:2 Mechanisms involved in nitrite inhibition of warmed-over flavor development in cooked meat

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Introduction

Nitrite has been shown to inhibit development of warmed-over flavor (WOF) in cooked meat. The mechanism of inhibition has not been characterized, although a number of suggestions have been made as to some possible factors involved (Pearson, Love and Shorland, 1977). Thus, the present study was designed to elucidate the mechanism(s) by which nitrite prevents WOF in cooked meat.

Materials and Methods

The study was divided into two stages. In the first stage, the reaction of nitrite alone and in association with reducing or non-reducing additives and synergists or chelators of heme pigments was utilized in a ground beef system. In the second stage of the study, meat pigment extracts (MPE) were used instead of the ground meat.

Meat system (stage 1)

Beef muscle (LD and BF) samples weighing about 2.0 kg were thawed overnight and ground twice, first through a plate with 0.91 cm holes and then through a plate with 0.48 cm holes. Each treatment consisted of 50 g meat that was mixed with 50 ml of distilled deionized water, in which the additives were dissolved to give final levels as follows: nitrite-156 mg/kg; L-ascorbate-250 mg/kg; adenosine diphosphate (ADP) - 213.6 mg/kg; sodium tripolyphosphate (TP) - 0.5% (w/w); and ethylenediaminetetraacetic acid (EDTA) - 2% (w/w). The samples containing nitrite were cured for 24 hr at 4°C.

Pigment extract system (stage 2)

Meat pigment extract was prepared by water extraction of ground beef as outlined by Igene et al. (1979). The extract was concentrated using a Virtis II freezedrier. The dried MPE was reconstituted with deionized distilled water to give a concentration of 1.386g of meat equivalent (raw meat) per ml. Muscle fibers (residue) were also prepared as described by Igene et al. (1979). Table 1 presents the abbreviations used in all subsequent tables and the corresponding treatments.

Cooking

All samples were placed in unsealed retortable pouches and heated in a boiling water bath to an internal temperature of 80°C. Storage was at 4°C for 0, 5, 12 and 15 days in stage 1 (meat) and for 0, 7 and 14 days in stage 2 (MPE).

Table 1. Abbreviations for treatments used in all subsequent tables.

	Level of Additives
Ground Meat (M) + H_2O (control)	0
M + Nitrite (N)	156 mg/kg
M + L-ascorbate (A)	250 mg/kg
M + Sodium Tripolyphosphate (TP)	0.50%
M + Adenosine Diphosphate (ADP)	213.6 mg/kg
M + Ethylenediaminetetraacetic Acid (EDTA)	2.0%
or	
Meat pigment extract (MPE) in place of M	(same as above)

TBA test

The distillation method of Tarladgis et al. (1960) was used to measure oxidative rancidity by the TBA test. The nitrite-treated samples required the use of a modified TBA test, in which sulfanilamide was added as described by Zipser and Watts (1962). The malonaldehyde-TBA colored complex was measured in a spectrophotometer at 532 nm, with results being expressed as mg malonaldehyde/kg of meat.

Iron determinations

Total iron was determined in the meat (stage 1) and MPE (stage 2) by the method of Schricker, Miller and Stouffer (1982), while heme and non-heme iron were measured using the procedures described by Igene et al. (1984).

Statistical Analysis

TBA values and non-heme iron levels were subjected to analysis of variance, with Tukey's test for multiple comparisons being used to determine the significance between and within treatments (Steel and Torrie, 1960).

Results and Discussion

Lipid oxidation in ground beef

Table 2 presents the TBA values for the different ground meat treatments. Results indicate that the control (meat + water) had the highest TBA values at all periods of storage. ADP and L-ascorbate also exhibited a progressive increase in the rate and extent of lipid oxidation during storage. All other treatments were essentially the same and exhibited significantly lower TBA values. Lipid oxidation in the control was 4-fold higher than for any of the treatments containing nitrite. Samples containing EDTA and TP also inhibited oxidation, apparently by chelating the metal ions, especially Fe^{+2}.

Table 2. 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,d]

Experimental Treatments	Effect of storage on TBA numbers Days in storage at 4°C			
	0	5	12	21
M	0.58 ± .09^{e}	1.55 ± .22^{f}	2.78 ± .24^{g}	2.83 ± .36^{g}
M + N	0.46 ± .04^{e}	0.48 ± .09^{e}	0.47 ± .03^{e}	0.54 ± .06^{e}
M + A	0.53 ± .15^{e}	0.60 ± .09^{e}	0.45 ± .14^{f}	1.10 ± .18^{f}
M + TP	0.48 ± .06^{e}	0.45 ± .04^{e}	0.45 ± .10^{e}	0.62 ± .13^{e}
M + ADP	0.60 ± .10^{e}	1.08 ± .13^{f}	1.91 ± .04^{g}	2.82 ± .80^{h}
M + EDTA	0.54 ± .12^{e}	0.36 ± .03^{e}	0.49 ± .02^{e}	0.49 ± .06^{e}
M + N + A	0.49 ± .13^{e}	0.40 ± .06^{e}	0.47 ± .09^{e}	0.47 ± .09^{e}
M + N + TP	0.45 ± .04^{e}	0.43 ± .08^{e}	0.44 ± .05^{e}	0.44 ± .05^{e}
M + N + EDTA	0.43 ± .04^{e}	0.37 ± .06^{e}	0.38 ± .03^{e}	0.44 ± .08^{e}
M + N + ADP	0.41 ± .03^{e}	0.41 ± .08^{e}	0.44 ± .05^{e}	0.46 ± .07^{e}
M + N + A + ADP	0.40 ± .05^{e}	0.42 ± .10^{e}	0.41 ± .06^{e}	0.42 ± .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 5% level.

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

d) See Table 1 for meaning of abbreviations.

Results with the ground meat system suggest that myoglobin (Mb) is not the principal prooxidant in meat, but that cooking results in release of Fe^{+2} from Mb, which catalyzes oxidation as proposed by Igene et al. (1979). On the other hand, nitrite appears to stabilize the porphyrin ring and prevents the release of Fe^{+2} during cooking. Further support for this theory comes from the fact that the metal chelators (EDTA and TP) also inhibited oxidation.

Lipid oxidation in MPE

Table 3 presents the results using the MPE, which are somewhat different from the ground meat (Table 2). MPE alone showed a significantly ($P < 0.01$) higher rate and extent of lipid oxidation than all other treatments, except for the sample containing TP, which did not inhibit oxidation (Table 3). The samples containing ADP, muscle residue (2nd control) and ascorbate also suffered from autoxidation. On the other hand, all treatments containing nitrite were protected from oxidation.

Results indicate that both the residual lipids and Fe^{2+} are involved in oxidation. Since the addition of nitrite inhibits oxidation, it appears that nitrite stabilizes the muscle membranes as shown by the fact that the muscle residue without added nitrite underwent some oxidation, although less than was the case for the control sample, which contained the muscle pigments. This agrees with earlier work and suggests that nitric oxide may react with the unsaturated fatty acids in the membranes (Cassens et al., 1976).

Interaction of nitrite with free Fe^{2+}

Table 4 presents the results of a study designed to elucidate the nature of the interaction of nitrite with Mb in the meat system. Results demonstrate that cooking significantly ($P < 0.05$) increased the proportion of non-heme iron in the control but had no effect upon the non-heme iron level in the samples treated with nitrite. Heating appears to catalyze oxidation in the fresh meat by releasing Fe^{+2} from the porphyrin ring (Igene et al., 1979).

Table 5 presents similar information showing the effects of cooking on the levels of Fe^{+2} in MPE. Results show that the level of Fe^{+2} in the control (untreated) samples significantly increased as a result of heating. Samples containing nitrite, ascorbate and ADP had an increased proportion of Fe^{+2} after heating. The sample treated with EDTA also had an increased amount of Fe^{+2} as a result of heating. Thus, EDTA was not effective in the MPE system.

Summary

Results indicate that nitrite may inhibit oxidation in meat during heating in one of three ways: (1) by chelating or sequestering of free metal ions in the meat; (2) by stabilizing the membrane lipids, probably by reacting with unsaturated fatty acids; and (3) by stabilization of the porphyrin ring and preventing its breakdown and consequent release of non-heme iron. Although all three methods appear to be involved, the latter mechanism seems to be the most important.

Table 3. Effect of time in storage and treatments on the mean TBA values of cooked meat pigment extracts[a,b,c,d] (S.D.).

Experimental Treatments	Storage effects on TBA numbers Days in storage at 4°C		
	0	7	14
MPE + H_2O only (control)	0.82 ± .06^{e}	1.17 ± .12^{e}	1.64 ± .07^{f}
MPE + N	0.47 ± .03^{e}	0.29 ± .05^{e}	0.44 ± .03^{e}
MPE + A	0.83 ± .13^{e}	0.78 ± .11^{g}	0.82 ± .06^{e}
MPE + TP	0.85 ± .05^{3}	1.41 ± .06^{f}	1.60 ± .03^{f}
MPE + ADP	0.79 ± .07^{e}	1.10 ± .10^{e}	1.51 ± .08^{f}
MPE + EDTA	0.77 ± .06^{e}	0.58 ± .04^{e}	0.77 ± .05^{e}
MPE + N + A	0.63 ± .06^{e}	0.46 ± .02^{e}	0.66 ± .05^{e}
MPE + N + TP	0.42 ± .03^{e}	0.27 ± .03^{e}	0.48 ± .07^{e}
MPE + N + EDTA	0.43 ± .04^{e}	0.38 ± .02^{e}	0.53 ± .02^{e}
MPE + N + ADP	0.41 ± .02^{e}	0.26 ± .01^{e}	0.54 ± .04^{e}
Muscle residue + H_2O	0.74 ± .05^{e}	0.77 ± .02^{e}	1.14 ± .03^{f}

a) MPE was prepared using beef LD muscle. Experimental treatments were replicated two times.

b) TBA number in the same row bearing the same letter are not significantly different from each other at 0.5% level.

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

d) See Table 1 for meaning of abbreviations.

Table 4. Effect of cooking and treatments on the level of non-heme iron (μgFe/g meat) in beef biceps femoris muscle[a,b,c,d]

Experimental Treatments	Effect of Cooking (Mean ± SD)		Average of Treatments Over time
	Uncooked	Cooked	
M	6.62 ± 1.15^{e}	10.80 ± .18^{g}	8.71 ± .09^{fg}
M + N	6.65 ± .91^{e}	6.80 ± .39^{e}	6.73 ± .08^{e}
M + A	6.52 ± .92^{e}	7.92 ± .81^{e}	7.22 ± .70^{e}
M + TP	7.35 ± .79^{e}	8.45 ± 1.07^{e}	7.90 ± .55^{ef}
M + ADP	6.19 ± 2.15^{e}	8.46 ± 1.52^{f}	7.33 ± 1.15^{ef}
M + EDTA	8.42 ± .91^{e}	8.21 ± .70^{e}	8.32 ± 11^{f}
M + N + A	7.04 ± 1.07^{e}	7.13 ± 1.90^{e}	7.09 ± .05^{ef}
M + N + TP	7.17 ± 1.23^{e}	8.10 ± 1.33^{e}	7.63 ± .47^{ef}
M + N + EDTA	6.94 ± .83^{e}	8.05 ± .93^{e}	7.50 ± .56^{ef}
M + N + ADP	6.96 ± 1.15^{e}	7.44 ± 1.59^{e}	7.20 ± .24^{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 5% level.

d) See Table 1 for meaning of abbreviations.

Table 5. Effect of cooking and treatments on the non-heme iron content (μgFe/g meat) in beef muscle pigment extract[a,b,c,d].

Experimental Treatments	Total iron (μgFe/g meat) in uncooked treatments	Non-heme Iron Content Effect of Cooking (Means ± S.D.)	
		Uncooked	Cooked
MPE only	12.0	2.74 ± .49fghi	3.09 ± .52jk
MPE + N	10.47	2.69 ± 46fgh	2.70 ± .53fgh
MPE + A	10.82	2.67 ± .44fg	3.14 ± .50jk
MPE + TP	9.11	2.77 ± .42fghi	3.10 ± .49jk
MPE + ADP	11.46	2.58 ± .33f	2.91 ± .46ghij
MPE + EDTA	12.00	2.92 ± .42ghij	3.27 ± .51k
MPE + N + A	10.83	2.14 ± .40e	2.57 ± .40f
MPE + N + TP	9.29	2.76 ± .36fghi	3.01 ± .27ijkl
MPE + N + EDTA	9.02	3.03 ± .43ijk	3.21 ± .43jk
MPE + N + ADP	10.10	2.49 ± .31f	2.98 ± .61hij

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

b) Values the same row bearing the same letter are not significantly different from each other at 5% levels.

c) Values in the same column bearing the same letter are not significantly different from each other at 5% levels.

d) See Table 2 for meaning of abbreviations.

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