.

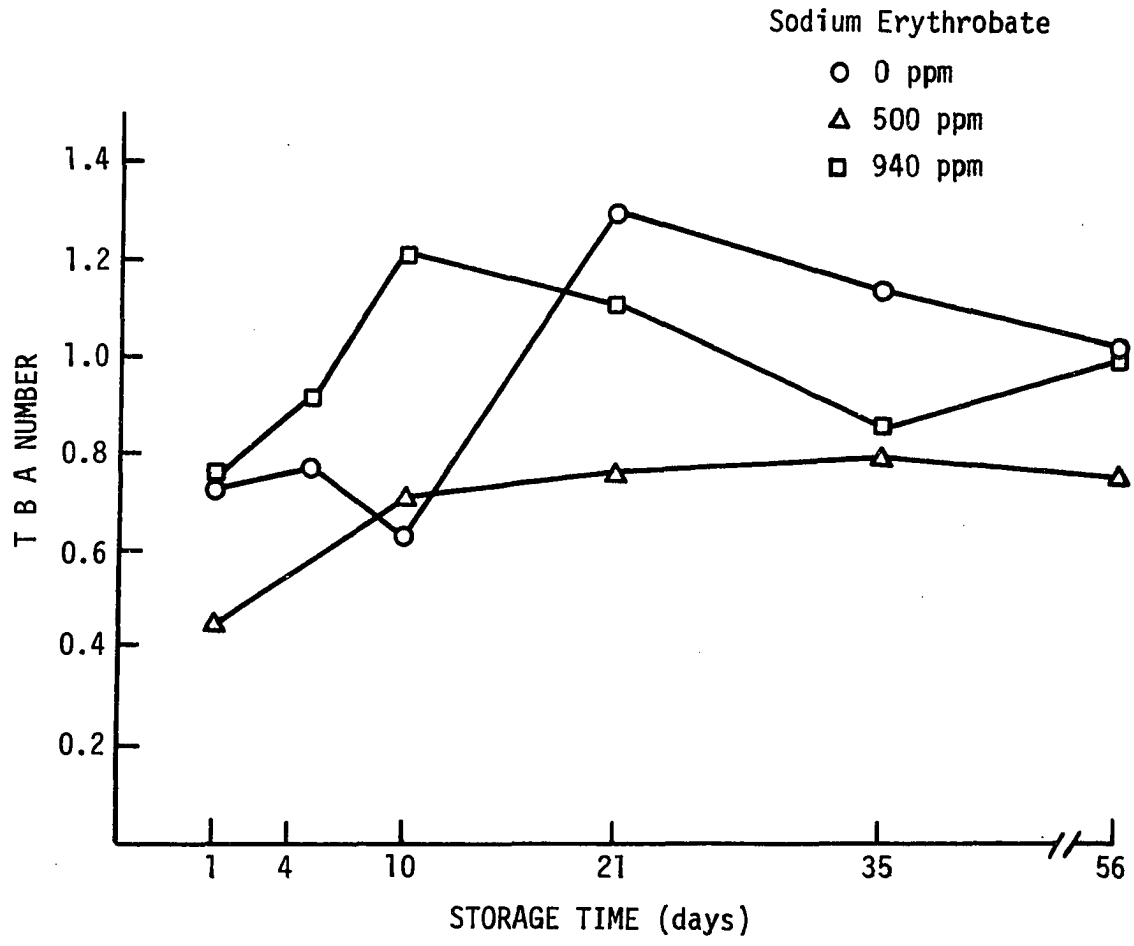


Figure 18. Effect of sodium erythrobate and storage time on TBA number

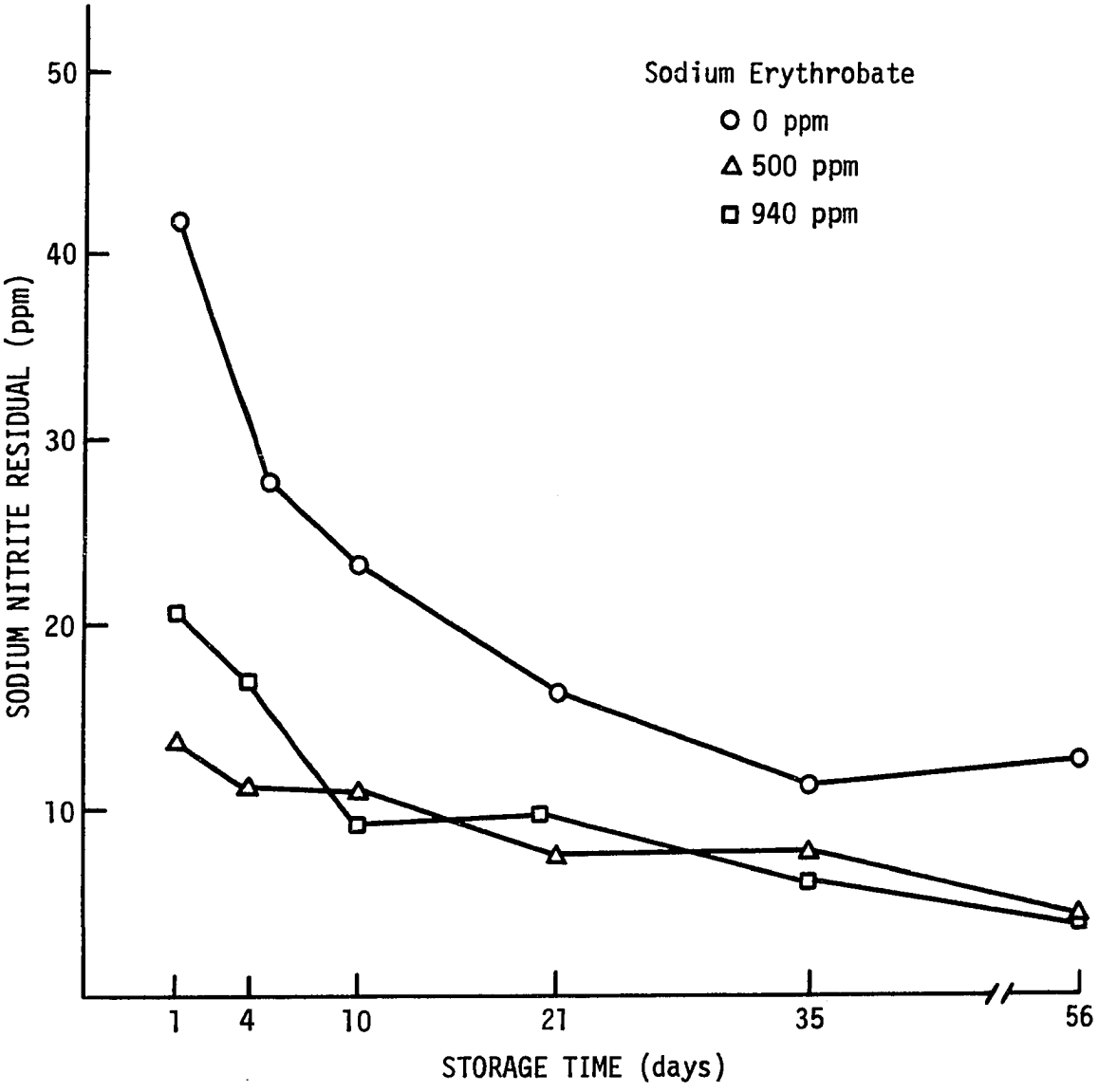


Figure 19. Effect of sodium erythrobate and storage time on residual nitrite

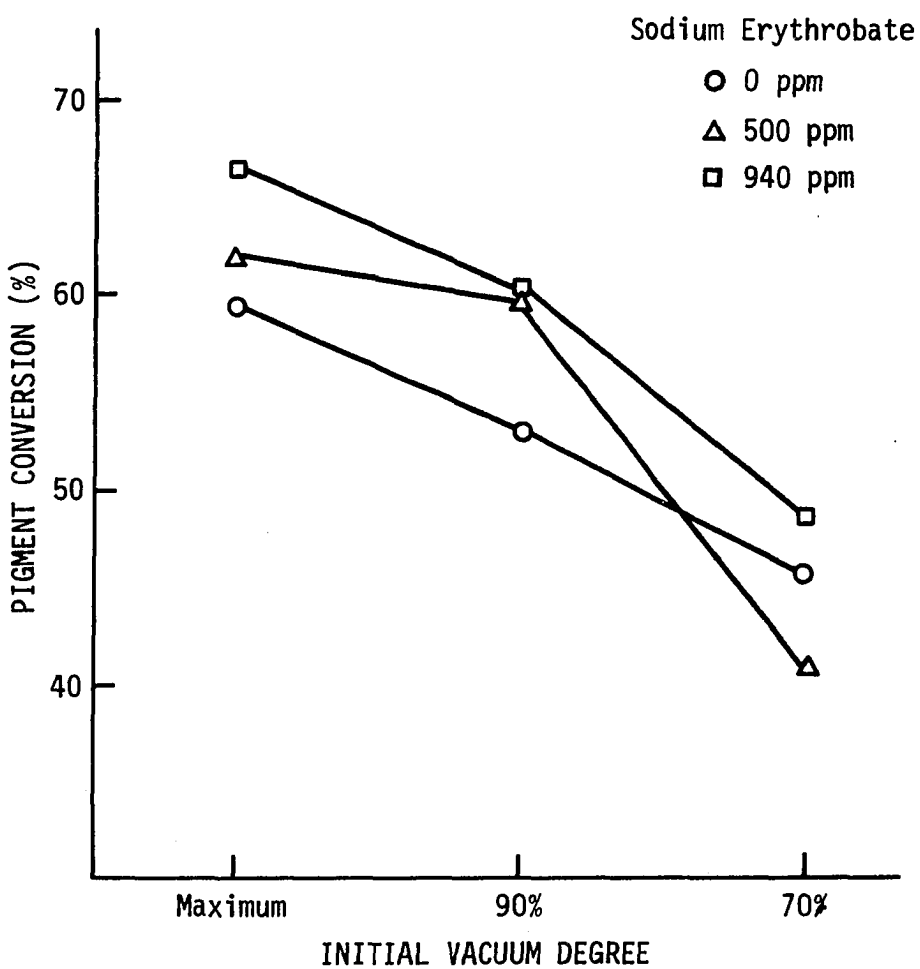


Figure 20. Effect of sodium erythrobate and initial vacuum degree on pigment conversion

Table 16. Effect of sodium erythrobate and initial vacuum degree on spectral ratio and residual nitrite[a]

Items	Initial vacuum degree	Levels of sodium erythrobate 0 ppm	500 ppm	940 ppm
K/S 570/K/S 650	Maximum	6.17±1.59	4.73±1.05	5.79±1.51
Residual nitrite (ppm)	Maximum	23.24±10.82	10.36±3.48	11.60±6.01
K/S 570/K/S 650	90%	3.65±1.14	4.23±0.95	4.46±1.59
Residual nitrite (ppm)	90%	21.40±10.79	9.65±3.44	11.29±6.66
K/S 570/K/S 650	70%	2.82±0.63	2.46±1.01	2.60±0.92
Residual nitrite (ppm)	70%	22.17±12.00	7.74±3.93	10.51±7.18

[a] $X \pm SD$ = mean ± standard deviation.

Residual nitrite was significantly lower in samples with erythrobate than in those without erythrobate. However, there is only a slight difference between the 500 ppm and 940 ppm erythrobate groups for residual nitrite.

The 940 ppm erythrobate level was noted to be better for color retention in all films at all three initial vacuum conditions as described by pigment conversion (Table 17). Acceptable color development was indicated for film 2 at all three initial vacuum and erythrobate levels, for films 3, 6, and 7 at the three erythrobate levels, but only at maximum and 90% initial vacuum levels, and for film 4 at only 500 ppm erythrobate with maximum and 90% initial vacuum. The pigment conversion of film 6 was 61.13% which is higher than any others in 70% initial vacuum and 940 ppm erythrobate samples. It might be experimental error because all the other values were unacceptable with color scores of 2.33 and a spectral ratio of 3.10.

Table 17. Effect of films, sodium erythrobate, and initial vacuum degree on pigment conversion[a]

Level of sodium erythrobate	Initial vacuum degree	Films				
		2	3	4	6	7
0 ppm	Maximum	67.11±6.09	65.54±5.98	30.13±20.68	67.01±5.05	69.39±7.17
	90%	62.22±10.89	60.91±12.69	25.97±9.88	61.48±11.61	56.07±16.07
	70%	56.33±14.02	52.97±17.17	24.45±8.60	50.96±16.33	44.09±19.96
500 ppm	Maximum	65.82±11.52	64.18±9.51	58.52±10.17	62.52±6.09	59.00±5.19
	90%	63.02±10.59	64.33±7.73	56.92±14.07	58.94±6.34	57.39±6.74
	70%	59.16±9.39	55.69±11.33	30.02±18.37	30.92±9.96	27.63±16.08
940 ppm	Maximum	72.01±4.91	68.03±4.44	51.04±17.34	70.36±4.35	71.38±4.48
	90%	67.84±5.13	61.87±6.93	46.69±16.29	67.68±8.27	57.01±11.29
	70%	59.90±5.15	47.71±13.61	44.07±13.11	61.13±7.69	30.14±17.79

[a]X ± SD = mean ± standard deviation.

Correlations among variables are given in Table 18. Nitric oxide heme pigment was significantly ($P<0.01$) correlated with color score, spectral ratio, pigment conversion, total pigment, terminal vacuum, and negatively ($P<0.01$) correlated with reflectance and TBA values. High correlations existed among all color characteristics. Terminal vacuum was also significantly ($P<0.01$) correlated with color traits. This means the higher terminal vacuum level resulting from high initial vacuum was more desirable. TBA number was negatively related ($P<0.01$) to cured color. The higher TBA numbers also caused cured pigment to be oxidized. Therefore, all the factors which reduced the TBA number also enhanced cured meat color development.

Conclusions

Sodium erythrobate accelerated cured color development, decreased residual nitrite more rapidly, and showed antioxidation at the 500 ppm erythrobate level. Initial vacuum as low as 90% of maximum with erythrobate can retain acceptable quality for all films except film 4 which had high oxygen permeability of 57 $ml/m^2/24$ hr. Good barrier films (less than 7.2 $ml/m^2/24$ hr) were necessary to provide good product stability in storage, even with erythrobate. If high initial vacuum was used, all films would retain better cured color and less rancidity compared to low initial vacuum.

Table 18. Correlations among variables studied

Variable	pH	Color score	Reflectance	K/S 570/K/S 650	Nitric oxide heme pigment (ppm)	Total pigment (ppm)	Pigment conversion (%)	Terminal vacuum (in.)	$NaNO_2$ residual (ppm)	TBA no.
pH	1.00									
Color score	-0.07	1.00								
Reflectance	-0.02	-0.04	1.00							
K/S 570/K/S 650	-0.04	0.64**	-0.08	1.00						
Nitric oxide heme pigment (ppm)	-0.01	0.29**	-0.23**	0.35**	1.00					
Total pigment (ppm)	0.05	0.05	-0.11	0.18*	0.45**	1.00				
Pigment conversion(%)	-0.02	0.25**	-0.18*	0.30**	0.87**	0.05	1.00			
Terminal vacuum (in.)	0.03	0.29**	-0.12	0.30**	0.38**	0.16*	0.35**	1.00		
$NaNO_2$ residual (ppm)	0.08	0.13	-0.08	0.11	0.12	0.00	0.07	0.07	1.00	
TBA no.	-0.05	-0.05	0.16*	-0.06	-0.22**	-0.12	-0.19*	-0.12	-0.10	1.00

*Significant at P<0.05 level.

**Significant at P<0.01 level.

GENERAL SUMMARY

The interrelationship between color and flavor, or more specifically metmyoglobin in raw meat or dissociation of cured meat pigment with rancidity development, become apparent. Two types of oxidative changes occur in meat, namely, oxidation of the fat resulting in rancidity, and oxidation of the heme pigments resulting in discoloration. The two are closely related. In fact, each can accelerate the other, but each can also proceed independently of the other. Together they account for a large part of the economic loss due to substandard or spoiled meat.

The main considerations in the packaging of cured meat products are the exclusion of oxygen and light. Any type of packaging which reduces contact with oxygen retards both rancidity and discoloration. Impermeable wrappings can prevent the oxygen reentry to the packages and vacuum can remove residual air within the packages.

The results of study I of this thesis indicated the necessity of good barrier films in the packaging of cured meat products. Maximum oxygen permeability of between 7.2 and 57 $ml/m^2/24$ hr is recommended. The maximum initial vacuum level (29 in. of Hg) should be used in packaging processed meat. If rigid types of material such as in films 6 and 7 are used, it is essential to use the maximum initial vacuum level in order to retain good color and reduce rancidity development.

Cured meat and meat products are manufactured by the addition of sodium nitrite, which is the compound primarily responsible for the unique characteristics of cured meats. The possible health hazard of using nitrite in meat products is due to potential formation of

carcinogenic nitrosamines in the product or in the body after consumption. Therefore, study II was conducted to evaluate the effect of interactions between packaging conditions and reduced nitrite levels for good color retention. Three levels of sodium nitrite were used. Increased amounts of nitrite (100 ppm and 156 ppm) showed similar results of better color retention and less rancidity development compared to the lower nitrite level (50 ppm). However, the products using 50 ppm sodium nitrite, good barrier films (7.2 $ml/m^2/24$ hr or less), and maximum initial vacuum (29 in. of Hg) retained acceptable cured meat color and reduced rancidity.

Three levels of sodium erythrobate were used in study III. The higher amounts of sodium erythrobate (500 ppm and 940 ppm) resulted in rapid cured color development, resistance to color fading, rapid nitrite depletion, and greater retardation of oxidative changes compared to zero amount of sodium erythrobate. If a high level of reducing agent, such as sodium erythrobate, was used it might be possible to reduce the nitrite level even lower than 50 ppm for cured meat products. This does not, however, take into account the microbial influence of nitrite.

It is hoped that these efforts will contribute to the commercial production of meat products which are completely free of nitroso compounds. Thorough investigation of the reaction mechanisms by sodium nitrite in meat and meat products is necessary. A possible substitute or replacement for nitrite should be considered. Good control and understanding of other factors such as light and temperature are also imperative.

REFERENCES

American Meat Institute. 1978. Nitrite. American Meat Institute, Washington, D.C.

American Meat Institute Foundation. 1971. Meat Sci. Rev. 5:1.

Andrews, M. M., Guthneck, B. T., McBride, B. H. and Schweigert, B. S. 1952. Stability of certain respiratory and glycolytic enzymes in animal tissue. J. Biol. Chem. 194:715.

Anonymous. 1972. Botulism. J. Food Sci. 37:985.

Antonini, E. and Brunori, M. 1971. Hemoglobin and myoglobin in their reactions with ligands. North-Holland Research Monographs, Frontiers of Biology, Vol. 21. North-Holland Publishing Company, Amsterdam.

AOAC. 1970. Official methods of analysis. 11th ed. Assoc. Official Anal. Chem., Washington, D.C.

Atkinson, J. L., Follett, M. J. and Ratcliff, P. W. 1969. Postmortem changes in oxygen uptake and NAD content in lamb muscularis semimembranosus. Nature 223:1372.

Bailey, M. E. and Swain, J. W. 1973. Influence of nitrite on meat flavor. Proc. Meat Ind. Res. Conf., 1973:29.

Bailey, M. E., Frame, R. W. and Naumann, H. D. 1964. Studies of the photooxidation of nitrosomyoglobin. J. Agr. Food Chem. 12:89.

Baltzer, J. 1969. The relation between bacterial contamination and growth on meats and the quality of meat products. Proc. Recip. Meat Conf. 22:294.

Bard, J. and Townsend, W. E. 1971. Meat curing. Pages 452-483 in Price, J. F. and Schweigert, B. S., eds. The Science of Meat and Meat Products. W. H. Freeman and Company, San Francisco, California.

Barnes, J. M. and Magee, P. N. 1954. Some toxic properties of dimethylnitrosamine. Brit. J. Int. Med. 11:167.

Bauernfeind, J. C. and Pinkert, D. M. 1970. Food processing with added ascorbic acid. Advances in Food res. 18:219.

Bembers, M. and Satterlee, L. D. 1975. Physical-chemical characterization of normal and PSE porcine muscle myoglobin. J. Food Sci. 40: 40.

Bendall, J. R. and Taylor, A. A. 1972. Consumption of oxygen by the muscles of beef animals and related species. 2. Consumption of oxygen by postrigor muscle. J. Sci. Food Agric. 23:707.

Bender, A. E. and Ballance, P. E. 1961. A preliminary examination of the flavor of meat extract. J. Sci. Food Agr. 12:683.

Bills, D. D., Hildrum, K. I., Scanlan, R. A. and Libbey, L. M. 1973. Potential precursors of N-nitrosopyrrolidine in bacon and other fried foods. J. Agr. Food Chem. 21:876.

Birdsall, J. 1976. Plant commercial bacon study. Report presented to Expert Panel. American Meat Institute, Chicago, November, 1976.

Bowen, V. G. and Deibel, R. H. 1974. Effects of nitrite and ascorbate on botulinal toxin formation in wieners and bacon. Proc. Meat Ind. Res. Conf. 1974:63.

Bowling, R. A., Carpenter, Z. L., Smith, G. C. and Hoke, K. E. 1977. Vacuum packaging systems for subprimal beef cuts. J. Anim. Sci. 45: 1280.

Brooks, J., Haines, R. B., Moran, T. and Pace, J. 1940. The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Res. Food Invest. Spec. Report No. 49. H. M. Stationary Office, London.

Brown, W. D. 1973. Possible substitutes for nitrite in cured foods. Proc. Meat Ind. Conf. 1973:21.

Brown, C. L., Hedrick, H. B. and Bailey, M. E. 1974. Characteristics of cured ham as influenced by levels of sodium nitrite and sodium ascorbate. J. Food Sci. 39:977.

Brunori, M., Giacometti, G. M., Antonini, E. and Wyman, J. 1973. Heme proteins: Quantum yield determined by the pulse method. Proc. Nat. Acad. Sci. 70:3141.

Buchanan, R. L. and Solberg, M. 1972. Interaction of sodium nitrate, oxygen and pH on growth of Staphylococcus aureus. J. Food Sci. 37: 81.

Bulman, C. and Ayres, J. C. 1952. Preservative effect of various concentrations of curing salt in comminuted pork. Food Technol. 6:255.

Cassens, R. G., Sebranek, J. G., Kubberod, G. and Woolford, G. 1974. Where does the nitrite go? Food Prod. Development 8:50.

Chang, S. S. and Peterson, R. J. 1977. Symposium: The basis of quality in muscle food. Recent developments in the flavor of meat. J. Food Sci. 42:298.

Chang, C. K. and Taylor, T. G. 1975. Kinetics of oxygen and carbon monoxide binding to synthetic analogs of the myoglobin and hemoglobin active sites. Proc. Nat. Acad. Sci. 72:1166.

Cheah, K. S. 1976. Formation of nitrosylmyoglobin in bacon involving lactate dehydrogenase. J. Food Technol. 11:181.

Chien, J. C. W. 1969. Reactions of nitric oxide with methemoglobin. J. Amer. Chem. Soc. 91:2166.

Cho, I. C. and Bratzler, L. J. 1970. Effect of sodium nitrite on flavor of cured pork. J. Food Sci. 35:668.

Christiansen, L. N., Johnston, R. W., Kautter, D. A., Howard, J. W. and Aunan, W. J. 1973. Effect of nitrite and nitrate on toxin production by Clostridium botulinum and on nitrosamine formation in perishable canned comminuted cured meat. Appl. Microbiol. 25:357.

Christiansen, L. N., Tompkin, R. B., Shaparis, A. B., Kueper, T. V., Johnston, R. W., Kautter, D. A. and Kolari, O. J. 1974. Effect of sodium nitrite on toxin production Clostridium botulinum in bacon. Appl. Microbiol. 27:733.

Christiansen, L. N., Tompkin, R. B., Shaparis, A. B., Johnston, R. W. and Kautter, D. A. 1975. Effect of sodium nitrite and nitrate on Clostridium botulinum growth and toxin production in a summer style sausage. J. Food Sci. 40:488.

Correa, P., Haenszel, W., Cuello, C., Tannenbaum, S. and Archer, M. 1975. A model for gastric cancer epidemiology. Lancet 1:58.

Counsell, J. N. 1971. Meat processing with ascorbic acid. Process Biochem. 6:25.

Cross, C. K. and Ziegler, P. 1965. A comparison of the volatile fractions from cured and uncured meat. J. Food Sci. 30:610.

Deibel, R. H. and Evans, J. B. 1957. American Meat Institute Foundation Bulletin 32.

Dethmers, A. E., Rock, H., Fazio, T. and Johnston, R. W. 1975. Effect of added sodium nitrite and sodium nitrate on sensory quality and nitrosamine formation in thuringer sausage. J. Food Sci. 40:491.

DeVore, D. P. and Solberg, M. 1974. Oxygen uptake in post-rigor bovine muscle. J. Food Sci. 39:22.

DeVore, D. P. and Solberg, M. 1975. A study of the rate-limiting factors in the respiratory oxygen consumption of intact post-rigor bovine muscle. J. Food Sci. 40:651.

Dugan, L. R., Jr. 1961. Development and inhibition of oxidative rancidity in foods. Food Technol. 15:10.

Duncan, C. L. and Foster, E. M. 1968. Effect of sodium nitrite, sodium chloride and sodium nitrate on germination and outgrowth of anaerobic spores. Appl. Microbiol. 16:406.

Dutton, A. H. and Heath, D. F. 1956. Demethylation of dimethylnitrosamine in rats and mice. Nature 178:644.

Dymicky, M., Fox, J. B. and Wasserman, A. E. 1975. Color formation in cooked model and meat systems with organic and inorganic compounds. J. Food Sci. 40:306.

Eakes, B. D., Blumer, T. N. and Monroe, R. J. 1975. Effect of nitrate and nitrite on color and flavor of country-style hams. J. Food Sci. 40:973.

Eddy, B. P. and Ingram, M. 1956. A salt-tolerant, denitrifying Bacillus strain which blows canned bacon. J. Appl. Bacteriol. 19:62.

Ellis, R., Currie, G. T., Thornton, F. E., Bollinger, N. C. and Gaddis, A. M. 1968. Carbonyls in oxidizing fat. II. The effect of the prooxidant activity of sodium chloride on pork tissue. J. Food Sci. 33:555.

Emerick, R. J., Nelson, D. L. and Olson, D. E. 1963. Effect of nitrate and some of its reduction products on carotene stability. J. Agr. Food Chem. 11:140.

Ender, F. and Ceh, L. 1968. Occurrence of nitrosamines in foodstuffs for human and animal consumption. Food Cosmet. Toxicol. 6:569.

Epstein, S. S. 1972. Environmental pathology, a review. Am. J. Pathol. 66:352.

Ertle, N. L. 1969. Sausage shelf-life as affected by packaging. Proc. Meat Ind. Res. Conf. 1969:175.

Fiddler, W., Pensabene, J. W., Piotrowski, E. G., Doerr, R. C. and Wasserman, A. E. 1973. Use of sodium ascorbate or erythrobate to inhibit formation of N-nitrosodimethylamine in frankfurters. J. Food Sci. 38:1084.

Fox, J. B. 1966. The chemistry of meat pigments. J. Agr. Food Chem. 14:207.

Fox, J. B. and Ackerman, S. A. 1968. Formation of nitric oxide myoglobin: Mechanisms of the reaction with various reductants. J. Food Sci. 33:364.

Fox, J. B. and Nicholas, R. A. 1974. Nitrite in meat. Effect of various compounds on loss of nitrite. Agr. Food Chem. 22:302.

Fox, J. G. and Thomson, J. S. 1963. Formation of bovine nitrosyl-myoglobin. I. pH 4.5-6.5. Biochem. J. 2:465.

Fox, J. B., Jr., Townsend, W. E., Ackerman, S. A. and Swift, C. E. 1967. Cured color development during frankfurter processing. Food Technol. 21:386.

George, P. and Stratmann, C. J. 1952. The oxidation of myoglobin to metmyoglobin by oxygen. Biochem. J. 51:418.

Giddings, G. G. 1977. Symposium: The basis of quality in muscle foods. The basis of color in muscle foods. J. Food Sci. 42:289.

Giddy, C. 1966. The changes in meat pigments during sausage making processes. J. Sci. Food Agric. 17:14.

Grant, N. W. 1955. The respiration enzymes of meat. 1. Identification of active enzymes. Food Res. 20:250.

Greenberg, R. A. 1972. Nitrite in the control of Clostridium botulinum. Proc. Meat Ind. Res. Conf. 1972:25.

Greenberg, R. A. 1975. Update on nitrite, nitrate, and nitrosamines. US-MIRC 1975:71.

Greenberg, M., Birnkraut, W. B. and Schiftner, J. J. 1945. Outbreak of sodium nitrite poisoning. Am. J. Publ. Health 35:1217.

Greenblatt, M. 1973. Ascorbic acid of aminopyrine nitrosation in NZO/BL mice. J. Nat. Cancer Inst. 50:1055.

Greene, B. E. 1969. Lipid oxidation and pigment changes in raw beef. J. Food Sci. 34:110.

Greenwood, D. A. 1940. American Meat Institute Annual Meeting Report 1940:135.

Grever, A. B. G. 1973. Minimum nitrite concentration for inhibition of Clostridia in cooked meat products. Proc. Int. Symp. Nitrite Meat Products, Zeist, Netherlands, 1973:103.

Hadden, J. P., Ockerman, H. W., Cahill, V. R., Parrett, N. A. and Borton, R. J. 1975. Influence of sodium nitrite on the chemical and organoleptic properties of comminuted pork. J. Food Sci. 40:626.

Heath, D. F. 1962. The decomposition and toxicity of dialkylnitrosamines in rats. Biochem. J. 85:72.

Hedler, L. and Marquardt, P. 1968. Occurrence of diethylnitrosamine in some samples of food. Food Cosmet. Toxicol. 6:341.

Herring, H. K. 1973. Effect of nitrite and other factors on the physico-chemical characteristics and nitrosamine formation in bacon. US-AMIF 1973:47.

Hollenback, C. M. and Monaham, R. 1953. Application of ascorbic acid in meat curing. Proc. Meat Ind. Res. Conf. 5:106.

Hornsey, H. C. 1956. The colour of cooked cured pork. I. Estimation of the nitric oxide - haem pigments. J. Sci. Food Agric. 7:534.

Hornsey, H. C. 1964. The coloration and discoloration of cured meats. Int. Food Indust. Congress. A. G. Bishop and Sons, Ltd., St. Mary Cray, England.

Hornstein, I. and Crowe, P. F. 1960. Flavor study on beef and pork. J. Agr. Food Chem. 8:494.

Howard, A., Duffy, P., Else, K. and Brown, W. D. 1973. Possible substitutes for nitrite for pigment formation in cured meat products. J. Agr. Food Chem. 21:894.

Hunt, M. C., Smith, R. A., Kropf, D. H. and Tuma, H. J. 1975. Factors affecting showcase color stability of frozen lamb in transparent film. J. Food Sci. 40:637.

Hustad, G. O., Cerveny, J. G., Trenk, H., Deibel, R. H., Kautter, D. A., Fazio, T., Johnston, R. W. and Kolari, O. F. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. Appl. Microbiol. 26:22.

Ingold, K. V. 1962. Metal catalysis. Pages 93-121 in Schultz, H. W., Day, E. A. and Sinnhuber, R. O., eds. Symposium on Foods: Lipids and Their Oxidation. Avi Publishing Co., Westport, Connecticut.

Ingram, M. 1962. Microbiological principles in prepacking meats. J. Appl. Bact. 25:259.

Ingram, M. 1973. The microbiological effects of nitrite. Proc. Int. Symp. on Nitrite in Meat Prod., Zeist, Netherlands, 1973:63.

Jacobsen, M. F. 1973. How sodium nitrite can affect your health. Center for Science in the Public Interest, Washington, D.C.

Johnson, B. Y. 1974. Chilled vacuum-packed beef. CSIRO Fed. Res. Quarterly 34:14.

Johnston, M. A. and Loynes, R. 1971. Inhibition of Clostridium botulinum by sodium nitrite as affected by bacteriological media and meat suspensions. Can. Inst. Food Technol. J. 4:179.

Judd, D. B. and Wyszecki, G. 1963. Color in Business, Science and Industry. 2nd ed. John Wiley and Sons, Inc., New York.

Kagen, L. J. and Gurevich, R. 1967. Localization of myoglobin in human skeletal muscle using fluorescent antibody technique. J. Histochem. and Cytochem. 15:436.

Kamm, J. J., Dashman, T., Conrey, A. H. and Burns, J. J. 1973. Protective effect of ascorbic acid on hepatotoxicity caused by sodium nitrite and aminopyrin. Proc. Nat. Acad. Sci. 70:747.

Kearns, D. R. 1971. Physical and chemical properties of singlet molecular oxygen. Chem. Reviews 71:395.

Keller, J. D. and Kinsella, J. E. 1973. Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. J. Food Sci. 38:1200.

Kemp, J. D., Fox, J. D. and Moody, W. G. 1974. Cured ham properties as affected by nitrate and nitrite and fresh pork quality. J. Food Sci. 39:972.

Kemp, J. D., Langlois, B. E., Fox, J. D. and Varney, W. Y. 1975. Effect of curing ingredients and holding times and temperatures on organoleptic and microbiological properties of dry-cured sliced ham. J. Food Sci. 40:634.

Koizumi, C. and Brown, C. D. 1971. Formation of nitric oxide myoglobin by nicotin amide adenine dinucleotides and flavins. J. Food Sci. 36: 1105.

Kraft, A. A. and Ayres, J. C. 1954. Effect of display case lighting on color and bacterial growth on packaged fresh beef. Food Technol. 8: 569.

Kubberd, G., Cassens, R. G. and Greaser, M. L. 1974. Reaction of nitrite with sulfhydryl groups of myosin. J. Food Sci. 39:1228.

Kueper, T. V. and Trelease, R. D. 1974. Variables affecting botulinum toxin development and nitrosamine formation in fermented sausages. Proc. Meat Ind. Res. Conf. 1974:69.

Labbe, R. G. and Duncan, C. L. 1970. Growth from spores of Clostridium perfringers in the presence of sodium nitrite. Appl. Microbiol. 19: 353.

Lea, C. H. 1957. Deteriorative reactions involving phospholipids and lipoproteins. J. Sci. Food Agric. 8:1.

Ledward, D. A. 1970. Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. J. Food Sci. 35: 33.

Lee, S. H. and Cassens, R. G. 1976. Nitrite binding sites on myoglobin. J. Food Sci. 41:969.

Lee, S. H., Cassens, R. G. and Fennema, O. R. 1976. Effect of muscle type on residual nitrite in cured meat. J. Food Sci. 41:100.

Lentz, C. P. 1971. Effect of light and temperature on color and flavor of prepackaged frozen beef. Can. Inst. Food Tech. J. 4:166.

Lijinsky, W. and Epstein, S. S. 1970. Nitrosamines as environmental carcinogens. Nature 225:21.

Likhachev, A. Ya. 1968. The combined effect of carcinogens. Vop. Onkol. 14(10):114.

Liu, H. P. and Watts, B. M. 1970. Catalysts of lipid peroxidation in meats. 3. Catalysts of oxidative rancidity in meats. J. Food Sci. 35:596.

Love, J. D. 1972. A comparison of myoglobin and nonheme iron as pro-oxidants in cooked meat and dispersions of phospholipid. Ph.D. Thesis, Michigan State University, East Lansing.

Love, J. D. and Pearson, A. M. 1974. Metmyoglobin and nonheme iron as prooxidants in cooked meat. J. Agric. Food Chem. 22:1032.

Lundberg, W. O. 1962. Mechanisms. Pages 31-50 in Schultz, H. W., Day, E. A. and Sinnhuber, R. O., eds. Symposium on Foods: Lipids and Their Oxidation. Avi Publishing Co., Westport, Connecticut.

Lynch, R. E., Lee, G. R. and Cartwright, G. E. 1976. Inhibition by superoxide dismutase of methemoglobin formation from oxyhemoglobin. J. Biol. Chem. 251:1015.

Madsen, H. C. 1976. Impact of the loss of nitrites on animal agriculture. Proc. Meat Ind. Res. Conf. 1976:35.

Magee, P. N. 1969. Growth and trophic factors in carcinogenesis. Envir. Res. 2:380.

Magee, P. N. and Barnes, J. M. 1967. Carcinogenic nitroso compounds. Adv. Cancer Res. 10:163.

McLeod, R. A., Jones, R. E. E. and Roberts, E. 1963. Glycolytic enzymes in the tissues of a salmonoid fish. Can. J. Biochem. Physiol. 41: 1971.

Miller, J. A. and Miller, E. C. 1971. Chemical carcinogenesis: Mechanism and approaches to its control. J. Nat. Cancer Inst. 47:5.

Mirvish, S. S., Wallcave, L., Eagen, M. and Shubik, P. 1972. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. Science 177:65.

Moore, E. G. and Gibson, Q. H. 1976. Cooperativity in the dissociation of nitric oxide from hemoglobin. J. Biol. Chem. 251:2788.

Neill, J. M. and Hastings, A. B. 1925. The influence of the tension of molecular oxygen upon certain oxidations of hemoglobin. J. Biol. Chem. 63:479.

Nordin, H. R. 1969. The depletion of added sodium nitrite in ham. J. Can. Inst. Food Technol. 2:79.

O'Brien, M. T. 1978. USDA acts on the bacon dilemma: Alternatives promise a reprieve. Food Prod. Dev. 12:32.

Ockerman, H. W., Hadden, J. P. and Cahill, V. R. 1973. The influence of nitrite on cured pork flavor. Proc. 18th European Meat Res. Conf. 1973:1493.

Pearson, A. M., Love, J. C. and Shorland, F. B. 1977. Warmed-over flavor in meat, poultry, and fish. Adv. in Food Res. 23:i.

Pensabene, J. W., Fiddler, W., Gates, R. A., Fagan, J. C. and Wasserman, A. E. 1974. Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. J. Food Sci. 39:314.

Perigo, J. A. and Roberts, T. A. 1968. Inhibition of clostridia by nitrite. J. Food Tech. 3:91.

Perigo, J. A., Whiting, E. and Bashford, T. E. 1967. Observations on the inhibition of vegetative cells of Clostridium sporogens by nitrite which has been autoclaved in a laboratory medium, discussed in the context of sublethally processed cured meats. J. Food Technol. 2:377.

Phillips, U. E. J. 1971. Naturally occurring nitrate and nitrite in foods in relation to infant methaemoglobinaemia. Food Cosmet. Toxicol. 9:219.

Phillips, W. E. 1968. Change in the nitrate and nitrite content of fresh and processed spinach during storage. J. Agr. Food Chem. 16:88.

Pierson, M. D., Collins-Thompson, D. L. and Ordal, Z. J. 1970. Microbiological, sensory and pigment changes of aerobically and anaerobically packaged beef. Food Technol. 24:129.

Ramsbottom, J. M. 1971. Packaging. Pages 513-537 in Price, J. F. and Schweigert, B. S., eds. The Science of Meat and Meat Products. 2nd ed. W. H. Freeman and Company, San Francisco, California.

Ramsbottom, J. M., Goeser, P. A. and Schultz, H. W. 1951. How light decolors meat: What to do about it. Food Indus. 23:120.

Rhee, K. S. 1978. Factors affecting oxygen uptake in model systems used for investigating lipid peroxidation in meat. J. Food Sci. 43:6.

Rifkind, J. M. 1973. Hemoglobin and myoglobin. Pages 25-50 in Eichhorn, G., ed. Inorganic Biochemistry. Vol. 2. Elsevier Scientific Pub. Co., Amsterdam.

Rifkind, J. M. 1974. Copper and autoxidation of hemoglobin. Biochemistry 13:2475.

Roberts, T. A. 1971. The inhibition of bacteria in canned pasteurized hams by sodium nitrite. Proc. 17th European Meat Res. Workers.

Rubin, A. A., Zitowitz, L. and Hausker, L. 1963. Acute circulatory effects of diazoxide and sodium nitrite. J. Pharmacol. Exp. Ther. 140:46.

Sander, J. 1974. Formation of N-nitroso compounds in laboratory animals. A short review. Proc. Int. Symp. on Nitrite in Meat Prod. 1974:243.

Sanderson, A., Pearson, A. M. and Schweigert, B. S. 1966. Effect of cooking procedure on flavor components of beef carbonyl compounds. J. Agr. Food Chem. 14:245.

Sato, K. and Hegarty, G. R. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36:1098.

Sato, K., Hegarty, G. R. and Herring, H. K. 1973. The inhibition of warmed-over flavor in cooked meats. J. Food Sci. 38:398.

Satterlee, L. D. and Hansmeyer, W. 1974. The role of light and surface bacteria in the color stability of prepackaged beef. J. Food Sci. 39:305.

Satterlee, L. D. and Zachariah, N. Y. 1972. Porcine and ovine myoglobin: Isolation, purification, characterization and stability. J. Food Sci. 37:909.

Sebranek, J. G. 1974. Studies on the ultimate fate and distribution of nitrite in a cured meat product. Ph.D. Thesis, University of Wisconsin, Madison.

Sebranek, J. G., Cassens, R. G., Hoekstra, W. G., Winder, W. C., Podebradsky, E. V. and Kielsmeier, E. W. 1973. ^{15}N tracer studies of nitrite added to a comminuted meat product. J. Food Sci. 38:1220.

Sebranek, J. G., Schroder, B. G., Rust, R. E. and Topel, D. G. 1977. Influence of sodium erythrobate on color development, flavor, and overall acceptability of frankfurters cured with reduced levels of sodium nitrite. J. Food Sci. 42:1120.

Seideman, S. C., Carpenter, Z. L., Smith, G. C. and Hoke, K. E. 1976a. Effect of degree of vacuum and length of storage on the physical characteristics of vacuum packaged beef wholesale cuts. J. Food Sci. 41:732.

Seideman, S. C., Vanderzant, C., Smith, G. C., Hanna, M. O. and Carpenter, Z. L. 1976b. Effect of degree of vacuum and length of storage on the microflora of vacuum packaged beef wholesale cuts. J. Food Sci. 41:738.

Shank, J. L., Silliker, J. H. and Harper, R. H. 1962. The effect of nitric oxide on bacteria. Appl. Microbiol. 10:185.

Simon, S., Ellis, D. E., MacDonald, B. D., Miller, D. G., Waldman, R. C. and Westerberg, D. O. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. J. Food Sci. 38:919.

Smith, A. 1921. Introduction to Organic Chemistry. Century Company, New York.

Solberg, M. and Franke, W. C. 1971. Photo sensitivity of fresh meat color in the visible spectrum. J. Food Sci. 36:990.

Spencer, R. 1971. Nitrite in curing - microbiological implications. Proc. 17th European Meeting Meat Res. Workers 1971:194.

Stoychev, M. and Djejeva, G. 1971. Influence of nitrates, nitrites and polyphosphates on breathing activity of Str. faecalis. Proc. 17th European Meeting Meat Res. Workers 1971:240.

Stryer, L. 1975. Biochemistry. W. H. Freeman and Company, San Francisco, California.

Swain, J. W. 1972. Volatile flavor constituents of pork cured with and without nitrite. University of Missouri, Columbia.

Sweet, C. W. 1973. Activity of antioxidants in fresh fish. J. Food Sci. 38:1260.

Tannenbaum, S. R. 1976. Relative risk of nitrate and nitrite ingestion. Proc. Meat Ind. Res. Conf. 1976:25.

Tannenbaum, S. R., Sinskey, A. J., Weisman, M. and Bishop, W. 1974. Nitrite in human saliva. Its possible relation to nitrosamine formation. J. Nat. Cancer Inst. 53:79.

Tannenbaum, S. R., Fett, D., Young, V. R., Land, P. D. and Bruce, W. R. 1978. Nitrite and nitrate are formed by endogenous synthesis in the human intestine. Science 200:1487.

Tappel, A. L. 1962. Heme compounds and lipoxidase as biocatalysts. Pages 122-129 in Schultz, H. W., Day, E. A. and Sinnhuber, R. O., eds. Symposium on Food: Lipids and Their Oxidation. Avi Publishing Co., Westport, Connecticut.

Tarladgis, B. G. 1962. Interpretation of the spectra of meat pigments. II. Cured meats. The mechanism of color fading. J. Sci. Food Agric. 13:485.

Tarladgis, B. G., Watts, B. G., Younathan, M. T. and Dugan, L., Jr. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44.

Townsend, W. E. and Bratzler, L. J. 1958. Effects of storage conditions on the color of frozen packaged retail beef cut. Food Technol. 12: 663.

Treinin, A. and Hayon, E. 1970. Adsorption spectra and reaction kinetics of NO_2, N_2O_3 and N_2O_4 in aqueous solution. J. Am. Chem. Soc. 92:5821.

Van Roon, P. S. 1973. Inhibitors in cooked meat products. Proc. Int. Symp. on Nitrite in Meat Prod., Zeist, Netherlands, 1973:117.

Von Elbe, J. H., Klement, J. T., Amundson, C. H., Cassens, R. G. and Lindsay, R. C. 1974. Evaluation of betalain pigments as sausage colorants. J. Food Sci. 39:128.

Walters, C. L. and Taylor, A. McM. 1965. The reduction of nitrite by skeletal muscle mitochondria. Biochem. Biophys. Acta 96:522.

Walters, C. L., Taylor, A. McM., Casselden, R. J. and Ray, N. 1968. Investigation of specific reducing systems in relation to meat curing. Brit. Food Mfg. Ind. Res. Ass. Rep. 139, Leatherhead.

Walters, C. L., Burger, I. H., Jewell, G. G., Lewis, D. F. and Parke, D. V. 1975. Mitochondria enzyme pathways and their possible role during curing. Z. Lebensm. Unters. Forsch. 158:193.

Wasserman, A. E. and Talley, F. 1972. The effect of sodium nitrite on the flavor of frankfurters. J. Food Sci. 37:536.

Watts, B. M. 1962. Meat products. Pages 202-214 in Schultz, H. W., Day, E. A. and Sinnhuber, R. O., eds. Symposium on Food: Lipids and Their Oxidation. Avi Publishing Co., Westport, Connecticut.

Watts, B. M., Kendrick, J., Zipsen, M. W., Hutchins, B. and Saleh, B. 1966. Enzymatic reducing pathways in meat. J. Food Sci. 31:855.

Westerberg, D. O. 1971. Packaging films and meat color. Proc. Recip. Meat Conf. 24:360.

Westerberg, D. O. 1973. Cured meat flavor and the role of nitrite in its development. Recip. Meat Conf. 26:45.

White, J. W., Jr. 1975. Relative significance of dietary sources of nitrate and nitrite. J. Agric. Food Chem. 23:886.

Wodicka, V. O. 1956. The nature and properties of the pigment of cured meats. Ph.D. Thesis, Rutgers - The State University, New Brunswick, New Jersey.

Woolford, G., Cassens, R. G., Greaser, M. L. and Sebranek, J. G. 1976. The fate of nitrite: Reaction with protein. J. Food Sci. 41:585.

Yonetani, T., Yamamoto, H., Erman, J. E., Leigh, J. S., Jr. and Reed, G. H. 1972. Electromagnetic properties of hemoproteins. 5. Optical and electron paramagnetic resonance characteristics of nitric oxide derivatives of metalloporphyrin-apohemo protein complexes. J. Biol. Chem. 247:2447.

Younathan, M. T. and Watts, B. M. 1959. Relationship of meat pigments to lipid oxidation. Food Res. 24:728.

Younathan, M. T. and Watts, B. M. 1960. Oxidation of tissue lipids in cooked pork. Food Res. 25:538.

Yueh, M. H. and Strong, F. M. 1960. Some volatile constituents of cooked beef. J. Agr. Food Chem. 8:491.

Zachariah, N. Y. and Satterlee, L. D. 1973. Effect on light, pH and buffer strength on the autoxidation of porcine, ovine and bovine myoglobins at freezing temperatures. J. Food Sci. 38:418.

Zaika, L. L., Zell, T. E., Smith, J. L., Palumbo, S. A. and Kissinger, J. C. 1976. The role of nitrite and nitrate in Lebanon bologna, a fermented sausage. J. Food Sci. 41:1457.

Zipsen, M. W., Kwon, T. W. and Watts, B. M. 1964. Oxidative changes in cured and uncured frozen cooked pork. J. Agric. Food Chem. 12: 105.

ACKNOWLEDGMENTS

The author wishes to express his sincerest appreciation to Dr. D. G. Topel for his guidance and encouragement throughout the entire graduate program. Special appreciation is extended to Dr. J. G. Sebranek for his assistance, encouragement, and advice concerning these investigations and for the many hours spent in reading and evaluating this manuscript.

Gratitude is expressed to Professor R. E. Rust, Dr. H. E. Snyder, and Dr. J. N. Hathcock for their advice and suggestions while serving as members of the author's committee; to Dr. D. L. Kuhlers for his assistance with the statistical analysis; and to Sarah Brown and G. E. Carstens for their excellent technical assistance.

Appreciation is sincerely extended to:

The American Can Company for support on this research project.

The Townsend Engineering Company for financial support of this author's graduate study.

The National Science Council, Taiwan Sugar Cooperation, and the Animal Industry Research Institute of TSC of the Republic of China (Taiwan) for their help and understanding during the past four years.

The staff and graduate students of the Meat Laboratory at Iowa State University for their willing assistance, constructive criticism, and unending friendship.

A sincere feeling of gratitude is felt for my mother and wife for their encouragement, support, and continual patience.