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Antioxidant activity of preformed cooked cured-meat pigment in a β-carotene/linoleate model system

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Antioxidative efficacy of preformed cooked cured-meat pigment (CCMP, at concentrations of 2.2, 6.2 and 10.0 μM) was investigated in a β-carotene/linoleate model system. Results were compared to those for butylated hydroxyanisole (BHA), metmyoglobin (MMb) and nitrosylmyoglobin (NOMb) at the same concentrations of 2.2, 6.2 and 10.0 μM. CCMP at a concentration of 2.2 μM exhibited a pro-oxidant effect, but at 6.2 and 10 μM it exhibited a significant ($P < 0.05$) antioxidant activity. The antioxidant effect of CCMP (at 6.2 and 10 μM) was less than that of BHA but more pronounced than that of NOMb at the same concentration. MMb exhibited a pro-oxidant effect at 2.2, 6.2 and 10.0 μM concentrations. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Preservation of meat by salt curing has been known to man since antiquity. Rock salt, which contains nitrate impurities, and smoke were used by the Greeks to preserve meats, and these techniques were later passed on to the Romans. Nowadays, a wide selection of cured-meat products is available (Pierson & Smoot, 1982). Although a variety of compounds may be added to the cure, standard meat-curing ingredients include sodium nitrite, sodium chloride, sugars, ascorbates, polyphosphates and spices (Shahidi, 1991).

Although it is added to meat in very minimal quantities, sodium nitrite is the key ingredient because it imparts multiple functional properties to cured products. For example, when added in combination with sodium chloride, sodium nitrite inhibits spore germination of *Clostridium botulinum*, it develops a cured-meat flavour and colour, and prevents warmed-over flavour development by suppressing lipid auto-oxidation (Hadden *et al.*, 1975; Shahidi *et al.*, 1985; Shahidi & Pegg, 1992). Although sodium nitrite and its possible precursor sodium nitrate have been used to cure meat for centuries, their addition to meat is a concern because of potential adverse health effects (Buege *et al.*, 1980). Reaction of nitrite with free amino acids in meat, followed by decarboxylation, or directly with amines in meat or spices and amines in the gastric fluid can result in the formation of *N*-nitrosamines (Gray & Randall, 1979; Shahidi & Pegg, 1990). Many of these compounds have been shown to exhibit mutagenic, embryopathic and/or teratogenic properties in experimental animals (Walters, 1980; Shahidi *et al.*, 1985, 1987; Vösgen, 1992). Shahidi & Pegg (1992) noted that the most reliable means of overcoming the problem of *N*-nitrosamine formation in cured meat is the total elimination of nitrite from the curing process. However, it is unlikely that a single compound will be found that can perform all the functions of sodium nitrite. Therefore, any alternative meat-curing system must contain a colorant, an antioxidant/sequestrant and an antimicrobial agent (Shahidi *et al.*, 1988; Shahidi & Pegg, 1992).

Substitutes for sodium nitrite in producing the desired colour have been reported. Shahidi *et al.* (1984) were able to synthesize the natural cooked cured-meat pigment (CCMP) from haemin, an iron(III) protoporphyrin recovered from bovine red blood cells, and sodium nitrite. Shahidi *et al.* (1985) later demonstrated a novel method for synthesis of CCMP using haemin and nitric oxide (NO) as the nitrosating agent. They noted that CCMP so prepared had a purity of greater than 97%, and the pigment was capable of imparting a characteristic cured colour to meat.

The present study was carried out to investigate the effects of preformed CCMP on the oxidative stability of lipids in a β-carotene/linoleate model system.

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MATERIALS AND METHODS

Fresh pork shoulder meat was procured from a local supermarket, and most of its surface fat was trimmed off. The meat was ground twice with a meat grinder (Omega, Type T12) using a 0.79 and then a 0.48 cm plate. Ground pork was stored at −60°C until used.

Food-grade sodium tripolyphosphate (STPP) was obtained from Albright and Wilsons Americas (a Division of Tenneco Canada Inc., Toronto, ON). Ethylenediaminetetraacetic acid (EDTA), sodium ascorbate, myoglobin, β-carotene, Tween 80 and butylated hydroxyanisole (BHA) were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium nitrite, sodium carbonate, haemin and linoleic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ethanol and chloroform used in this study were ACS grade. Nitric oxide and nitrogen were obtained from Canadian Liquid Air Ltd. (St. John's, NF).

CCMP was prepared according to the method of Shahidi *et al.* (1994). Nitrosylmyoglobin (NOMb) was produced according to the method of Fox and Thomson (1963), and the buffered β-carotene/linoleate model system was prepared as explained by Ben-Aziz *et al.* (1971). The β-carotene/linoleate assay was carried out at room temperature (22 ± 2°C). Absorption measurements were made in a 1 cm borosilicate cuvette using a photodiode array spectrophotometer (Model 8452A; Hewlett Packard, Mississauga, ON). Buffered β-carotene/linoleate system (1.5 ml) and an appropriate volume of the pigment (in solution) to be examined were transferred to the cuvette and the volume was then adjusted to 2 ml with deionized water. The concentrations of β-carotene, linoleic acid, EDTA and Tween 80 in the assay medium were 25 μg, 562.5 μg, 375 μg and 0.5 μl ml^{-1}, respectively. The absorbance was measured at 460 nm in 1 min intervals up to 5 min. A blank sample, containing all reactants except β-carotene, was measured for background subtraction. Deionized water (0.5 ml) was used in the control sample. The amount of β-carotene bleached (in μg) was calculated using a conversion factor obtained from a standard line.

The CCMP, NOMb and metmyoglobin (MMb) were tested for their effects on β-carotene bleaching at 2.2, 6.2 and 10 μM levels. Sodium ascorbate (SA) was tested at levels of 50, 100 and 550 ppm and STPP was tested at 50, 100 and 500 ppm levels, in both the presence and absence of CCMP (10 μM). The lower amount of STPP used (compared to the 3000 ppm application limit) was due to solubility limitations. BHA was used for comparative purposes.

Analysis of variance and Tukey's studentized range test (Snedecor & Cochrane, 1980) were performed to determine differences in mean values based on data collected from triplicate experiments. Significance was determined at a 95% level of probability.

RESULTS AND DISCUSSION

The antioxidant efficacy of CCMP at the three levels tested, in the presence or absence of STPP and/or SA was assessed. Data obtained were compared to those of systems containing NOMb, MMb or BHA. The method of Ben-Aziz *et al.* (1971) was chosen because of its simplicity, convenience and adaptability for use of small sample volumes. In this procedure, linoleic acid was allowed to oxidize in the presence of haem pigments, SA, STPP and β-carotene. As autoxidation proceeds, linoleic acid is oxidized to form free radicals. The free radicals so formed oxidize β-carotene molecules and subsequently β-carotene loses its characteristic yellow colour. Compounds that possess the ability to inactivate free radicals will hinder the progression of free radical chain reactions, thus much of the β-carotene in the reaction mixture will remain intact. On the other hand, compounds that are capable of accelerating free radical formation will result in a high β-carotene bleaching. Therefore, the pro- or antioxidative nature of substances can be evaluated by monitoring the β-carotene bleaching in a β-carotene/linoleate model system. Ben-Aziz *et al.* (1971) reported that the reduction in absorbance values (at 460 nm) of β-carotene/linoleate systems containing haem pigments over time is due to the bleaching of β-carotene. The antioxidant activity as reflected in the ability of each compound (or system) to inhibit bleaching of β-carotene was measured and compared with that of the control containing no antioxidant.

The antioxidant activity of CCMP, NOMb, MMb and BHA in a β-carotene/linoleate model system is shown in Table 1. The behaviour of CCMP in a β-carotene/linoleate model system changed from pro-oxidative to antioxidative, depending on the concentration. Therefore, the critical concentration of CCMP that determines its pro- or antioxidant activity in a β-carotene/linoleate model system lies within the concentration range tested in this study. Kanner *et al.* (1979) made a similar observation for NOMb. At 2.2 μM concentration, the ability of CCMP to quench free radicals may be inadequate to inhibit lipid autoxidation. However, CCMP at 6.2 and 10 μM showed a strong antioxidative effect. As in the case of NOMb, CCMP has its iron atom in the ferrous oxidation state. The high stability of CCMP does not permit the release of iron(II) and its subsequent oxidation to iron(III) ions which can act as powerful pro-oxidants. According to Kanner *et al.* (1979), iron(II) porphyrin nitric oxide compounds can act in the early stages of the reaction to neutralize substrate free radicals and thereby inhibit lipid oxidation. They also suggested that the NO group might interact with free radicals leaving iron porphyrin in the system. In the case of CCMP, the NO group may quench free radicals in the medium. On the other hand, haemin, an iron(III) protoporphyrin, may be generated upon dissociation and oxidation of CCMP

Table 1. Effect of haem pigments and BHA on β-carotene bleaching in a β-carotene/linoleate model system as reflected by cumulative loss of β-carotene (μg)

Compound	Reaction time (min)			
	1	2	3	4
Control (H_2O)	1.41 ± 0.02^{d}	1.80 ± 0.07^{de}	2.08 ± 0.11^{d}	2.41 ± 0.20^{d}
Concentration 2.2 μM				
BHA	0.20 ± 0.05^{fg}	0.27 ± 0.10^{f}	0.30 ± 0.07^{f}	0.34 ± 0.03^{h}
MMb	9.18 ± 0.07^{a}	10.71 ± 0.86^{a}	11.28 ± 0.90^{a}	11.54 ± 0.65^{a}
NOMb	1.09 ± 0.18^{de}	1.51 ± 0.13^{e}	1.82 ± 0.25^{de}	1.99 ± 0.35^{de}
CCMP	5.68 ± 0.45^{b}	7.05 ± 0.38^{b}	7.85 ± 0.35^{b}	8.40 ± 0.34^{b}
Concentration 6.2 μM				
BHA	0.18 ± 0.07^{fg}	0.25 ± 0.11^{f}	0.31 ± 0.10^{f}	0.36 ± 0.10^{h}
MMb	6.16 ± 0.64^{b}	7.35 ± 0.78^{b}	7.92 ± 0.80^{b}	8.20 ± 0.79^{b}
NOMb	0.82 ± 0.01^{def}	1.17 ± 0.13^{ef}	1.36 ± 0.11^{def}	1.49 ± 0.10^{defg}
CCMP	0.45 ± 0.08^{efg}	0.49 ± 0.01^{f}	0.88 ± 0.11^{ef}	1.02 ± 0.07^{efgh}
Concentration 10.0 μM				
BHA	0.11 ± 0.01^{g}	0.21 ± 0.02^{f}	0.31 ± 0.10^{f}	0.35 ± 0.08^{h}
MMb	4.74 ± 0.12^{c}	5.96 ± 0.11^{c}	6.54 ± 0.11^{c}	6.52 ± 0.35^{c}
NOMb	0.69 ± 0.14^{efg}	1.10 ± 0.32^{ef}	1.35 ± 0.24^{def}	1.52 ± 0.28^{def}
CCMP	0.41 ± 0.04^{efg}	0.49 ± 0.01^{f}	0.54 ± 0.03^{f}	0.57 ± 0.06^{fgh}

Results are mean values of three determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($P > 0.05$) different from one another.
BHA, butylated hydroxyanisole; MMb, metmyoglobin; NOMb, nitrosylmyoglobin; CCMP, cooked cured-meat pigment.

and act as a hydroperoxide decomposer. Haemin has also been shown to act as a free radical quencher at low concentrations. Thus haemin is able to act either as an initiator or a terminator in the oxidation process in the β-carotene/linoleate model system with the latter overshadowing the degree of initiation.

MMb showed a pro-oxidant effect for all concentrations tested. The pro-oxidant activity decreased with increasing concentration of MMb (Table 1). The result is in agreement with that reported by Hirano and Olcott (1971). These authors have observed a reduction in oxygen uptake by linoleate emulsions, which was monitored using a polarographic oxygen analyser, with increasing concentrations of MMb. They have reported that oxygen uptake by a linoleate emulsion containing 10^{-5} M MMb was lower than that of a linoleate emulsion containing 10^{-6} M or 10^{-7} M MMb. Furthermore, they suggested that the positive catalytic effect of low levels of MMb on linoleate emulsions may depend primarily upon their ability to decompose peroxides with the formation of chain-initiating free radicals. When present in large amounts, porphyrin compounds may become free radical scavengers and possibly interfere with the autoxidation reactions.

Table 2 shows the effect of CCMP (at 10 μM) on β-carotene bleaching in the presence of SA at 50, 100 and 550 ppm levels. The CCMP exhibited a stronger antioxidative effect in the presence of SA than in its absence. CCMP and SA may have acted synergistically to protect β-carotene against oxidation. Shahidi *et al.* (1987) suggested that SA and CCMP may retard lipid oxidation, most probably by keeping the haem pigment in its catalytically inactive state. Sato and Hegarty (1971) and Pearson *et al.* (1977) envisaged that SA could upset the balance between ferrous and ferric ions or that it could act as an oxygen scavenger.

The effect of CCMP (10 μM) on β-carotene bleaching in a β-carotene/linoleate model system containing STPP at 50, 100 and 500 ppm is shown in Table 2. The presence of STPP at 100 and 500 ppm in the assay medium had a counteracting effect on the antioxidative activity of CCMP. Moreover, the antioxidative effect of CCMP decreased with increasing concentration of STPP. According to Tims and Watts (1958), STPP may inhibit oxidation in meat model systems by sequestering metal ions, especially non-haem irons. However, in the present study iron(II) of CCMP might oxidize to iron(III) due to pH effects brought about by STPP addition. Since ferric haem ions are more powerful catalysts (Kanner & Harel, 1985), they may favour substrate free radical decomposition causing an increase in new free radical generation and leading to enhanced β-carotene destruction.

At a 500 ppm concentration, STPP alone showed a significant ($P < 0.05$) pro-oxidative effect while at 50 and 100 ppm it exhibited an antioxidative effect (Table 2). At low concentrations, STPP may inhibit lipid oxidation by sequestering metal ions and at high concentrations may alter the pH buffering capacity of the reaction mixture to a point where free radical chain reactions are favoured, causing a high β-carotene destruction exceeding its sequestering effect.

As shown in Table 2, CCMP in the presence of both SA and STPP, exhibited an antioxidative effect and the effect was not significantly ($P > 0.05$) different from that of the CCMP alone. This effect may be attributed to the antioxidative nature of CCMP backed up by SA, but possibly counteracted by STPP.

Table 2. Effect of cooked cured-meat pigment (CCMP), sodium ascorbate (SA), sodium tripolyphosphate (STPP) and their combinations on β-carotene destruction in a β-carotene/linoleate model system as reflected by cumulative loss of β-carotene (μg)

Experiment no.	Treatment	Reaction time (min)			
		1	2	3	4
1	Control (H_2O)	1.41 ± 0.02^{a}	1.80 ± 0.07^{a}	2.08 ± 0.11^{a}	2.41 ± 0.20^{b}
2	CCMP 10.0 μM	0.41 ± 0.04^{cdef}	0.49 ± 0.01^{defg}	0.54 ± 0.03^{fg}	0.57 ± 0.06^{fg}
3	SA 50 ppm	0.39 ± 0.08^{cdefg}	0.51 ± 0.00^{cdef}	0.59 ± 0.04^{efg}	0.67 ± 0.06^{def}
4	SA 100 ppm	0.30 ± 0.04^{defg}	0.39 ± 0.04^{fgh}	0.48 ± 0.01^{fgh}	0.56 ± 0.02^{fg}
5	SA 550 ppm	0.27 ± 0.09^{efg}	0.33 ± 0.07^{h}	0.37 ± 0.11^{h}	0.39 ± 0.08^{g}
6	STPP 50 ppm	0.39 ± 0.08^{cdefg}	0.62 ± 0.01^{bcd}	0.86 ± 0.04^{bc}	1.09 ± 0.01^{c}
7	STPP 100 ppm	0.51 ± 0.07^{bcd}	0.77 ± 0.04^{b}	1.01 ± 0.01^{b}	1.20 ± 0.01^{c}
8	STPP 500 ppm	1.25 ± 0.20^{a}	1.78 ± 0.09^{a}	2.19 ± 0.08^{a}	2.72 ± 0.16^{a}
9	2+3	0.19 ± 0.02^{g}	0.28 ± 0.05^{h}	0.36 ± 0.04^{h}	0.41 ± 0.03^{g}
10	2+4	0.27 ± 0.05^{efg}	0.35 ± 0.04^{gh}	0.43 ± 0.03^{gh}	0.48 ± 0.01^{fg}
11	2+5	0.27 ± 0.06^{efg}	0.36 ± 0.08^{gh}	0.47 ± 0.02^{fgh}	0.53 ± 0.02^{fg}
12	2+6	0.40 ± 0.04^{cdefg}	0.52 ± 0.06^{cdef}	0.60 ± 0.07^{efg}	0.65 ± 0.03^{def}
13	2+7	0.55 ± 0.03^{bc}	0.65 ± 0.03^{bc}	0.76 ± 0.05^{de}	0.80 ± 0.03^{de}
14	2+8	0.64 ± 0.02^{b}	0.77 ± 0.05^{b}	0.82 ± 0.06^{cd}	0.86 ± 0.02^{d}
15	2+3+6	0.31 ± 0.10^{defg}	0.41 ± 0.06^{efgh}	0.47 ± 0.04^{fgh}	0.50 ± 0.06^{fg}
16	2+4+7	0.36 ± 0.05^{cdefg}	0.51 ± 0.04^{cdef}	0.59 ± 0.04^{efg}	0.60 ± 0.07^{efg}
17	2+5+8	0.47 ± 0.04^{bcde}	0.56 ± 0.03^{cde}	0.63 ± 0.08^{ef}	0.70 ± 0.03^{def}

Results are mean values of three determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($P > 0.05$) different from one another.

CONCLUSIONS

The CCMP exhibited a concentration-dependent antioxidant effect in a β-carotene/linoleate model system. The antioxidant properties of CCMP might arise from the ability of its NO group to quench free radicals or the capacity of the molecule to neutralize substrate-free radicals in the early stages of autoxidation. The presence of SA enhanced the antioxidant properties of CCMP, perhaps by upsetting the balance between ferrous and ferric ions in the system or by acting as an oxygen scavenger. The presence of STPP had a countereffect on the antioxidant properties of CCMP, perhaps due to pH effects in conversion of iron(II) of the CCMP to iron(III). Iron(III), in turn, might favour substrate-free radical decomposition.

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