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Mechanisms by Which Nitrite Inhibits the Development of Warmed-Over Flavour (WOF) in Cured Meat

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ABSTRACT

The mechanism by which nitrite functions as an antioxidant in cured meat products was studied using ground beef and meat (beef) pigment extracts (MPE). Nitrite, L-ascorbate, sodium tripolyphosphate, ADP and EDTA were reacted with ground beef and MPE, after which the samples were heated and stored at 4°C. Lipid oxidation was assessed by the TBA method. The results suggested that nitrite functions as an antioxidant in three possible ways: (1) by the formation of a strong complex with heme pigments, thereby preventing the release of non-heme iron and its subsequent catalysis of lipid oxidation; (2) by interacting directly with the liberated non-heme iron (Fe^{2+}) from denatured heme pigments and (3) to a lesser extent, by stabilization of the unsaturated lipids within the membranes. Stabilization of the porphyrin ring, preventing release of Fe^{2+} during the cooking process, appears to be the most important mechanism.

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INTRODUCTION

Watts (1954) reported that nitrite delays the development of oxidative rancidity, Tarladgis (1961) proposed that heme compounds from muscle are active catalysts of lipid oxidation in the oxidized (Fe^{3+}) form while nitrite forms cured meat pigments and retains the heme in the reduced (Fe^{2+}) form, which is inactive as a catalyst for lipid oxidation. Sato & Hegarty (1971), Cassens *et al.* (1976) and Goutefongea *et al.* (1977) have suggested that nitrite reacts with lipids in the membrane, leading to membrane stabilization and retardation of lipid oxidation. Pearson *et al.* (1977) have reviewed the factors influencing WOF in both cured and fresh meat.

Zipser *et al.* (1964) proposed that nitrite forms a stable complex with iron porphyrins in heat-denatured meat, thereby inhibiting the development of WOF. Igene & Pearson (1979) reacted nitrite with purified unsaturated phospholipids and demonstrated that nitrite significantly reduced 2-thiobarbituric acid (TBA) numbers whilst improving sensory scores. It was suggested that nitrite functions by converting the meat pigments to catalytically inactive forms, while stabilizing the unsaturated fatty acids (Igene & Pearson, 1979; Igene *et al.*, 1979).

Kanner (1979) has shown *S*-nitrosocysteine to be a potent antioxidant, which may be generated in the meat curing process. Kanner *et al.* (1984) have also proposed that the antioxidant effects of nitrite in cured meat result from the formation of nitric oxide, which interacts with metals, especially with heme iron and non-heme compounds.

Heme compounds, especially metmyoglobin and methemoglobin, are catalysts of lipid oxidation in meat (Tappel, 1952; Watts, 1954, 1962; Tappel, 1962; Greene *et al.*, 1971; Love & Pearson, 1974; Igene *et al.*, 1979). Studies by Sato & Hegarty (1971), Yamauchi (1972*a*), Love & Pearson (1974) and Igene *et al.* (1979) provided evidence for the direct involvement of non-heme iron in WOF. Igene *et al.* (1979) demonstrated that non-heme iron is the major pro-oxidant in cooked meat and is released from heme pigments (myoglobin) during cooking. This has been recently confirmed by Schricker *et al.* (1982), Schricker & Miller (1983) and Chen *et al.* (1984).

In spite of research on the rôle of nitrite as an antioxidant in cured meat, the mechanisms involved in the reaction between nitrite and pro-oxidants in meat remain to be elucidated (MacDonald *et al.*, 1980).

Pearson & Gray (1983) have proposed that nitrite stabilizes the heme pigments so that they do not release Fe^{2+} ion, thus preventing catalysis of WOF.

The major objective of this research was to study the mechanism(s) by which nitrite functions as an antioxidant in cured meat. In addition, the effects of L-ascorbate as a reducing agent, sodium tripolyphosphate as a metal sequestrant, ethylenediaminetetraacetic acid (EDTA) as a metal chelator, or adenosine diphosphate (ADP) as a pro-oxidant, alone or in combination with nitrite, were also evaluated.

MATERIALS AND METHODS

Sources of meat

The beef muscles in this study were taken from a liveweight Charolais heifer slaughtered at the Michigan State University Meat Laboratory. Portions of the *longissimus dorsi* (LD) and *biceps femoris* (BF) muscles were excised from the carcass at 24 h post mortem, frozen and stored at −18 °C until used for analysis.

Organization of the study

The study was divided into two stages. The reactions of nitrite alone and in association with reducing or non-reducing additives and synergists or chelators of non-heme iron using ground beef were investigated in the first stage of the study. The second stage was essentially the same as the first except that meat pigment extracts were used instead of ground meat.

In the first stage, beef samples (LD and BF muscles), weighing about 2·0 kg, were thawed overnight at 4 °C and, after removing all visible fat and connective tissue, were cut into pieces, ground first through a plate with 0·9 cm holes and then through a plate with 0·48 cm holes. Eleven experimental treatments were used, as shown in Table 1. Each consisted of 50 g of meat mixed with 50 ml distilled, deionized water in which the additives were dissolved. Each treatment was replicated twice. The final level of additives in the treatments were: nitrite, 156 mg/kg; L-ascorbate, 250 mg/kg; adenosine diphosphate (ADP), 213·6 mg/kg; sodium tripolyphosphate, 0·5% (w/w) and ethylenediaminetetraacetic acid–disodium salt (EDTA)-2% (w/w), respectively. The samples were held at 4 °C for 24 h in order to allow curing to occur.

TABLE 1
Design and Formulation of Experimental Treatments Using Ground Beef[a]

Number	*Experimental treatment composition*
1	Ground meat (M) + distilled, deionized water (control)
2	M + nitrite (N)
3	M + L-Ascorbate (A)
4	M + Sodium tripolyphosphate (TP)
5	M + Adenosine diphosphate (ADP)
6	M + Ethylenediaminetetraacetic acid (EDTA)
7	M + N + A
8	M + N + TP
9	M + N + EDTA
10	M + N + ADP
11	M + N + A + ADP

[a] Samples were cooked to 80 °C in unsealed retortable pouches and were replicated twice each, using beef LD and BF muscles.

The second stage involved the use of 1386 g of ground beef (LD) muscle and preparation of meat pigment extracts (MPE) according to the method of Igene *et al.* (1979). The filtered meat pigment extracts were combined and concentrated in a Virtis II freeze-drier. Following reconstitution of the pigment powder in distilled deionized water, each millilitre of extract represented 1·386 g of raw meat. Muscle fibers, hereafter referred to as the residue, which served as a matrix for reaction of pigment extract with nitrite, were prepared using beef LD muscle as described by Igene *et al.* (1979). Thus, the second stage of the study consisted of eleven experimental treatments to evaluate the rôle of nitrite, with or without reducing or chelating agents, upon the meat pigment extracts (Table 2).

Samples were prepared by first adding nitrite, L-ascorbate, ADP, EDTA or sodium tripolyphosphate of the designated composition to 100 ml pigment extract. The final concentrations of additives were the same proportions as used for the ground beef (stage 1). The treated samples were held overnight at 4 °C. Thereafter, 50 ml each of the pigment–additive mixtures were thoroughly mixed with 100 g of meat residue and left for another 24 h at 4 °C before cooking and evaluation of lipid oxidation by the TBA method.

TABLE 2
Design and Formulation of Experimental Treatments using Meat Pigment Extracts and Meat Residues[a]

Number	*Experimental treatment composition*[b]
1	Meat pigment extract (MPE) + residue only (first control)
2	MPE + N + residue
3	MPE + A + residue
4	MPE + TP + residue
5	MPE + ADP + residue
6	MPE + EDTA + residue
7	MPE + N + A + residue
8	MPE + N + TP + residue
9	MPE + N + EDTA + residue
10	MPE + N + ADP + residue
11	Residue + distilled, deionized water (second control)

[a] MPE was prepared using beef LD muscle. Samples were heated to 80 °C in unsealed retortable pouches. Treatments were replicated twice for TBA analysis.
[b] Abbreviations are the same as in Table 1.

Cooking

The treated samples from both stages were placed in unsealed retortable pouches and heated in a boiling water bath to an internal temperature of 80 °C, unless otherwise indicated. They were then stored at 4 °C and tested at intervals of 0, 5, 12 and 21 days for stage 1 and 0, 7 and 14 days for stage 2, respectively.

TBA test

The distillation method of Tarladgis *et al.* (1960) was utilized to measure the development of oxidative rancidity by the TBA test. Since nitrite interferes with the distillation step by nitrosation of malonaldehyde (Hougham & Watts, 1958; Younathan & Watts, 1959), the method of Zipser & Watts (1962) was modified by adding 2 ml of sulfanilamide to the samples containing nitrite before distillation. The pigment was measured at 532 nm and TBA numbers were expressed as milligrams of malonaldehyde per kilogram of meat.

Determination of total iron, non-heme and heme iron

The concentrations of total iron, heme and non-heme iron in the ground beef samples (stage 1) and in the meat pigment extracts (stage 2) were determined. Total iron was determined in the ground meat, as well as in the meat pigment extracts, by digesting 5 g or 5 ml (as appropriate) in concentrated nitric acid and, later, in hydrogen peroxide, as outlined by Schricker *et al.* (1982). The digest was analyzed for iron using an atomic absorption spectrophotometer (Instrumentation Laboratory aa/ae Spectrophotometer 95). Non-heme iron was determined for both the ground beef and the meat pigment extracts before and after cooking. Meat samples weighing 2–3 g or 1–3 ml of the pigment extract were placed in 15-ml centrifuge tubes and heated in boiling water for 1 h. Non-heme iron was separated from heme iron by a modification of the procedure outlined by Igene *et al.* (1979). Non-heme iron was analyzed separately by first precipitating the bound heme iron with 5 ml 40 % trichloroacetic acid (TCA) and vigorously shaking the tubes in a vortex mixer. Thereafter, the tubes were centrifuged for 20 min. Following centrifugation, the supernatant was removed for determination of free non-heme iron.

Statistical methods

The TBA values and non-heme iron values were subjected to the analysis of variance using a Control Data Corporation (CDC) 6500 computer. Significance between and within treatments was determined using Tukey's test for multiple comparisons according to Steel & Torrie (1960).

RESULTS AND DISCUSSION

Lipid oxidation in cured and uncured ground beef

Results in Table 3 give means and standard deviations for TBA values of the eleven different treatments designed to evaluate the rôle of nitrite as an antioxidant, either alone or in combination with L-ascorbate, tripolyphosphate, EDTA or ADP. Analysis of variance indicated highly significant ($P < 0{\cdot}001$) differences between treatments. Time of storage at 4 °C was significant ($P < 0{\cdot}05$), as was the time–treatment interaction ($P < 0{\cdot}001$).

TABLE 3

TBA Numbers of Oxidizing Cooked Ground Beef as Influenced by the Presence or Absence of Nitrite. Values Given are Means ± Standard Deviations (±SD)[a,b,c]

Experimental treatments[d]	*Days in storage at 4°C*			
	0	*5*	*12*	*21*
1 M	0·58 ± 0·09^{e}	1·55 ± 0·22^{f}	2·78 ± 0·24^{g}	2·83 ± 0·36^{g}
2 M + N	0·46 ± 0·04^{e}	0·48 ± 0·09^{e}	0·47 ± 0·03^{e}	0·54 ± 0·06^{e}
3 M + A	0·53 ± 0·15^{e}	0·60 ± 0·09^{e,h}	0·90 ± 0·14^{f,h}	1·10 ± 0·18^{f}
4 M + TP	0·48 ± 0·06^{e}	0·45 ± 0·04^{e}	0·45 ± 0·10^{e}	0·62 ± 0·13^{e,f}
5 M + ADP	0·60 ± 0·10^{e}	1·08 ± 0·13^{f}	1·91 ± 0·04^{g}	2·82 ± 0·80^{g}
6 M + EDTA	0·54 ± 0·12^{e}	0·36 ± 0·03^{e}	0·47 ± 0·02^{e}	0·49 ± 0·06^{e}
7 M + N + A	0·49 ± 0·13^{e}	0·40 ± 0·06^{e}	0·44 ± 0·09^{e}	0·47 ± 0·09^{e}
8 M + N + TP	0·45 ± 0·04^{e}	0·43 ± 0·08^{e}	0·40 ± 0·05^{e}	0·44 ± 0·05^{e}
9 M + N + EDTA	0·43 ± 0·04^{e}	0·37 ± 0·06^{e}	0·38 ± 0·03^{e}	0·44 ± 0·08^{e}
10 M + N + ADP	0·41 ± 0·03^{e}	0·41 ± 0·08^{e}	0·44 ± 0·05^{e}	0·46 ± 0·07^{e}
11 M + N + A + ADP	0·40 ± 0·05^{e}	0·42 ± 0·10^{e}	0·41 ± 0·06^{e}	0·42 ± 0·09^{e}

[a] Values represent pooled data from ground beef of LD and BF muscles, each with duplicate replications.

[b] TBA numbers in the same row bearing the same letter are not significantly different from each other at the 5% level.

[c] TBA numbers in the same column bearing the same letter are not significantly different from each other at the 5% level.

[d] See Table 1 for meaning of abbreviations.

Treatment 1 (control), containing ground beef and deionized water (1:1), consistently had the highest TBA values ($P < 0{\cdot}001$), followed by treatments 5 and 3, which contained ADP and L-ascorbate, respectively. In all other treatments (2, 4, 6, 7, 8, 9, 10 and 11) the rate and extent of lipid oxidation were essentially the same and significantly ($P < 0{\cdot}01$) lower than that of treatments 1, 5 and 3, respectively. After 21 days' storage at 4°C, lipid oxidation was at least five times higher in the control than that for any of the treatments containing nitrite. Treatments 4 and 6, in which ground beef was combined with tripolyphosphate and EDTA, respectively, behaved essentially like those containing nitrite alone or in association with other additives (2, 7, 8, 9, 10 and 11).

The results demonstrate the effectiveness of nitrite as an antioxidant, either when used alone or in combination with other additives (Table 3). Numerous workers (Zipser *et al.* 1964; Cho & Bratzler, 1970; Sato & Hegarty, 1971; Bailey & Swain, 1973; Fooladi *et al.*, 1979; Igene *et al.*,

TABLE 4
Effect of Time in Storage and Treatments on the Mean TBA Values of MPE Cooked Model Meat Systems[a,b,c]

Experimental treatments[d]	*Days in storage at 4°C*		
	0	*7*	*14*
1 MPE + H_2O only (control)	$0{\cdot}82 \pm 0{\cdot}06^{e}$	$1{\cdot}17 \pm 0{\cdot}12^{f}$	$1{\cdot}64 \pm 0{\cdot}07^{f}$
2 MPE + N	$0{\cdot}47 \pm 0{\cdot}03^{e}$	$0{\cdot}29 \pm 0{\cdot}05^{e}$	$0{\cdot}44 \pm 0{\cdot}03^{e}$
3 MPE + A	$0{\cdot}83 \pm 0{\cdot}13^{e}$	$0{\cdot}78 \pm 0{\cdot}11^{e}$	$0{\cdot}82 \pm 0{\cdot}06^{e}$
4 MPE + TP	$0{\cdot}85 \pm 0{\cdot}05^{e}$	$1{\cdot}41 \pm 0{\cdot}06^{f}$	$1{\cdot}60 \pm 0{\cdot}03^{f}$
5 MPE + ADP	$0{\cdot}79 \pm 0{\cdot}07^{e}$	$1{\cdot}10 \pm 0{\cdot}10^{f}$	$1{\cdot}51 \pm 0{\cdot}08^{f}$
6 MPE + EDTA	$0{\cdot}77 \pm 0{\cdot}06^{e}$	$0{\cdot}58 \pm 0{\cdot}04^{e}$	$0{\cdot}77 \pm 0{\cdot}05^{e}$
7 MPE + N + A	$0{\cdot}63 \pm 0{\cdot}06^{e}$	$0{\cdot}46 \pm 0{\cdot}02^{e}$	$0{\cdot}66 \pm 0{\cdot}05^{e}$
8 MPE + N + TP	$0{\cdot}42 \pm 0{\cdot}03^{e}$	$0{\cdot}27 \pm 0{\cdot}03^{e}$	$0{\cdot}48 \pm 0{\cdot}07^{e}$
9 MPE + N + EDTA	$0{\cdot}43 \pm 0{\cdot}04^{e}$	$0{\cdot}38 \pm 0{\cdot}02^{e}$	$0{\cdot}53 \pm 0{\cdot}02^{e}$
10 MPE + N + ADP	$0{\cdot}41 \pm 0{\cdot}02^{e}$	$0{\cdot}26 \pm 0{\cdot}01^{e}$	$0{\cdot}54 \pm 0{\cdot}04^{e}$
11 Muscle residue + H_2O	$0{\cdot}74 \pm 0{\cdot}05^{e}$	$0{\cdot}77 \pm 0{\cdot}02^{e}$	$1{\cdot}14 \pm 0{\cdot}03^{f}$

[a] MPE was prepared using beef LD muscle. Experimental treatments were replicated twice.
[b] TBA numbers in the same row bearing the same letter are not significantly different from each other at the 5% level.
[c] TBA numbers in the same column bearing the same letter are not significantly different from each other at the 5% level.
[d] See Table 2 for meaning of abbreviations.

1979) have shown that nitrite effectively inhibits oxidation in cooked cured meat products. Both 0·5% tripolyphosphate and 2% EDTA were equally effective in the inhibition of lipid oxidation. Tims & Watts (1958) first demonstrated that phosphates prevent lipid oxidation in cooked meat. According to Tims & Watts (1958), Sato & Hegarty (1971) and Yamauchi (1972*b*) phosphates sequester Fe^{+2} or other metal catalysts of lipid oxidation, thereby inhibiting the development of WOF.

The effectiveness of EDTA in preventing lipid oxidation in cooked meat (Table 3) confirms the studies of Igene *et al.* (1979) and Tay *et al.* (1983), who have demonstrated that 2% EDTA chelates the non-heme iron released on cooking meat and in post-rigor raw meat, thereby inhibiting lipid oxidation. Since the TBA numbers in treatments 2, 4, 6, 7, 8, 9, 10 and 11 were not significantly different from each other, it may be concluded that nitrite also functions as an antioxidant in meat systems similar to polyphosphate or EDTA by reducing the pro-oxidant activity of

metal ions, chiefly Fe^{+2}. This agrees with the findings of Igene *et al.* (1979), who demonstrated that non-heme iron is released by the meat pigments during cooking whereas EDTA effectively prevents oxidation by chelating the non-heme iron. Evidence suggests that nitrite can react with trace metals in lean meat (Sato & Hegarty, 1971; Love & Pearson, 1974), leading to the conclusion that myoglobin *per se* is not the principal pro-oxidant of lipid oxidation in meat systems, but that non-heme iron released from myoglobin during cooking catalyzes oxidation. The observation that Fe^{2+} ions are released from heme pigments during cooking has recently been confirmed by Schricker *et al.* (1982), Schricker & Miller (1983) and Chen *et al.* (1984).

The rapid increase in lipid oxidation during storage following the heating of both meat (Table 3) and MPE (Table 4) may be associated with changes in membrane structure that expose the phospholipids to oxygen, as suggested by earlier work from our laboratory (Wilson *et al.*, 1976; Igene & Pearson, 1979). Although alteration of the lipid–protein complex by heating (Igene *et al.*, 1979) may play an important rôle in the development of WOF, it does not explain the mechanism by which nitrite inhibits WOF.

Lipid oxidation as influenced by nitrite and other additives

Mean TBA values for experimental treatments using meat pigment extracts (MPE), instead of ground meat, are shown in Table 4. Pigment-free muscle fibers (residue) served as the matrix on which myoglobin and the various additives were reacted. As with ground beef, analysis of variance indicated significant ($P < 0{\cdot}001$) differences among treatments. Time in storage at 4 °C was significantly different ($P < 0{\cdot}001$), as well as the interaction of time in storage × treatments ($P < 0{\cdot}001$).

The effects of the additives on oxidation in MPE (Table 4) were somewhat different from those found for intact meat (Table 3). Treatment 1 caused essentially the same effects as before, both in the rate and extent of lipid oxidation which were significantly ($P < 0{\cdot}01$) higher than in all other treatments except treatment 4, which contained tripolyphosphate (TP). Unlike the meat, however, TP did not inhibit oxidation in MPE. Next in order of the decreasing extent of lipid oxidation were treatments 5 (MPE + ADP) and 11 (muscle residue + deionized water) serving as the second control, both of which acted as pro-oxidants. Contrary to the

meat system, however, ascorbate alone (treatment 3) did not act as a pro-oxidant in MPE (Table 4). Treatments 2, 7, 8, 9 and 10 exhibited the lowest levels of lipid oxidation and were not significantly different from each other.

Lipid oxidation in treatment 1 (first control) was about three times greater than that in treatments 2, 7, 8, 9 and 10, which consisted of nitrite alone or in combination with other additives. On the other hand, the extent of lipid oxidation was only twofold higher in treatment 11 (second control) than in treatments 2, 7, 8, 9 or 10. Two factors, therefore, seem to be involved in lipid oxidation for treatments 1 and 11. In treatment 1, both muscle lipids and pigments appear to play contributory rôles (Igene & Pearson, 1979; Igene *et al.*, 1979) while, in treatment 11, only the lipids seem to be of relevance since the muscle fibers (residue) were devoid of all pigments but contained the lipids (Igene *et al.* 1979).

The somewhat higher TBA numbers in treatment 6 (MPE + EDTA) than in treatments 2, 7, 8, 9 and 10, although not statistically significant (Table 4), may be related to the chelating effect of EDTA on the pro-oxidant metals released during cooking, especially Fe^{2+} ions. When TBA values of treatments 1 and 2 are compared, the significant ($P < 0{\cdot}05$) difference (Table 4) may be ascribed to the effect of meat pigments (myoglobin). However, the difference between treatment 2 (nitrite only) and treatment 11 (second control) can be attributed to nitrite effectively inhibiting lipid oxidation by stabilizing the membrane lipids, since the pigments had been removed in treatment 11.

Igene & Pearson (1979) suggested that nitrite actually functions as an antioxidant through the formation of a stable complex with the phospholipid components by stabilizing the membranes, as well as by forming a stable complex with the pigments. Specifically, nitrite was demonstrated to be effective as an antioxidant against phosphatidyl ethanolamine (PE), the major phospholipid component responsible for the development of WOF. Nitrite stabilization of lipid-containing membranes becomes pertinent since Igene *et al.* (1981) demonstrated an increased proportion of unsaturated fatty acids for cooked meat, especially in the PE fraction, as the drippings contained largely triglycerides. Yamauchi (1973) also demonstrated an increase in the amount of free fatty acids released on heating the mitochondrial fraction from skeletal muscle. Evidence also suggests that nitric oxide from nitrite reacts with the unsaturated fatty acids in meat (Cassens *et al.*, 1976; Frouin *et al.*, 1975).

Interaction of nitrite and heme pigments and the levels of non-heme iron

Resolution of the rôles played by heme and non-heme iron as catalysts of lipid oxidation in meat products is very important in understanding the factors responsible for the development of off-flavors. Robinson (1924) first implicated the porphyrins (hemoglobin, myoglobin and cytochromes) as the catalysts of lipid oxidation in meat and attributed catalysis to their iron content. It is, therefore, important that the distribution of various forms of iron in muscle tissue be elucidated. Such information could aid in assessing their relative importance as catalysts of lipid oxidation. Iron is present in several forms in meat, although knowledge about the combinations in which it exists is incomplete (Love, 1983). Recently, Hazell (1982) reported that the iron in beef, lamb, pork and chicken leg muscle is distributed between five main fractions: (1) an insoluble fraction, (2) ferritin, (3) hemoglobin, (4) myoglobin and (5) a low molecular weight fraction. Myoglobin was shown to be the predominant iron compound in beef and lamb, while most iron in pork and chicken was associated with the insoluble fraction. More than 70% of the iron in beef was associated with the hemoproteins (hemoglobin and myoglobin) whereas less than 30% of that in chicken was in this form. Tay *et al.* (1983) divided the iron of pork muscle into soluble protein-bound iron, free iron and residual iron (insoluble) and indicated that only the free iron fraction varies significantly between the pre-rigor and post-rigor states. The major form of iron is myoglobin, which constitutes more than 70% of the total (Igene *et al.*, 1979; Hazel, 1982; Tay *et al.*, 1983).

The nature of the interaction between nitrite and myoglobin in the curing process is demonstrated by the data in Table 5. While cooking significantly ($P < 0{\cdot}05$) increased the proportion of non-heme iron in the control (treatment 1), the levels of non-heme iron remained unchanged in treatment 2, which contained nitrite. A similar pattern in the levels of non-heme iron was also observed in all other treatments containing nitrite (7, 8, 9 and 10). The significant increase in non-heme iron following the cooking of uncured meat was first observed by Igene *et al.* (1979) and later confirmed by Schricker & Miller (1983) and Chen *et al.* (1984) and apparently results from the release of non-heme iron from the heme complex as a consequence of heating. It has been suggested that oxidative cleavage of the porphyrin ring is probably involved (Schricker *et al.*, 1982).

According to Schmid & McDonagh (1979), *in vivo* degradation of heme

in humans and mammals involves oxidation of the porphyrin ring by molecular oxygen at the α-bridge, resulting in cleavage and subsequent release of iron. The theory that conversion of heme iron to non-heme iron in meat involves oxidation of the porphyrin ring is strengthened by the observation of Igene *et al.* (1979) showing that H_2O_2 significantly increased the amount of non-heme iron. It is suggested, therefore, that the low level of non-heme iron observed in cured cooked meat (Table 5) is probably due to the stabilizing effect of nitrite on myoglobin. The low TBA values for the treatments containing nitrite (Tables 3 and 4) provide further verification for the stabilizing effect of nitrite.

There was a significant ($P < 0{\cdot}05$) increase in the level of non-heme iron following cooking of ground beef in combination with ADP (Table 5—treatment 5), which is probably due to the oxidative effect of ADP on the porphyrin ring. This is reinforced by the TBA results in Tables 3 and 4 for the treatments in which ADP was either combined with ground beef or with the meat pigment extract. As previously observed, ADP did not

TABLE 5
Effect of Cooking Treatments. Levels of Non-Heme Iron (μgFe/g) in Beef *Biceps femoris* Muscle[a,b,c]

Experimental treatments[d]	*Levels of non-heme iron (Mean ± SD)*	
	Uncooked	*Cooked*
1 M	$6{\cdot}62 \pm 1{\cdot}15^{e}$	$10{\cdot}8 \pm 0{\cdot}18^{h}$
2 M + N	$6{\cdot}65 \pm 0{\cdot}91^{e}$	$6{\cdot}80 \pm 0{\cdot}39^{e}$
3 M + A	$6{\cdot}52 \pm 0{\cdot}92^{e}$	$7{\cdot}92 \pm 0{\cdot}81^{eg}$
4 M + TP	$7{\cdot}35 \pm 0{\cdot}79^{e}$	$8{\cdot}45 \pm 1{\cdot}07^{eg}$
5 M + ADP	$6{\cdot}19 \pm 2{\cdot}15^{e}$	$8{\cdot}46 \pm 1{\cdot}52^{fg}$
6 M + EDTA	$8{\cdot}42 \pm 0{\cdot}91^{e}$	$8{\cdot}21 \pm 0{\cdot}70^{eg}$
7 M + N + A	$7{\cdot}04 \pm 1{\cdot}07^{e}$	$7{\cdot}13 \pm 1{\cdot}90^{e}$
8 M + N + TP	$7{\cdot}17 \pm 1{\cdot}23^{e}$	$8{\cdot}10 \pm 1{\cdot}33^{eg}$
9 M + N + EDTA	$6{\cdot}94 \pm 0{\cdot}83^{e}$	$8{\cdot}05 \pm 0{\cdot}93^{eg}$
10 M + N + ADP	$6{\cdot}96 \pm 1{\cdot}15^{e}$	$7{\cdot}44 \pm 1{\cdot}59^{e}$

[a] Treatments were replicated four times.
[b] Values in the same row bearing the same letter are not significantly different from each other at the 5% level.
[c] Values in the same column bearing the same letter are not significantly different from each other at the 5% level.
[d] See Table 1 for meaning of abbreviations.

behave as an antioxidant, but as a pro-oxidant. However, when nitrite was combined with ADP, the pro-oxidant effect was removed (Tables 3 and 4). Non-heme iron levels in the treatments containing ascorbate, tripolyphosphate and EDTA did not change significantly following cooking (Table 5—treatments 3, 4 and 6); thus, these compounds—which normally act as reducing agents, synergists or chelators—do not have any oxidative effects on the porphyrin ring at the levels added.

Interaction of nitrite with meat pigment extracts (MPE)

The effect of cooking in relation to changes in the level of non-heme iron in the meat pigment extracts is shown in Table 6. Non-heme iron in the control sample (uncured—treatment 1) significantly ($P < 0{\cdot}05$) increased following cooking, but there was no measurable increase on reacting nitrite with MPE. This observation is similar to that for ground beef

TABLE 6

Effect of Cooking and Treatments. Non-Heme Iron Content (μgFe/g Meat) in Beef Muscle Pigment Extract[a,b,c]

Experimental treatments[d]	*Total iron (μgFe/g meat) in uncooked treatments*	*Non-heme iron content*	
		Uncooked (*Means* ± *SD*)	*Cooked*
1 MPE only	12·0	$2{\cdot}74 \pm 0{\cdot}49^{fghi}$	$3{\cdot}09 \pm 0{\cdot}52^{jk}$
2 MPE + N	10·5	$2{\cdot}69 \pm 0{\cdot}46^{fgh}$	$2{\cdot}70 \pm 0{\cdot}53^{fgh}$
3 MPE + A	10·8	$2{\cdot}67 \pm 0{\cdot}44^{fg}$	$3{\cdot}14 \pm 0{\cdot}50^{jk}$
4 MPE + TP	9·11	$2{\cdot}77 \pm 0{\cdot}42^{fghi}$	$3{\cdot}10 \pm 0{\cdot}49^{jk}$
5 MPE + ADP	11·5	$2{\cdot}58 \pm 0{\cdot}33^{f}$	$2{\cdot}91 \pm 0{\cdot}46^{ghij}$
6 MPE + EDTA	12·0	$2{\cdot}92 \pm 0{\cdot}42^{ghij}$	$3{\cdot}27 \pm 0{\cdot}51^{k}$
7 MPE + N + A	10·8	$2{\cdot}14 \pm 0{\cdot}40^{e}$	$2{\cdot}57 \pm 0{\cdot}40^{f}$
8 MPE + N + TP	9·29	$2{\cdot}76 \pm 0{\cdot}36^{fghi}$	$3{\cdot}01 \pm 0{\cdot}27^{ijkl}$
9 MPE + N + EDTA	9·02	$3{\cdot}30 \pm 0{\cdot}43^{ijk}$	$3{\cdot}21 \pm 0{\cdot}43^{jk}$
10 MPE + N + ADP	10·1	$2{\cdot}49 \pm 0{\cdot}31^{f}$	$2{\cdot}98 \pm 0{\cdot}61^{hij}$

[a] Experiments were replicated five times. MPE was prepared using beef LD.

[b] Values within columns and rows bearing the same letter are not significantly different from each other at the 5% level.

[c] Values in the same column bearing the same letter are not significantly different from each other at the 5% level.

[d] See Table 2 for meaning of abbreviations.

subjected to the same treatments (Table 5). Chen *et al.* (1984) reported a similar effect of heating on beef pigment extracts and concluded that nitrite prevents the release of heme iron, apparently by stabilizing the porphyrin ring.

When nitrite was combined with other additives, such as ascorbate, tripolyphosphate, EDTA and ADP, it behaved differently with MPE upon cooking. Addition of ascorbate to MPE (Table 6—treatment 3) resulted in a significant ($P < 0{\cdot}05$) increase in the non-heme iron content of cooked samples in comparison with controls (treatment 1). This is in contrast to results for ground beef (Table 5, treatment 7) in which non-heme iron levels in uncooked and cooked samples remained virtually unchanged.

Ascorbic acid has been shown to be useful not only in developing and maintaining cured meat color but also in improving odor and flavor (Pearson *et al.*, 1983). Deng *et al.* (1978) reported that ascorbic acid behaved as an antioxidant in fish at concentrations of 1000 ppm or higher but acted as a pro-oxidant at 100 ppm or lower. There is a dearth of scientific information on the behavior of ascorbic acid in oxidizing systems at a concentration of 250 ppm—the concentration used in this study. The rôle of ascorbic acid, both as a lipid antioxidant and as a stabilizing agent in the curing process, varies with concentration and environmental factors. Substances such as non-heme iron, α-tocopherol, citric acid and amino acids, which are naturally present in muscle tissue, may change ascorbic acid from an antioxidant to a pro-oxidant state (Liu & Watts, 1970).

In the present study (Table 3, treatment 3), ascorbic acid caused a significantly ($P < 0{\cdot}05$) greater degree of lipid oxidation in ground beef than those treatments containing nitrite. This suggests that ascorbic acid acts as a pro-oxidant for heme iron at 250 ppm (Tables 5 and 6). Further support for this view is found by the higher TBA values for ascorbic acid in Table 3. This situation is reversed only when ascorbate is combined with nitrite.

SUMMARY

The results of this study suggest that nitrite may inhibit lipid oxidation in cured meat products by stabilizing the heme pigments or by the stabilization of the unsaturated lipids within the membranes. Both

mechanisms probably operate simultaneously. The results further indicate that the reaction of nitrite with myoglobin and subsequent heating leads to stabilization of the heme pigments, thereby preventing the release of free Fe^{2+} ions during cooking. Evidence also indicates that nitrite functions as an antioxidant by reducing the pro-oxidant activity of metal ions, chiefly Fe^{2+}, through complex formation.

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