MECHANISM OF NITRITE STABILIZATION OF MEAT LIPIDS AND HEME PIGMENTS

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INTRODUCTION

The mechanism by which nitrite prevents or retards the oxidation of meat lipids is not fully understood. Literature data suggest that more than one mechanism may be involved. Gray and Pearson (1987) have reviewed the proposed mechanisms which include: (a) formation of a strong complex between heme pigments and nitrite, thereby preventing the release of ferrous iron with its attendant catalysis of the propagation stage of lipid oxidation; (b) stabilization of the unsaturated lipids within the membranes; and (3) "chelation" of metal ions such as ferrous ions, thus rendering them unavailable for catalysis of oxidation reactions. Evidence is available indicating that all three mechanisms are involved in nitrite inhibition of oxidation in meats, although the first mechanism appears to be the most important.

While a number of studies have focused on the reaction of nitrite with unsaturated fatty acids, very few have addressed the mechanism involved in the stabilization of the polyunsaturated fatty acids in muscle membranes by nitrite. The major objective of this study was to provide information in support of this mechanism, as well as further evidence that nitrite stabilizes the heme pigments and thus prevents the release of nonheme into the meat system.

MATERIALS AND METHODS

Stabilization of meat lipids with nitrite

Cured and uncured (nitrite-free) pork samples from three pigs were prepared in the MSU Meat Laboratory to ensure that the cured and uncured samples came from the same animals. The cured samples had target levels of 156 mg/kg nitrite, 550 mg/kg ascorbate, 2.0% salt, 0.67% sucrose and 0.5% sodium tripolyphosphate added. The uncured samples did not contain nitrite but had the same target levels for the other additives. Mitochondrial and microsomal fractions were isolated from the pork samples by sequential centrifugation (Buckley et al., 1989) and dispersed in a KCl/lactic acid buffer (pH 5.5) for use in the peroxidation assay. Phospholipids were extracted from the cured and uncured pork samples using the dry column method of Marmer and Maxwell (1981). Liposomes for the peroxidation assay were prepared by dispersing a known quantity of the extracted phospholipids in the KCl/lactic acid buffer and adding 0.05% Triton X - 100 to emulsify the system.

Phospholipids from the uncured pork samples and ethyl esters of several polyunsaturated fatty acids were reacted with dinitrogen trioxide as described by Ross et al. (1987). Liposomes were again prepared by dispersing the lipids in the buffer systems described above. The oxidative stability of the microsomes, mitochondria, phospholipids and fatty acid ethyl esters was evaluated using the metmyoglobin/hydrogen peroxide-induced peroxidation assay of Harel and Kanner (1985). The peroxidation assay was carried out at 35`C and samples were taken at various time intervals. The extent of oxidation was monitored by a TBA procedure (Buckley et al., 1989).

To confirm that nitrite reacted with

the unsaturated fatty acids, the lipids were heated with a secondary amine (morpholine) in a sealed ampule. The samples were analyzed for the presence of N-nitrosomorpholine as described by Ross et al. (1987).

Stabilization of heme pigments by nitrite

Three pork loins were obtained from a local supermarket, trimmed of excess fat and ground. The pigments were removed from the ground pork using the distilled water-extraction procedure of Tichivangana and Morrissey (1984). Enough pork loin was extracted to provide approximately 2.5 kg water-extracted muscle fibers for each replicated experiment. The following additives were dispersed in 30 ml distilled water and added to 300 g aliquots of the muscle fibers: (i) control (no additives); (ii) hydrogen peroxide (80 µmoles); (iii) metmyoglobin (5 mg/g or 80 µmoles); (iv) nitric oxide myoglobin (80 µmoles); (v) metmyoglobin (80 µmoles)/H_2O_2 (80 umoles) and; (vi) nitric oxide myoglobin/H_2O_2 (80 µmoles). The fibers and reactants were thoroughly mixed, divided into three portions and subjected to the following heat treatments: (i) raw (no heat); (ii) short heat treatment - samples were placed in cooking bags, heated in a water bath (100`C) to an internal temperature of 70`C, removed immediately and placed in an ice bath to cool; (iii) prolonged heat treatment - samples were heated as in (ii) above, but were maintained at 70`C for 30 minutes before being placed in an ice bath.

All samples were analyzed for lipid oxidation immediately after cooking and after storage at 4`C for 24, 48 and 72 hours using the TBA procedure of Tarladgis et al. (1960), as modified by Crackel et al. (1988). The free iron content of the samples was determined after 72 hours by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

The peroxidation assay performed in these studies was based on the interaction of hydrogen peroxide with metmyoglobin, leading rapidly to the generation of an active species which promotes membrane-bound lipid peroxidation. Results from the peroxidation assays indicate that the microsomal and mitochondrial lipids from the cured pork samples were oxidized less rapidly than those from the nitrite-free samples (Figure 1). At the end of the peroxidation assay (210 minutes), TBA values for the microsomes and mitochondria from cured samples were approximately 2.2 times smaller than those for the uncured samples. Phospholipids from the cured pork samples also oxidized less rapidly than those from the uncured samples when subjected to the same peroxidation conditions (Figure 2). Thus it is apparent that nitrite stabilizes the polyunsaturated fatty acids in the phospholipids. To lend further credence to this observation, the phospholipids from the uncured pork samples were reacted with dinitrogen trioxide and subjected to the metmyoglobin/hydrogen peroxide-initiated peroxidation assay. These phospholipids were significantly ($p<0.01$) more stable than the phospholipids obtained from the uncured pork (Figure 2).

Similar trends were obtained for a series of ethyl esters of polyunsaturated fatty acids that had been reacted with dinitrogen trioxide (data not included for purposes of brevity). All of the reacted samples had lower TBA values than the unreacted samples.

To confirm that the stabilization of the lipids was due to the interaction of nitrite or dinitrogen trioxide with the double bonds of

the unsaturated fatty acids, the lipids samples were heated with a secondary amine (morpholine) in order to form the corresponding N-nitroso compound. Results clearly indicated that phospholipids extracted from cured pork were capable of nitrosating morpholine whereas those from the uncured pork samples could not. When the latter phospholipids were reacted with dinitrogen trioxide and then heated with morpholine, a significantly ($p<0.05$) greater amount of N-nitrosomorpholine was produced compared to that produced by the phospholipids from uncured pork. Similarly, reaction of the fatty acid ethyl esters (linoleic, linolenic and arachidonic) with dinitrogen trioxide produced compounds capable of nitrosating morpholine upon heating. Results of these studies thus imply that nitrite reacts with unsaturated lipids to form nitro-nitrosite derivatives, thus stabilizing the lipids against peroxidative changes.

Stabilization of heme pigments

The second phase of the study was designed to investigate the stabilization of the heme pigments in meat by nitrite, thus preventing the release of nonheme iron during cooking and storage. Water-extracted pork samples were treated with metmyoglobin and nitric oxide myoglobin, with and without hydrogen peroxide, and stored at 4`C. TBA results indicated that lipid oxidation was significantly ($p<0.05$) higher in samples containing metmyoglobin/hydrogen peroxide compared to the control samples and those containing nitric oxide myoglobin and metmyoglobin (Table 1). Nonheme iron analysis revealed that the amount of iron released from metmyoglobin/hydrogen peroxide-treated samples was significantly ($p<0.05$) higher than the amount released from metmyoglobin alone. Therefore, it appears that hydrogen peroxide in the presence of metmyoglobin does exert some effect on lipid oxidation. Hydrogen peroxide may "activate" metmyoglobin, thereby increasing heme-catalyzed lipid oxidation as suggested by Kanner and Harel (1985) and Rhee et al. (1987).

Raw samples treated with nitric oxide myoglobin alone or in combination with hydrogen peroxide showed no increase in lipid oxidation over the 72 hour storage period. TBA values for the uncooked samples containing nitric oxide myoglobin and nitric oxide myoglobin/hydrogen peroxide after 72 hours were 0.39 and 0.45, respectively. Short- and long-term heating did not accelerate lipid oxidation in either sample. Thus, it appeared that nitric oxide myoglobin acted as a specific antioxidant in these systems. Morrissey and Tichivangana (1985) also reported that nitric oxide myoglobin maintained its antioxidant properties in the presence of strong prooxidants such as metmyoglobin and free metal ions.

Another possible explanation for the low amount of lipid oxidation in the samples containing nitric oxide myoglobin is that neither heating nor hydrogen peroxide caused any breakdown of nitric oxide myoglobin. Nonheme iron contents of the samples containing nitric oxide myoglobin/hydrogen peroxide remained in the range 1.6 to 1.8 µg nonheme iron/g muscle fiber even after heating. This level was not significantly different from that of the control sample which contained 1.4 µg nonheme iron/g muscle fiber after long-term heating. Hydrogen peroxide also had no apparent effect on the nonheme iron content of the muscle fibers. Therefore, it can be concluded that heating or hydrogen peroxide did not produce any measurable decomposition of nitric

oxide myoglobin.

SUMMARY

Results of this study confirm previous observations that several mechanisms pertaining to the antioxidant role of nitrite in cured meats are operative. It has been demonstrated that nitrite stabilizes unsaturated lipids toward peroxidative attack by forming a nitro-nitrosite derivative, as suggested by Liu et al. (1988). This study also provides further indirect evidence that the peroxidative reactions in meat systems are initiated in the membranes. Therefore, stabilization of the membrane-bound lipids through the formation of a nitro-nitrosite derivative should contribute to the enhanced oxidative stability of cured meats. Evidence that phospholipids, microsomes and mitochondria from cured pork are less susceptible to peroxidation than their uncured counterparts has been provided in this investigation.

Additional evidence in support of nitrite stabilization of heme pigments has also been presented. The addition of nitrite oxide myoglobin to water-extracted muscle fibers did not result in any significant increase in nonheme iron when the model system was heated (short- and long-term heating) and then stored at 4°C for 72 hours. In contrast, when metmyoglobin was added to the muscle fibers and subjected to the same heat treatments, there was a significant increase in the nonheme iron content. It has been suggested that nonheme iron is the catalyst of the propagation stage of the oxidation process and will decompose preformed lipid hydroperoxides (Asghar et al., 1988). Thus, nitrite also functions as an antioxidant by reacting with the heme pigments and preventing the release of free iron as a consequence of exposure to heat and hydrogen peroxide.

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REFERENCES

Asghar, A., Gray, J.I., Buckley, D.J., Pearson, A.M. & Booren, A.M. (1988): Perspectives in warmed-over flavor. Food Technol. 42(6):102.

Buckley, D.J., Gray, J.I., Asghar, A., Price, J.F., Crackel, R.L., Booren, A.M., Pearson, A.M., & Miller, E.R. (1989): Effects of dietary antioxidants and oxidized oil on membranal lipid stability and pork products quality. J. Food Sci. (in press).

Crackel, R.L., Gray, J.I., Pearson, A.M., Booren, A.M. & Buckley, D.J. (1988): Some further observations on the TBA test as an index of lipid oxidation in meats. Food Chem. 28:187.

Gray, J.I. & Pearson, A.M. (1987): Rancidity and warmed-over flavor: Chapter 6. In "Advances in Meat Research. Vol. 3. Restructured Meat and Poultry Products." pp. 221-269. A.M. Pearson & T.R. Dutson (eds). Van Nostrand Reinhold Co., New York.

Harel, S. & Kanner, J. (1985): Muscle membranal lipid peroxidation initiated by hydrogen peroxide-activated metmyoglobin. J. Agric. Food Chem. 33:1186

Liu, R.H., Conboy, J.J. & Hotchkiss, J.H. (1988): Nitrosation by nitro-nitrosite derivatives of olefins: a potential mechanism for N-nitrosamine

formation in fried bacon. J. Agric. Food Chem. 36:984

Marmer, R.J. & Maxwell, R.J. (1981): Dry column method for the quantitative extraction and simultaneous class separation of lipdis from muscle tissue. Lipids 16:365.

Morrissey, P.A. & Tichivangana, J.Z. (1985): The antioxidant activities of nitrite and nitrosylmyoglobin in cooked meats. Meat Sci. 14:157.

Rhee, K.S., Ziprin, Y.A. & Ordonez, G. (1987): Catalysis of lipid oxidation in raw and cooked beef by metmyoglobin-H_2O_2, nonheme iron and enzyme systems. J. Agric. Food Chem. 35:1013.

Ross, H.D., Henion, J., Babish, J.G. & Hotchkiss, J.H. (1987): Nitrosating agents from the reaction between methyl oleate and dinitrogen trioxide: Identification and mutagenicity. Food Chem. 23:207.

Tarladgis, B.G., Watts, B.M., Younathan, M.T. & Dugan, L.R. (1960): A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Amer. Oil Soc. 37:44.

Tichivangana, J.Z. & Morrissey, P.A. (1984): Factors influencing lipid oxidation in heated fish muscle system. Ir. J. Food Sci. Technol. 8:47.

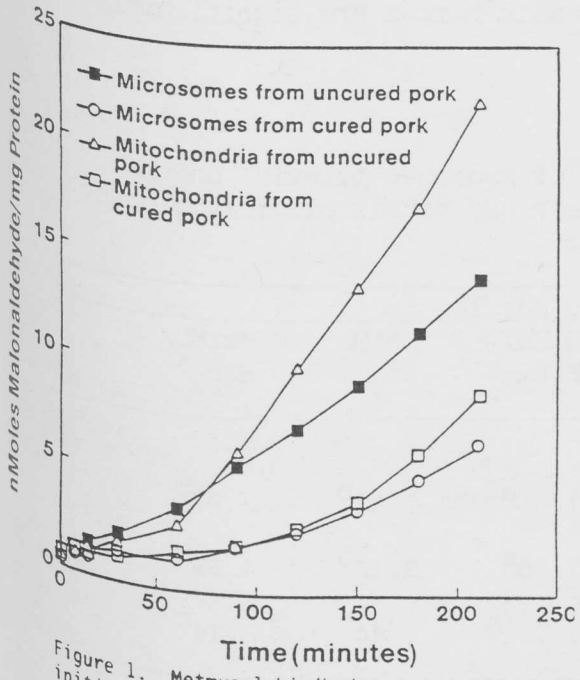

Figure 1. Metmyoglobin/hydrogen peroxide-initiated lipid peroxidation in microsomes and mitochondria from cured and uncured pork

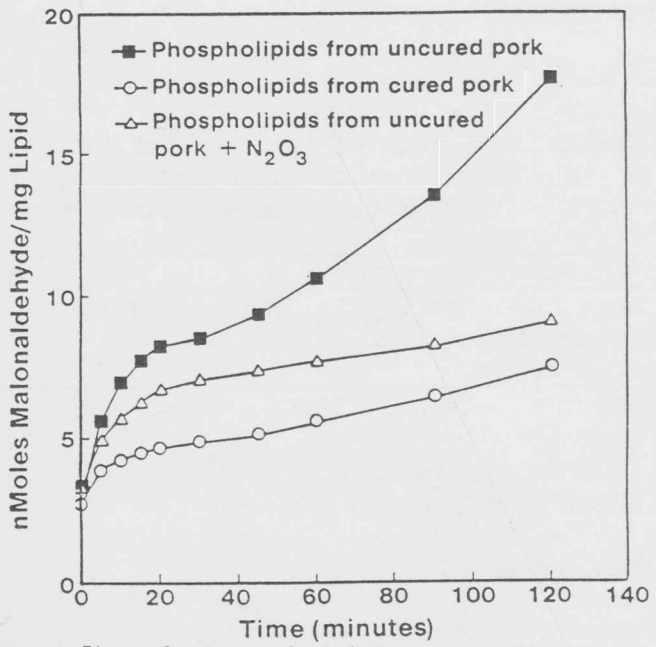

Figure 2. Metmyoglobin/hydrogen peroxide-initiated lipid peroxidation of phospholipids isolated from cured and uncured pork samples

Table 1. Effect of metmyoglobin and nitric oxide myoglobin on the oxidative stability of lipids in water-extracted muscle.

Storage Time, hr	TBA value (mg/malonaldehyde/kg muscle) Control	H_2O_2	NOMb	NOMb/ H_2O_2	MetMb	MetMb/ H_2O_2
Raw						
0	0.20	0.38	0.27	0.22	0.49	0.38
24	0.31	0.35	0.37	0.43	0.71	0.82
48	0.31	0.37	0.38	0.43	0.80	1.11
72	0.32^{a}	0.40^{a}	0.39^{a}	0.45^{a}	0.87^{b}	1.18^{c}
Short term heating						
0	0.32	0.39	0.32	0.32	0.55	0.66
24	0.89	0.88	0.46	0.51	1.25	1.47
48	1.23	0.97	0.56	0.63	1.60	2.02
72	1.48^{c}	1.11^{b}	0.56^{a}	0.74^{a}	1.95^{d}	2.49^{e}
Prolonged heating						
0	0.62	0.81	0.37	0.34	0.63	0.68
24	1.30	1.23	0.59	0.49	1.57	1.66
48	1.41	1.39	0.75	0.79	1.96	2.39
72	1.48^{b}	1.57^{b}	0.90^{a}	0.93^{a}	2.35^{c}	3.01^{d}

Means followed by different superscripts within rows are significantly different at $p<0.05$

Table 2. Effect of heating and addition of hydrogen peroxide on the release of iron from metmyoglobin and nitric oxide myoglobin added to water-extracted muscle

Heat Treatment	µg iron/g muscle Control	H_2O_2	NOMb	NOMb/ H_2O_2	MetMb	MetMb/ H_2O_2
Raw	1.79^{a}	1.45^{a}	1.54^{a}	1.64^{a}	2.02^{b}	3.87^{b}
Short term heating	1.33^{a}	1.54^{a}	1.57^{a}	1.70^{a}	3.12^{b}	4.98^{c}
Prolonged heating	1.36^{a}	1.49^{a}	1.63^{a}	$1.79\pm^{a}$	4.44^{b}	5.14^{c}

Means followed by different superscripts within rows are significantly different at ($p<0.05$)