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Role of Metal Ions and Heme Pigments in Autoxidation of Heat-Processed Meat Products

Fereidoon Shahidi & Caroline Hong

Department of Biochemistry, Memorial University of Newfoundland,
St. John's, Newfoundland, Canada A1B 3X9

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ABSTRACT

The effect of myoglobin (Mb), hemoglobin (Hb), hemin (Hm) and the preformed cooked cured-meat pigment (CCMP), as well as Fe(II), Fe(III), Cu(I) and Cu(II) on the oxidative stability of cooked comminuted pork was studied over a 21 day storage at 4°C. All these compounds, except CCMP, had pro-oxidant activity. The pro-oxidant effect of metal ions was more pronounced at their lower oxidation state, as measured by the 2-thiobarbituric acid (TBA) test. Addition of chelators, disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA*), and to a lesser extent sodium tripolyphosphate (STPP), inhibited the pro-oxidant activity of the above compounds, Addition of metal ions to the meats after cooking similarly enhanced the autoxidation of lipids in the above systems, thus reflecting the importance of processing conditions and equipment wear-out on the oxidative state of precooked meat products.*

INTRODUCTION

The deleterious effects of oxidation in cooked meat products are numerous. Oxidative reactions are responsible for changes in colour, flavour, texture and nutritional value, due to the destruction of vitamins A, D and E and essential fatty acids (e.g. linoleic acid), in both fresh and cooked muscles (Dziezak, 1986). Lipids in cooked meats are particularly susceptible to autoxidation reactions. These reactions are generally catalyzed by a number of factors such as oxygen, light, heat, heavy metals, pigments and alkaline conditions, as well as the degree of unsaturation.

Although heme pigments are generally known for their pro-oxidant activity (Robinson, 1924; Younathan & Watts, 1959; Tappel, 1962; Sato & Hegarty, 1971; Love & Pearson, 1974; Pearson *et al.*, 1977; Igene *et al.*, 1979; Shahidi *et al.*, 1988; Johns *et al.*, 1989), they have also been reported as having antioxidant properties (Kanner *et al.*, 1980; 1984; Shahidi *et al.*, 1987; Shahidi, 1989). The catalytic activity of heme compounds on deterioration of meat lipids arises, at least in part, from their decomposition upon cooking and liberation of free iron. Processing equipment is also a source of contamination from metals such as iron and copper. These may in turn accelerate the autoxidation of meat products, thus leading to the development of warmed-over flavour and rancidity in cooked meats upon storage. Morrissey and Tichivangana (1985) and Tichivangana and Morrissey (1985) reported that ferrous ion at 1–10 ppm levels acted as a strong pro-oxidant in cooked fish muscles. Similarly copper (II) and cobalt (II), at the same levels, had stronger catalytic effects than metmyoglobin. These observations are in agreement with the findings of Igene *et al.* (1979) who also reported that non-heme iron was the major catalyst responsible for autoxidation enhancement in muscle foods.

This study was undertaken to examine the effect of a number of iron-porphyrin compounds as well as iron and copper ions on the oxidative stability of cooked comminuted meats. The effect of addition of these compounds, immediately after cooking, to meat systems was also investigated. Addition of chelators, namely ethylenediaminetetraacetic acid (ETDA) and sodium tripolyphosphate (STPP) to meat systems containing metal ions or iron-porphyrin compounds, prior to heat processing, was also examined.

MATERIALS AND METHODS

All chemicals used in this study were reagent- or food-grade and were used without any further purification. Deionized, distilled water was used in all experiments.

Post-rigor pork loin was obtained from Newfoundland Farm Products, St. John's, Newfoundland. It was prepared by removing most of its exterior fat and was then ground twice using 7.9 mm and 4.8 mm grind plates, respectively. The fat content of the resultant meat was 10·2 ± 0·2% (AOAC, 1980).

Meat slurries were prepared by addition of 20% (w/w) water and additives, at levels given in the tables, to comminuted pork prior to heat processing to an internal temperature at 75 ± 1°C, unless otherwise specified.

They were then homogenized and transferred into Whirl-Pack plastic bags and were stored in a refrigerator at 4°C, until used.

The thiobarbituric acid reactive substances (TBARS) in the above meat systems, as a function of storage time at 4°C, were quantified after distillation of meat samples dispersed in acidified water, using the 2-thiobarbituric acid (TBA) reagent and the method of Tarladgis *et al.* (1960) and described previously (Shahidi *et al.*, 1987). The absorbance of TBARS-TBA coloured complex was read at its maximum intensity at 532 nm using a Beckman DU-8 spectrophotometer. The compound 1,1,3,3-tetramethoxypropane was used as a standard, affording a value of 8·1 for the conversion of the absorbance readings to the so-called TBA numbers (mg malonaldehyde equivalents per kilogram sample, i.e. ppm).

In another set of experiments, addition of metal ions or iron-porphyrin compounds was done immediately after the heat processing of meat samples. The oxidative state of cooked meats was evaluated as described above.

RESULTS AND DISCUSSION

The TBA numbers of meat samples cooked with 30 ppm metal ions, as their chloride salts, namely Fe(II), Fe(III), Cu(I) and Cu(II), as well as iron-porphyrin compounds, namely hemin (Hm), myoglobin (Mb), hemoglobin (Hb) and the preformed cooked cured-meat pigment (CCMP) are given in Table 1. The pro-oxidant activity of iron ions was greater than that for copper ions. Presence of vacant 3*d* orbitals in iron may be responsible for this difference. Furthermore, the potency of metal ions as pro-oxidants was more pronounced in their lower oxidation states (Table 1). Better binding of oxygen to these metal ions at their lower valence state may be responsible for their increased activity in autoxidation of meat lipids, despite the fact that metals are generally more effective free radical initiators in their higher oxidation state (Love, 1985). These results are in general agreement with those of Ellis *et al.* (1971) and Tichivangana and Morrissey (1985) who reported that ferrous ions at low concentrations are more active than ferric ions. Our results are also in agreement with those of Morrissey and Tichivangana (1985) who reported a higher pro-oxidant activity for ferrous as compared to that of cupric ions.

Addition of ferrous and ferric ions, as their chloride salts, to freshly cooked meats resulted in a similar increase in the TBA values of the samples (Table 2). Therefore, breakdown of the hydroperoxides by metal ions is enhanced regardless of whether they are present in meats prior to

TABLE 1

TBA Numbers of Cooked Comminuted Pork as affected by Metal Ions and Iron-Porphyrin Compounds[a]

Treatment number	*Additive(s)*	*Storage period (days)*			
		1	*7*	*14*	*21*
1	Control (No additive)	3·15	9·40	10·81	12·70
2	Cu(II)	3·20	9·92	12·45	12·80
3	Cu(I)	3·55	11·15	13·12	14·10
4	Fe(III)	4·60	11·14	14·38	16·10
5	Fe(II)	4·80	12·10	15·52	20·40
5	Hemin	4·35	11·65	14·90	14·85
6	Myoglobin	4·20	12·70	15·40	16·25
7	Hemoglobin	4·33	13·50	16·04	14·68
8	CCMP (12 ppm)	0·42	7·53	7·50	8·20
9	8 + SA (550 ppm)	0·42	1·53	2·20	3·25

[a] All additives were added to meats at 30 ppm levels, unless otherwise specified. Metal ions were used as their chloride salts. CCMP: preformed cooked cured-meat pigment; SA: sodium ascorbate.

cooking or are added to them after heat processing, Thus, contamination of meats with metal ions from cookware and processing equipment is of utmost importance in the stability of precooked meat products.

Presence of iron-porphyrin compounds namely hemin, myoglobin, hemoglobin and the preformed cooked cured-meat pigment (CCMP) enhanced the autoxidation of meat lipids and the formation of warmed-over flavour as reflected in the TBA values (Table 1). Again, heme pigments containing a Fe(II) atom were found to exert a more pronounced

TABLE 2

Effect of Addition of Iron Ions and Iron-Porphyrins to Freshly Cooked Comminuted Pork[a] (results given as TBA values)

Treatment number	*Additive(s)*	*Storage period (days)*			
		1	*7*	*14*	*21*
1	Control (No additive)	3·15	9·40	10·81	12·70
2	Fe(III)	4·82	10·89	14·52	15·25
3	Fe(II)	4·98	10·85	15·92	18·78
4	Hemin	3·86	9·72	14·28	14·90
5	Myoglobin	4·01	12·15	13·56	12·98

[a] Additives were included in freshly cooked meats at 30 ppm levels and were thoroughly homogenized.

pro-oxidant activity than hemin chloride. However, the cooked cured-meat pigment (CCMP) acted as an antioxidant in meats and gave reduced TBA values (Table 1). Furthermore, a synergistic effect was observed when CCMP together with sodium ascorbate was applied to meat (Table 1). Kanner *et al.* (1980, 1984) observed a similar antioxidant activity for nitrosyl myoglobin. Addition of myoglobin or hemin or freshly cooked meats had a similar effect in enhancing the autoxidation of the samples (Table 2). Thus, the release of iron from heme pigments in meats during the cooking process may not be the only mechanism by which iron-porphyrin compounds exert their pro-oxidant effects.

Addition of chelators, namely disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) or sodium tripolyphosphate (STPP), to meats containing metal ions or iron-porphyrin compounds resulted in a substantial decrease in the TBA values of the samples (Tables 3 and 4). The protection of meats against oxidation by Na_2EDTA (Table 3) was greater than that observed by STPP (Table 4). This is consistent with the relative chelating ability of these compounds. Furthermore, it is interesting to note that the pro-oxidant activity of iron-porphyrin compounds was considerably reduced when sequestering agents were present. While the presence of EDTA has the advantage of causing a substantial delay in the outgrowth of *C. botulinum* (Tompkin *et al.*, 1978), STPP improves the water-binding and texture properties of cooked meat products. These results are in good agreement with our previous observations (Shahidi *et*

TABLE 3

Effects of Na_2EDTA on the 2-Thiobarbituric Acid, TBA, Numbers of Cooked Comminuted Pork containing Selected Additives[a]

Treatment number	*Additive(s)*	*Storage period (days)*			
		1	*7*	*14*	*21*
1	Control (No additives)	2·90	4·01	10·94	12·95
2	1 + Na_2EDTA (500 ppm)	0·07	0·08	0·15	0·20
3	2 + Cu(II)	0·31	0.37	1·62	2·00
4	2 + Cu(I)	0·35	0·41	1·85	2·40
5	2 + Fe(III)	0·08	0·39	1·62	2·31
6	2 + Fe(II)	0·09	0·49	2·00	3·30
7	2 + Hemin	0·07	0·06	0·42	0·48
8	2 + Myoglobin	0·07	0·09	0·38	1·39
9	2 + Hemoglobin	0·10	0·10	0·24	0·91
10	2 + CCMP (12 ppm)	0·30	0·11	0·36	0·68

[a] All additives were added to meats at 30 ppm levels, unless otherwise specified. Metal ions were used as their chloride salts. CCMP: cooked cured-meat pigment.

TABLE 4
Effect of STPP on the 2-Thiobarbituric Acid, TBA, Numbers of Cooked Comminuted Pork containing Selected Additives[a]

Treatment number	*Additive(s)*	*Storage period (days)*			
		1	*7*	*14*	*21*
1	Control (No additive)	2·95	6·69	13·50	22·58
2	1 + STPP (3000 ppm)	0·22	0·96	1·21	2·38
3	2 + Cu(II)	0·38	0·80	0·98	3·13
4	2 + Cu(I)	0·32	0·70	0·98	3·25
5	2 + Fe(III)	0·37	1·40	2·16	4·65
6	2 + Fe(II)	0·27	0·80	3·68	5·50
7	2 + Hemin	0·33	0·68	0·54	1·03
8	2 + Myoglobin	0·28	0·67	0·76	2·14
9	2 + Hemoglobin	0·30	0·88	0·81	2·18
10	2 + CCMP (12 ppm)	0·30	0·54	0·85	1·89

[a] All additives were included in the meats at 30 ppm levels. Metal ions were used as their chloride salts. CCMP: cooked cured-meat pigment.

al., 1987) and those reported by other researchers (Watts, 1950; Tims & Watts, 1958; Igene *et al.*, 1985).

In conclusion, both metal ions and iron-porphyrin compounds were found to have a potent pro-oxidant effect in cooked meats, except for the cooked cured-meat pigment (CCMP) which had a weak antioxidant effect. The CCMP also acted synergistically with sodium ascorbate. Autoxidation of meat lipids was enhanced to a greater extent by metal ions and heme pigments at their lower valence states. Chelating agents, EDTA and to a lesser extent STPP, effectively reduced the pro-oxidant activity of the above compounds.

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