S-nitrosocysteine (RSNO), an Effective Antioxidant in Cured Meat

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ABSTRACT

S-nitrosocysteine (RSNO), a compound which has been shown to be generated during the curing process of meat, was found to act as an antioxidant. The antioxidative activity of RSNO in an aqueous linoleate model system, in the presence of myoglobin, was compared with that of other known antioxidants, such as BHT and α-tocopherol. The results indicated that RSNO has an antioxidative activity only slightly lower than that of BHT. During the initial stage of the reaction, RSNO acted not only as an inhibitor of linoleic acid oxidation, but also as a hydroperoxide decomposer. The high inhibitory effect of added RSNO (1mM/kg meat) on lipid oxidation in ground cooked turkey meat was demonstrated in the product itself.

INTRODUCTION

Nitrite plays an important role both in color development and as a preservative exerting an anticlostridial effect in cured meat. It is also recognized that the addition of nitrite during the curing process decreases lipid oxidation (1-4). The use of sodium nitrite as a food preservative is under close scrutiny because its presence may result in the formation of N-nitrosoamines, carcinogens in laboratory animals (5).

Recently, it was reported that almost all the added nitrite in cured meat was found as nitrosothiols and nitric-oxide myoglogin, as well as nitrite, and gaseous nitrogen compounds (6). Mirna and Hofmann (7) have reported that meat contains about 20 mM/kg sulfydryl groups which could theoretically react with ten times the quantity of nitrite added for the curing process. A specific interaction between nitrite and the sulfydryl groups of myosin, and the conversion of 20% of the nitrite to nitrosothiols, were demonstrated by Kubberod et al. (8).

It has been pointed out by several investigators (9-11) that RSNO acts as an anticlostridial compound and seems to be one of the few compounds which generates during the curing process and inhibits the growth of *Clostridium botulinum*.

EXPERIMENTAL PROCEDURES

Butylated hydroxytoluene (BHT) and sodium nitrite were purchased from British Drug Houses Ltd., Poole, England, and α-tocopheol, L-cysteine, 2-thiobarbituric acid (TBA), myoglobin (type I) and β-carotene from Sigma Chemical Co., St. Louis, MO. Linoleic acid was obtained from Fluka AG, Buchs, Switzerland.

S-nitrosocysteine was synthesized in an acidic solution of 1N HCl containing 0.7 M of cysteine and sodium nitrite. An inert atmosphere of nitrogen was maintained in the flask during the reaction, which was allowed to proceed for 20 min at room temperature. The RSNO concentration was measured spectrophotometrically using an extinction coefficient of $E_{335} = 800$ (cm^2/M) (7).

The assay for antioxidative activities of RSNO at 25 C was carried out according to the coupled linoleate-carotene bleaching method of Ben-Aziz et al. (12), and by diene-conjugation of linoleate affected by myoglobin in the presence of the antioxidants (13). Both methods were carried out using a Varian 634 recording spectrophotometer.

Lipid oxidation in cooked red turkey meat (2 min at 70 C, and 2 C during storage) was determined by the TBA method (14). It was found that RSNO did not affect the reaction between malonaldehyde generated from 1,1,3,3,-tetraethoxy-propane and TBA.

The color of cooked turkey meat was determined directly by a Gardner Tristimulus Colorimeter, model Xl 10. The instrument was calibrated by a white plate, L = 91.6; a = -1.8; b = +1.8.

RESULTS AND DISCUSSION

The antioxidative activity of RSNO in an aqueous linoleate model system in the presence of myoglobin was compared with that of other known antioxidants, such as BHT, and α-tocopherol, by two different methods (12-13). The results are presented herein. It was found that RSNO has an antioxidative activity only slightly lower than that of BHT (Figs. 1, 2).

Marcuse (15) reported that a number of amino acids, with the exception of cysteine, had a potential antioxidative effect. Cysteine was found to be normally prooxi-

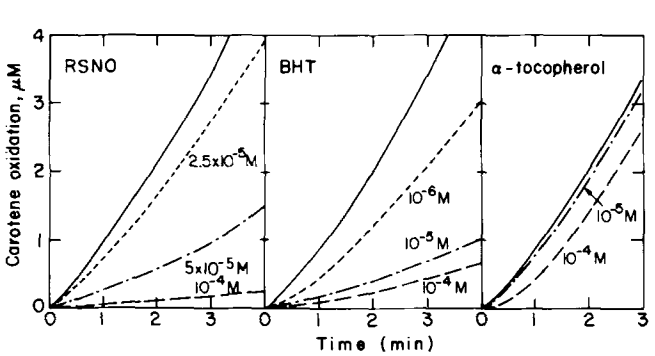

FIG. 1. Carotene oxidation as affected by myoglobin (45 μg/ml) in the presence of RSNO, BHT and α-tocopherol.

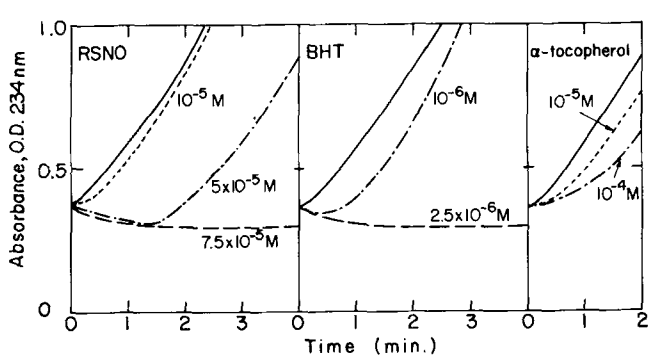

FIG. 2. Diene-conjugation of linoleate as affected by myoglobin (45 μg/ml) in the presence of RSNO, BHT and α-tocopherol.

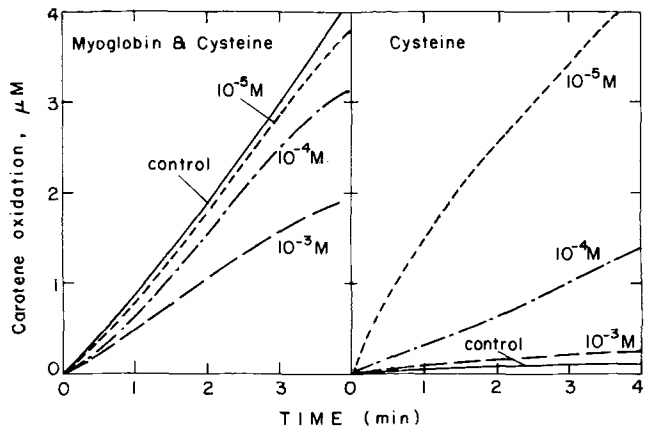

FIG. 3. Carotene oxidation as affected by myoglobin (45 μg/ml) in the presence of cysteine and cysteine alone.

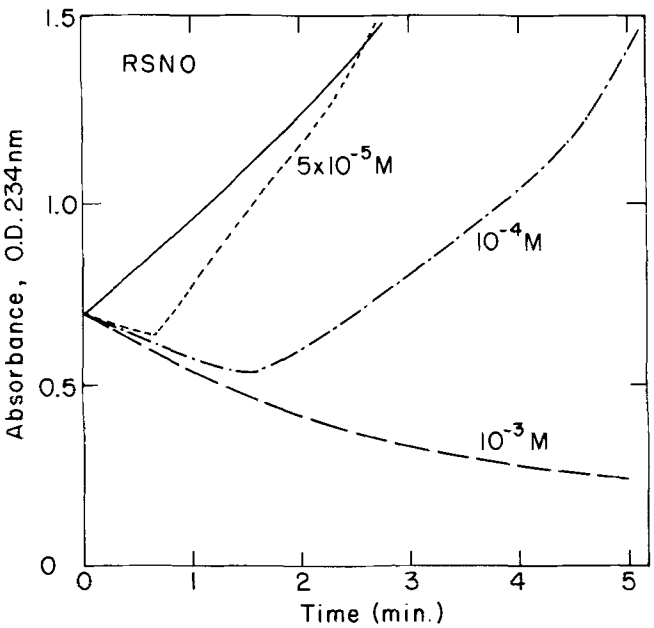

FIG. 4. Diene-conjugation of partially oxidized linoleate as affected by myoglobin (45 μg/ml) in the presence of RSNO.

dative, but it can become antioxidative under certain conditions (16-16). As S-nitrosocysteine is synthesized from cysteine, it was interesting to determine the cysteines activity in the linoleate model system. At low concentration, cysteine alone was found to act as a prooxidant; however, at high concentration in the presence of myoglobin, cysteine acts as an antioxidant (Fig. 3). The inversion activity of cysteine seems to be similar to that of ascorbic acid, as previously shown (18). Nevertheless, compared with cysteine RSNO has a higher inhibitory effect on the activity of myoglobin.

The inhibitory effect of RSNO was found to depend on the initial concentration of the hydroperoxides in the model system. Increasing the hydroperoxide concentration in the system was found to decrease the inhibitory effect of RSNO. During the initial stage of the oxidation reaction, RSNO acted not only as an inhibitor of linoleic acid oxidation, but also as hydroperoxide decomposer. This was evident especially when the concentration of RSNO was raised to 10^{-3}M (Fig. 4).

The dual action of RSNO as a hydroperoxide decomposer and an inhibitor of carotene bleaching may be explained by two types of mechanisms: (a) RSNO is a homolytic decomposer of hydroperoxides to nonradical compounds such as ketones or alcohols; (b) RSNO is a heterolytic decomposer of hydroperoxides to free radicals, but at the same time it reacts with the free radicals generated during the reaction and causes a rapid "termination" of free radicals. In practice many hydroperoxide decomposers act as free radical quenchers (19-20).

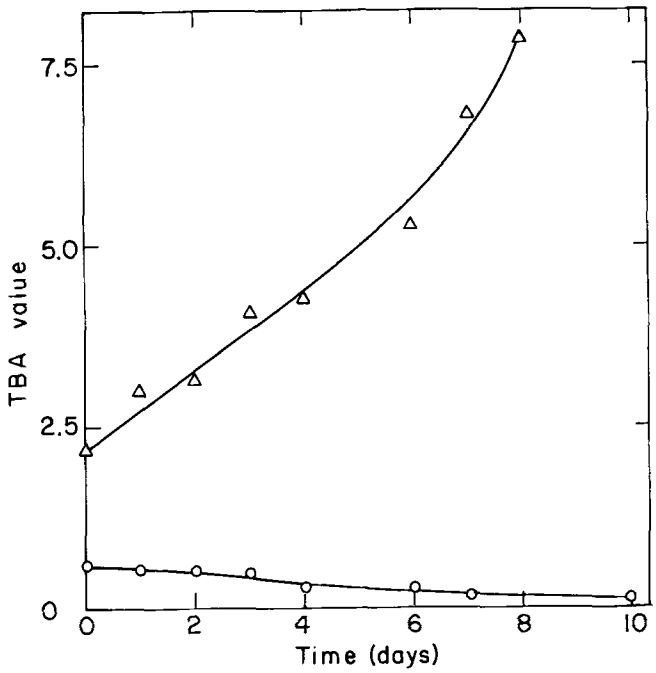

FIG. 5. Lipid oxidation in red turkey meat: △, control; ○, with RSNO (1mM/kg meat).

TABLE I

Color Development in Cured Red Turkey Meat[a]

Treatment	Reflectance L	a	b
Control	60.2	4.7	12.5
RSNO 0.5mM[b]	52.8	10.0	8.7
RSNO 1.0mM	52.3	11.0	8.5
RSNO 2.0mM	52.1	11.8	8.9
Nitrite 2.0mM	52.3	10.4	8.4

[a]Abbreviations: L = lightness; a = redness; b = yellowness.
[b]mM/kg meat.

It has been reported that aromatic and aliphatic amines show an antioxidative activity, presumably due to nitroxide groups formed during lipid oxidation (21-22). The antioxidative activity of RSNO may also be due to the presence of nitroxide group. S-nitrosocysteine also inhibited the prooxidative activity of ferric ions and lipoxygenase in an aqueous linoleate model system.

Nitrite itself has no antioxidative effect and in acidic media acts as a prooxidant; hence the low levels of lipid oxidation which have been observed in cooked cured meat (1-4) may now be partially explained by the antioxidative effect of RSNO generated during the curing process. The effect of added RSNO (1mM/kg meat) on lipid oxidation in ground cooked red turkey meat was investigated. The results (Fig. 5) demonstrate the high inhibitory effect of added RSNO on lipid oxidation in the product itself. It was also found that the addition of RSNO to the meat product led to the development of the typical pink color of cured meat. The color developed by 0.5mM RSNO was very similar to that initiated by 2.0mM nitrite (Table I).

S-nitrosocysteine may possibly replace nitrite in the curing process, since it provides greater control over the curing process and fulfills three functional activities: as an anticlostridial compound, as an antioxidant, and as a color-developing agent. The influence of RSNO on the flavor of the cured product is not known and further research must be done to establish the safety of RSNO for edible products. The possible influence of RSNO on the formation of N-nitrosoamines and other parameters concerning its activity in models and cured products is now being investigated in our laboratory.

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REFERENCES

1. Cross, C.K., and P. Ziegler, J. Food Sci. 30:610 (1965).
2. Bailey, M.E., and J.W. Swaine, "Proc. Meat Ind. Res. Conf.," Chicago, IL 1973. p. 29.
3. Greene, B.E., and L.G. Price, J. Agric. Food Chem. 23:164 (1975).
4. Hadden, J.P., H.W. Okerman, N.A. Cahill, N.A. Parrett, and J.R. Borton, J. Food Sci. 40:626 (1975).
5. Shank, R.C., Toxic. Appl. Pharmacol. 31:361 (1975).
6. Emi-Miwa, M., A. Okitani, and M. Fujumaki, Agric. Biol. Chem. 40:1387 (1976).
7. Mirna, A., and K. Hofmann, Fleishwirtschaft 10:1361 (1969).
8. Kubberod, G., R.C. Cassens, and M.L. Greaser, J. Food Sci. 39:1228 (1974).
9. Van Roon, P.S., in "Proc. Int. Symp. Nitrite in Meat Prod.," Edited by B. Krol and B.J. Tinbergen, Padoc, Zeist, Wageningen, (1973).
10. Ineze, K., J. Farkas, Y. Uihalyi, and E. Zukal, Appl. Microbiol. 27:202 (1974).
11. Moran, D.M., S.R. Tennenbaum, and M.C. Archer, Ibid. 30:838 (1975).
12. Ben-Aziz, A., S. Grossman, I. Ascarelli, and P. Budowski, Anal. Biochem. 34:88 (1970).
13. Ben-Aziz, A., S. Grossman, I. Ascarelli, and P. Budowski, Phytochemistry 10:1445 (1971).
14. Witte, C.V., F.G. Krause, and M. Bailey, J. Food Sci. 35:582 (1970).
15. Marcuse, R., JAOCS 39:97 (1962).
16. Scarborough, D.A., and B.M. Watts, Food Technol. 3:152 (1949).
17. Karel, M., S.S. Tennenbaum, D.H. Wallace, and H. Maloney, J. Food Sci. 31:892 (1966).
18. Kanner, J., H. Mendel, and P. Budowski, Ibid. 42:60 (1977).
19. Uri, N., Israel J. Chem. 8:125 (1970).
20. Hiatt, R.R., Crit. Rev. Food Technol. 7:1 (1975).
21. Van Der Veen, J., T.J. Weil, T.E. Kennedy, and H.S. Olcott, Lipids 5:509 (1970).
22. Lin, J.S., and H.S. Olcott, J. Agric. Food Chem. 23:798 (1975).

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