Catalysis of Lipid Oxidation in Raw and Cooked Beef by Metmyoglobin–H_2O_2, Nonheme Iron, and Enzyme Systems

Ki Soon Rhee,* Yolanda A. Ziprin, and Gloria Ordonez

The effects of addition of metmyoglobin (MetMb)–H_2O_2, nonheme iron (Fe^{2+}), and the components of microsomal (or mitochondrial) enzymic lipid peroxidation system on lipid oxidation in water-extracted beef muscle residues stored at 4 °C were studied. Metmyoglobin–H_2O_2 at molar ratios ranging from 1:0.1 to 1:2 (0.26 mM MetMb with 0.026–0.52 mM H_2O_2) catalyzed the oxidation of beef muscle lipids in both raw and cooked systems. Metmyoglobin alone had little or extremely low catalytic activity. The catalytic activity of MetMb–H_2O_2 was highest at the molar ratio of approximately 1:0.25 in the raw residue system and at the molar ratio of 1:1.5 or 1:2 in the cooked system. The catalytic effect of MetMb–H_2O_2 was ascribed to "activated MetMb" and the nonheme iron released from MetMb. Activated MetMb, nonheme iron, and enzyme systems all played a major role in the catalysis of lipid oxidation in beef.

Lipid oxidation (peroxidation) is a major nonmicrobial cause of quality deterioration in muscle foods. Heme iron (meat pigments) and nonheme iron are the catalysts of lipid oxidation that have been studied most extensively in red meat. Before the early 1970s, myoglobin was viewed as the major catalyst of lipid oxidation in meat (Tappel, 1952, 1953; Younathan and Watts, 1959). However, Liu and Watts (1970) suggested that both heme and nonheme iron may be important catalysts of lipid oxidation in cooked meat although heme iron may be more important. Their suggestion was based on the observations that lipid oxidation still occurred in the cooked beef muscle samples where heme pigments were degraded by treatment with 30% H_2O_2 but the extent of lipid oxidation was much greater in the untreated cooked meat than in the 30% H_2O_2-treated meat (Liu and Watts, 1970). In contrast, Sato and Hegarty (1971), Love and Pearson (1974), and Igene et al. (1979) proposed that nonheme iron plays a major role in accelerating lipid oxidation in cooked meat and that heme iron does not catalyze the oxidation. The latter researchers reported that metmyoglobin (MetMb), the dialysate of aqueous beef muscle extracts, or EDTA-treated beef muscle extracts, when added to the water-washed beef muscle residue, did not accelerate the oxidation of beef muscle lipids upon heating and refrigeration (2 days) of the samples, whereas nonheme iron added to the muscle residue accelerated the oxidation. However, Kanner and Harel (1985) and Harel and Kanner (1985b) recently reported that MetMb activated by H_2O_2 can initiate the oxidation of lipids in microsomes isolated from poultry skeletal muscles. Heated MetMb was found to maintain its capacity to be activated by H_2O_2 (Harel and Kanner, 1985b).

For the catalysis of lipid oxidation in uncooked red meat, the meat pigment myoglobin has been implicated in many studies as playing an important role (Greene, 1969; Govindarajan et al., 1977; Verma et al., 1984; Rhee and Ziprin, 1987a). In any case, there has been no direct evidence of heme pigments being a major catalyst of lipid oxidation in actual uncooked muscle tissues, rather than in pure or simple systems. Tichivangana and Morrissey (1985) reported that MetMb added to water-washed beef or lamb muscle residue exerted virtually no catalytic activity in raw muscle residue systems. Liu (1970a,b) determined the effects of pH and additives on linoleate oxidation catalyzed by MetMb, a nonheme iron chelate (Fe^{2+}-EDTA), and raw beef homogenates and concluded, on the basis of responses to additives and pH, that the catalytic activity of raw beef homogenates was due to both heme iron and nonheme iron.

Nonheme iron, as other heavy metals with two or more valency states, exerts a prooxidative effect on lipid oxidation primarily through catalytic decomposition of preformed lipid hydroperoxides to free radicals. Likewise, the most important mechanism involved in lipid oxidation catalyzed by heme compounds had been considered to be the decomposition of preformed hydroperoxides, until Kanner and Harel (1985) and Harel and Kanner (1985b) showed the ability of H_2O_2-activated MetMb to initiate membranal lipid oxidation.

While lipid oxidation in red meat has been commonly regarded as nonenzymic reactions, our previous studies (Rhee et al., 1984; Rhee and Ziprin, 1987a) established the presence of an enzymic lipid peroxidation system associated with beef or pork muscle microsomes. Enzymic lipid peroxidation in beef microsomes was dependent on NADPH or NADH, with a higher reaction rate shown with NADPH than with NADH, and required ADP and Fe^{2+} or Fe^{3+} for a maximum rate; the reaction occurred readily even at pH 5.5, a common ultimate pH for beef muscles (Rhee et al., 1984). Luo and Hultin (1986) reported that an enzymic lipid peroxidation system in fish muscle mitochondria was similar to that in fish muscle microsomes (Lin and Hultin, 1976; Player and Hultin, 1977) in terms of cofactor requirements and the optimal pH. It is quite possible that an enzymic lipid peroxidation system could be present in beef muscle mitochondria and its cofactor requirements might be similar to the requirements of the beef muscle microsomal system. Both microsomal and mitochondrial lipid peroxidation systems may be involved in initiation of lipid oxidation in skeletal muscles of meat animals.

The present study was undertaken to determine the characteristics of MetMb–H_2O_2 catalysis of lipid oxidation in raw and cooked beef muscle systems. In addition, an attempt was made to determine the relative contributions of heme iron, nonheme iron, and enzymic lipid peroxidation systems to catalysis of lipid oxidation in raw and cooked beef muscle systems.

MATERIALS AND METHODS

Water-Extracted Muscle Residue. Beef top round (semimembranosus) samples were obtained from the Texas A&M University Meat Science and Technology Center at 24–48 h postmortem or from a local retail store and trim-

Meats and Muscle Biology Section, Department of Animal Science, Texas A&M University, College Station, Texas 77843.

0021-8561/87/1435-1013$01.50/0

Table I. TBA Number of Water-Extracted Beef Muscle Residue Samples (Raw or Cooked) Stored at 4 °C as Affected by the Addition of Metmyoglobin (MetMb) Alone, MetMb-H_2O_2, and Components of Enzymic Lipid Peroxidation Systems

	TBA no., mg malonaldehyde/kg residue (n = 2)[a]					
	raw residue			cooked residue		
treatment[b]	0 day	3 days	6 days	0 day	3 days	6 days
none (control)	*0.27*[e]	*0.28*[f]	*0.29*[f]	0.64[c]	*1.24*[g]	*1.37*[g]
MetMb	*0.49*[d,e]	*0.60*[e]	*0.52*[f]	0.89[b,c]	*1.50*[g,f]	*1.82*[f]
MetMb + H_2O_2 (1:0.1)[c]	1.79[b]	*1.16*[d]	*1.04*[e]	1.23[b]	1.77[f]	2.55[e]
MetMb + H_2O_2 (1:0.25)	2.63[a]	3.35[a]	4.13[a]	1.36[b]	2.26[e]	3.06[d]
MetMb + H_2O_2 (1:0.5)	0.64[d,e]	2.08[c]	2.80[c]	1.13[b]	2.64[d]	4.00[b]
MetMb + H_2O_2 (1:1)	0.87[c,d]	2.10[c]	2.69[c]	2.34[a]	3.97[a]	4.85[a]
Fe^{2+}	*1.14*[c]	*1.23*[d]	*1.31*[d]	2.09[a]	3.15[c]	3.53[c]
Fe^{2+} + NADPH + ADP	1.80[b]	2.43[b]	3.12[b]	2.19[a]	3.54[b]	4.00[b]

[a] Means within the same column followed by a common superscript letter are not different ($P > 0.05$). Means within the same experiment/sample category that are italicized are not different ($P > 0.05$). [b] Levels of additives: 4 mg of MetMb/g or 0.26 mM MetMb based on the moisture content (83%) of the meat residue mixtures; 3 μg of Fe^{2+}/g; 0.3 μmol of NADPH/g; 0.3 μmol of ADP/g. [c] Molar ratio of MetMb to H_2O_2.

med of all visible external fat and connective tissue. The tissue was chopped (25 s with 300 g of muscle for each chopping operation) by a Kitchen-Aid food processor (Model KFP 400; Hobart Corp.) with a plastic work bowl and stainless steel blades. The chopped muscle was extracted at 4 °C with several volumes of deionized-distilled water (Love and Pearson, 1974; Igene et al., 1979). The muscle slurry was filtered through six layers of cheesecloth on a Buchner funnel connected to an aspirator and the muscle residue extracted repeatedly (four to five times) with water until it was virtually devoid of the reddish meat color. The muscle residue remaining from each experiment was mixed thoroughly before further treatment. It was presumed that washing the chopped or ground (not homogenized) muscle with water only would not remove significant amounts of microsomes or mitochondria.

Treatment of Muscle Residue. Ten-gram portions of the water-extracted muscle residue were placed in scintillation vials and mixed with 30 ppm of the antibiotic chlortetracycline to inhibit microbial growth and with the additive(s) of each treatment category (see Tables I and II). The level of added MetMb (4 mg/g of muscle residue) was similar to the concentrations of heme pigments (total pigments: myoglobin plus hemoglobin) reported for beef muscles (Rickansrud and Henrickson, 1967; Rhee and Ziprin, 1987a; Rhee et al., 1986), and the amount of added nonheme iron (3 μg/g of muscle residue) was similar to nonheme iron concentrations of beef muscles (Rhee and Ziprin, 1987a,b) as determined by the modified Schricker method (Rhee and Ziprin, 1987b). The amount of added ADP (0.3 μmol/g) was similar to ADP concentrations reported for the beef longissimus muscles at or after 24 h postmortem (Calkins et al., 1982). Published data on NADPH concentrations in postmortem beef muscles were not available, but the combined concentration of NADPH and NADH in beef muscles was assumed to approach that equivalent (in terms of effectiveness) to 0.3 μmol of NADPH/g, which was used in the present study. NADH is less effective than NADPH for enzymic lipid peroxidation in mammalian muscle microsomes but present at higher levels than NADPH in muscle tissues in general.

All additives were dissolved together in distilled-deionized water, allowed to stand for 3 min, and then added to meat residue in a ratio of 1 mL/10 g of residue. Distilled-deionized water containing chlortetracycline alone was added to the control. Metmyoglobin (equine muscle MetMb; salt-free and lyophilized; 95–100%), ADP (equine muscle ADP; 98%, grade V), NADPH (98%, type X), nonheme iron (iron standard stock), and chlortetracycline (anhydrous, 90%) were purchased from Sigma Chemical Co., and all other chemicals used were of either reagent grade or certified ACS grade.

Table II. Nonheme Iron Content of Raw and Cooked Beef Muscle Residue Samples as Affected by Added Metmyoglobin (MetMb)-H_2O_2

	nonheme Fe, μg/g muscle residue (n = 6)[a,b]			
	raw residue		cooked residue	
treatment[c]	mean	SD	mean	SD
none	1.03[e]	0.30	1.12[d]	0.17
MetMb	2.22[d]	0.13	2.25[c]	0.35
MetMb + H_2O_2 (1:0.1)[d]	2.60[d]	0.64	2.79[c]	0.26
MetMb + H_2O_2 (1:0.5)	9.43[c]	0.80	8.95[b]	1.33
MetMb + H_2O_2 (1:1)	10.70[b]	0.45	11.54[a]	0.50
MetMb + H_2O_2 (1:2)	11.84[a]	0.90	12.19[a]	0.29

[a] Nonheme iron content was determined at day 0. [b] Means within the same column followed by a common superscript letter are not different ($P > 0.05$). [c] The level of MetMb added was 4 mg/g or 0.26 mM based on the moisture content (83%) of the meat residue mixture. [d] Molar ratio of MetMb to H_2O_2.

Meat samples in vials were stored either in the raw state or after cooking in an 80 °C water bath to an internal temperature of 70 °C. The samples to be cooked were placed in an ice bath immediately after the additive treatment. It required approximately 4 min to heat the meat sample (10 g in each vial) from the ice bath temperature to the desired internal temperature. The cooked samples were cooled rapidly in an ice bath. The vials containing raw or cooked meat samples were covered with oxygen-permeable poly(vinyl chloride) film and stored at 4 °C for 0, 3, or 6 days (the day 0 samples were not 0-h samples but were those samples analyzed on the day of muscle residue treatment).

Measurement of Lipid Oxidation. The distillation thiobarbituric acid (TBA) method of Tarladgis et al. (1960) was used with a modification involving the addition of antioxidants (propyl gallate and EDTA) at the initial blending step (Rhee, 1978). The TBA reagent was prepared in distilled water instead of an acidic medium (Tarladgis et al., 1964).

Nonheme Iron Assay. Nonheme iron content was determined by the method of Schricker et al. (1982) as modified by Rhee and Ziprin (1987b). The modification primarily consisted of mixing of the meat sample with 156 ppm of $NaNO_2$ before incubation in acidic medium and inclusion of a second blank to read the brownish color of the incubated liquid phase before adding the color reagent, in addition to the reagent blank.

Statistical Analysis. Where appropriate, data were analyzed by analysis of variance and means were separated by the Student–Newman–Keuls test using the SAS package (SAS, 1982).

RESULTS

Catalysis of Lipid Oxidation. Mean TBA values of beef muscle residue samples treated with MetMb alone, MetMb-H_2O_2 in different molar ratios, and the enzyme system, after 0, 3, and 6 days of storage at 4 °C, are shown in Table I.

In the raw system, little lipid oxidation or no increase in TBA values during 6 days of refrigeration was found for controls (muscle residue itself) or muscle residue samples treated with MetMb only. On the contrary, MetMb-H_2O_2 readily catalyzed the oxidation of beef

muscle lipids; the highest TBA values were obtained when MetMb and H_2O_2 were added in the molar ratio of 1:0.25, at the MetMb level of 4 mg/g of muscle residue mixture or 0.26 mM based on the moisture content (83%) of the raw muscle residue mixtures. The added nonheme iron (3 μg of Fe^{2+}/g) increased TBA values of beef muscle residue less than did MetMb–H_2O_2 at the molar ratio of 1:0.25 or 1:0.5 when samples were stored for 3 or 6 days. The added enzymic system cofactors increased TBA values more than did Fe^{2+} alone or MetMb–H_2O_2 at the ratio of 1:1, 1:0.5, or 1:0.1, but less than MetMb–H_2O_2 at the ratio of 1:0.25.

In the cooked system, lipid oxidation was slow for control samples with no additive (muscle residue alone) and samples treated with MetMb alone (without H_2O_2). Also, control and samples treated with MetMb alone were not significantly different in TBA values at day 0 or day 3. Lipid oxidation was accelerated by MetMb–H_2O_2 in the cooked system, too. However, unlike the case for the raw system, the catalytic activity of MetMb–H_2O_2 increased as the relative amount of H_2O_2 increased. An additional experiment for the molar ratios of MetMb–H_2O_2 showed that the catalytic activity of MetMb–H_2O_2 was highest at the molar ratio of 1:1.5 or 1:2 in the cooked system (data not shown in tabular form). The higher requirement of H_2O_2 in the cooked system, when compared to the raw system, could be due partially to some loss of H_2O_2 that might have occurred during the cooking process. The added nonheme iron markedly increased TBA values of cooked samples (Table I).

Water-extracted muscle residue with added Fe^{2+}, NADPH, and ADP might represent both microsomal and mitochondrial enzymic systems, because extraction of muscle samples with distilled water alone is unlikely to have removed a significant amount of microsomes or mitochondria and because it was reported that the microsomal and mitochondrial enzymic peroxidation systems in fish muscle were similar in cofactor requirements and the optimal pH (Lin and Hultin, 1976; Player and Hultin, 1977; Luo and Hultin, 1986). Although the cooked samples treated initially with the cofactors (Fe^{2+}, NADPH, ADP) had slightly higher TBA values than those treated with nonheme iron alone after 3 or 6 days of storage, the difference between the two treatments might be attributable to an increased nonenzymic lipid oxidation mediated by Fe^{2+}–ADP in the cooked samples initially treated with the cofactors. It is known that the catalytic activity of Fe^{2+} or Fe^{3+} can be increased by conversion of the iron to an iron chelate compound. It is also possible that the heating procedure and conditions used for cooking in the present study might not have completely inactivated the enzyme(s) involved.

Nonheme Iron from MetMb–H_2O_2 Treatment. Since 30% H_2O_2 destroyed heme pigments, releasing nonheme iron (Liu and Watts, 1970; Igene et al., 1979; Schricker and Miller, 1983), experiments were conducted to determine whether H_2O_2 at concentrations used in the present study would release iron from MetMb molecule. Nonheme iron concentrations in MetMb- or MetMb–H_2O_2-treated muscle residue samples, as determined at day 0, are shown in Table II. Muscle residue alone contained approximately 1 μg of nonheme iron/g even though finely comminuted muscle was exhaustively washed with water to obtain the residue. This amount of nonheme iron was apparently due to the protein-bound nonheme iron that was not extracted with water. In both raw and cooked systems, muscle residue samples treated with MetMb–H_2O_2 in the molar ratio of 1:0.1 was not significantly different in nonheme iron content from those treated with MetMb alone. However, muscle residue samples treated with MetMb–H_2O_2 in molar ratios of 1:0.5, 1:1, and 1:2 had significantly higher nonheme iron content than samples treated with MetMb alone. This was true for raw as well as cooked samples. However, differences in nonheme iron concentration between raw and cooked samples were not significant ($P > 0.05$).

Table III. Nonheme Iron Concentrations in MetMb and MetMb–H_2O_2 Solutions (without Muscle Residue) Held for 3 min after Preparation

solution	nonheme Fe,[a] μg	solution	nonheme Fe,[a] μg
MetMb	0.92	MetMb + H_2O_2 (1:0.5)	7.71
MetMb + H_2O_2 (1:0.1)	2.76	MetMb + H_2O_2 (1:1)	10.60
MetMb + H_2O_2 (1:0.25)	5.99	MetMb + H_2O_2 (1:2)	11.51

[a] In the additive solution equivalent to 1 g of final muscle residue mixture.

In order to confirm that nonheme iron was released from MetMb in MetMb–H_2O_2-treated muscle residue samples, MetMb and MetMb–H_2O_2 additive solutions (without meat residue) held for 3 min after preparation (see Treatment of Muscle Residue in the Materials and Methods) were assayed for nonheme iron concentrations without the acidic incubation step which was included in nonheme iron assays of muscle residue-containing samples. As shown in Table III, nonheme iron was indeed liberated from MetMb by H_2O_2.

In contrast to the results of the present study where purified MetMb (purchased) was added to water-extracted muscle residue prior to cooking, cooking or heating of intact beef muscles or muscle pigment extracts has been shown to increase the nonheme iron content (Igene et al., 1979; Schricker et al., 1982; Schricker and Miller, 1983; Chen et al., 1984; Rhee and Ziprin, 1987a). One of the reasons why cooking did not increase nonheme iron content of MetMb-added muscle residue samples might be that a considerable amount of nonheme iron could already have been released during the preparation/purification processes for the chemical; approximately 8% of the iron in the purchased MetMb was found to be nonheme iron. Results of our recent study (Rhee and Ziprin, 1987a) showed that about 6–16% of the iron in beef muscle heme pigments was released as nonheme iron during cooking of 50 g of ground muscle in a 90 °C water bath to an internal temperature of 70 °C. The short time (approximately 4 min in an 80 °C water bath) required to heat 10 g of ground muscle residue from an ice bath temperature to an internal temperature of 70 °C in the present study may be another reason for the insignificant nonheme iron increase due to heating in MetMb-treated samples.

DISCUSSION

Lipid oxidation catalyzing activity of MetMb–H_2O_2 in the raw system may be due primarily to MetMb activated by H_2O_2 (activated MetMb) and secondarily to the nonheme iron released from MetMb by H_2O_2. Kanner and Harel (1985) and Harel and Kanner (1985b), who investigated the effect of MetMb–H_2O_2 on lipid oxidation in microsomes isolated from poultry muscles, reported that little or no lipid oxidation occurred in the presence of H_2O_2 alone (up to 100 μM) or MetMb alone (30 μM) while the oxidation occurred readily in the presence of MetMb–H_2O_2. They suggested that the interaction of H_2O_2 with MetMb generated activated MetMb capable of initiating lipid oxidation. However, these researchers (Kanner and Harel, 1985; Harel and Kanner, 1985b) did not examine whether nonheme iron was released from MetMb in their

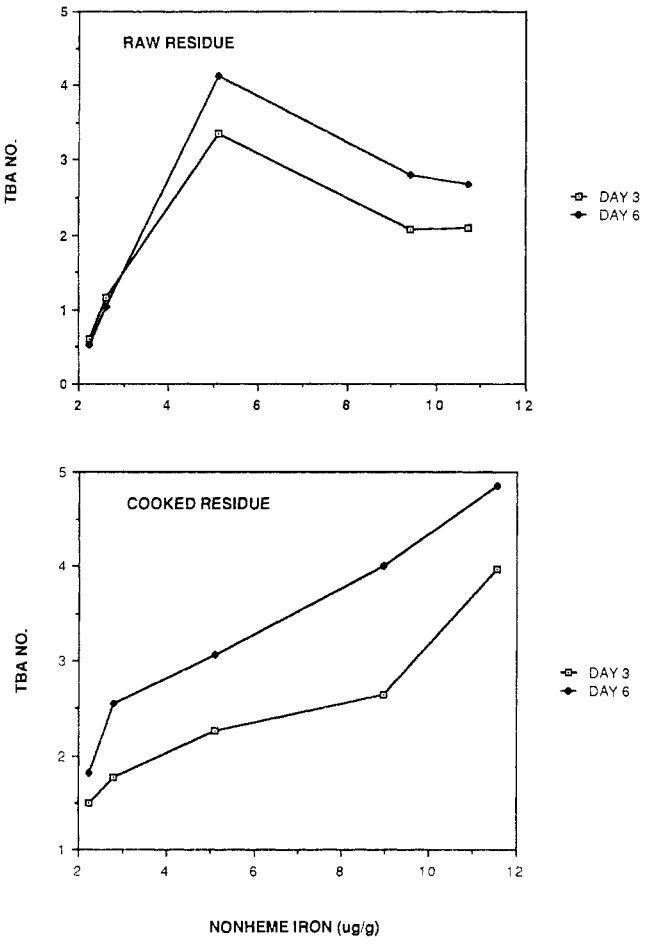

Figure 1. Nonheme iron concentration vs. TBA number for the MetMb–H_2O_2-treated muscle residue samples. (Nonheme iron concentrations for the 1:0.25 MetMb–H_2O_2 treated samples were extrapolated values from the Table II data.)

experimental system. The basis for proposing that activated MetMb may be more important than nonheme iron for the raw system in the present study is that the optimum amount of H_2O_2 for the lipid oxidation catalyzing activity of MetMb–H_2O_2 in the raw system (i.e., one-fourth the molar concentration of MetMb; Table I) was far below the H_2O_2 level causing the greatest nonheme iron release from MetMb (i.e., 2 times the concentration of MetMb; Table II). Additionally, when TBA numbers were plotted against nonheme iron concentrations, using the data in Tables I and II, the relationship was not linear in the raw system when the nonheme iron concentration increased beyond the level generated from the 1:0.25 MetMb–H_2O_2 treatment (Figure 1).

In the cooked system, the MetMb–H_2O_2 catalysis of lipid oxidation appeared to be due mainly to the nonheme iron released from MetMb by the H_2O_2 action (Figure 1), although activated MetMb may have played a role. Heated MetMb was reported to maintain its capacity to be activated by H_2O_2 (Harel and Kanner, 1985b).

An important aspect of the present study is its demonstration that the heme pigment system (MetMb–H_2O_2), regardless of how it may exert its prooxidative effect, plays a major role in the catalysis of lipid oxidation in raw and cooked meat. Although not quantitated in the present study, the amount of H_2O_2 is unlikely to be a limiting factor in postmortem skeletal muscles from red meat animals. The autoxidation of oxygenated heme pigments (oxymyoglobin and oxyhemoglobin) was reported to lead to the formation of MetMb and methemoglobin and $O_2^{\cdot-}$, which dismutates to H_2O_2 (Brown and Mebine, 1969; Mirsa and Fridovich, 1972; Gotoh and Shikama, 1976; Satoh and Shikama, 1981; Wallace et al., 1982). Since the oxidation of oxymyoglobin to MetMb is a common phenomenon in fabricated (cutup), postmortem red meat, especially upon storage, it is reasonable to assume that H_2O_2 could be produced from the pigment oxidation in quantities sufficient for the MetMb–H_2O_2 catalysis of lipid oxidation. Hydrogen peroxide may also be generated in muscle tissues by other systems, although enzymic production of H_2O_2 may be of minor significance compared to the nonenzymic production of H_2O_2 through the autooxidation of heme pigments (Harel and Kanner, 1985a). The finding from the present study that MetMb–H_2O_2 plays a major role in catalysis of lipid oxidation in raw beef, together with the reported findings that the oxidation of oxymyoglobin produces MetMb and H_2O_2 (Satoh and Shikama, 1981; Wallace et al., 1982), may explain the high positive correlations observed between discoloration (primarily MetMb formation) and lipid oxidation in uncooked red meat (Rhee and Smith, 1983; Rhee et al., 1983, 1985).

On the basis of results of the present study, research data from earlier reports (Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979; Tichivangana and Morrissey, 1985) dealing with the effect of added heme pigments on lipid oxidation in water-extracted beef muscle residues can be interpreted from an entirely different angle. The reason why added MetMb, the dialysates of meat pigment extracts, or meat pigment extracts with chelatable iron removed did not catalyze lipid oxidation in water-washed muscle residue samples may be that the component(s), such as H_2O_2, that are essential for heme pigments to be an active catalyst could be lacking in the lipid oxidation systems used in those studies.

Data from the present study indicate that activated MetMb, nonheme iron, and the enzymic lipid peroxidation systems may all play a major role in the catalysis of lipid oxidation in beef. Their relative contributions to the oxidation would likely be dependent on antemortem and postmortem events of the meat animal and carcass and, to some extent, on specific muscles selected.

ACKNOWLEDGMENT

Technical article No. 21,907, Texas Agricultural Experiment Station. Supported partially by the National Live Stock and Meat Board, Chicago, IL, and the Natural Fibers and Food Protein Commission of Texas, Dallas, TX. We thank Dr. Herbert O. Hultin at University of Massachusetts for helpful discussions.

Registry No. Fe, 7439-89-6.

LITERATURE CITED

Brown, W. D.; Mebine, L. B. *J. Biol. Chem.* **1969**, *244*, 6096.
Calkins, C. R.; Dutson, T. R.; Smith, G. C.; Carpenter, Z. L. *J. Food Sci.* **1982**, *47*, 1350–1353.
Chen, C. C.; Pearson, A. M.; Gray, J. I.; Fooladi, M. H.; Ku, P. K. *J. Food Sci.* **1984**, *49*, 581–584.
Gotoh, T.; Shikama, K. *J. Biochem.* **1976**, *80*, 397–399.
Govindarajan, S.; Hultin, H. O.; Kotula, A. W. *J. Food Sci.* **1977**, *42*, 571–577, 582.
Greene, B. E. *J. Food Sci.* **1969**, *34*, 110–113.
Harel, S.; Kanner, J. *J. Agric. Food Chem.* **1985a**, *33*, 1186–1188.
Harel, S.; Kanner, J. *J. Agric. Food Chem.* **1985b**, *33*, 1188–1192.
Igene, J. O.; King, J. A.; Pearson, A. M.; Gray, J. I. *J. Agric. Food Chem.* **1979**, *27*, 838–842.
Kanner, J.; Harel, S. *Arch. Biochem. Biophys.* **1985**, *237*, 314–321.
Lin, T. S.; Hultin, H. O. *J. Food Sci.* **1976**, *41*, 1488–1489.
Liu, H.-P. *J. Food Sci.* **1970a**, *35*, 590–592.
Liu, H.-P. *J. Food Sci.* **1970b**, *35*, 593–595.
Liu, H.-P.; Watts, B. M. *J. Food Sci.* **1970**, *35*, 596–598.
Love, J. D.; Pearson, A. M. *J. Agric. Food Chem.* **1974**, *22*, 1032–1034.

Luo, S. W.; Hultin, T. O. 46th Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 1986, Paper No. 438.
Mirsa, H. P.; Fridovich, I. *J. Biol. Chem.* **1972**, *247*, 6960.
Player, T. J.; Hultin, H. O. *J. Food Biochem.* **1977**, *1*, 153–171.
Rhee, K. S. *J. Food Sci.* **1978**, *43*, 1776–1779, 1781.
Rhee, K. S.; Smith, G. C. *J. Food Prot.* **1983**, *46*, 787–790.
Rhee, K. S.; Ziprin, Y. A. *J. Food Biochem.* **1987a**, *11*, 1–15.
Rhee, K. S.; Ziprin, Y. A. *J. Food Sci.* **1987b**, in press.
Rhee, K. S.; Smith, G. C.; Rhee, K. C. *J. Food Sci.* **1983**, *48*, 351–352, 359.
Rhee, K. S.; Dutson, T. R.; Smith, G. C. *J. Food Sci.* **1984**, *49*, 675–679.
Rhee, K. S.; Vanderzant, C.; Keeton, J. T.; Ehlers, J. G.; Leu, R. *J. Food Sci.* **1985**, *50*, 1388–1391.
Rhee, K. S.; Seideman, S. C.; Cross, H. R. *J. Agric. Food Chem.* **1986**, *34*, 308–312.
Rickansrud, D. A.; Henrickson, R. L. *J. Food Sci.* **1967**, *32*, 57–61.
SAS. *SAS User's Guide: Statistics*; SAS Institute: Cary, NC, 1982; pp 113–137.
Sato, K.; Hegarty, G. R. *J. Food Sci.* **1971**, *36*, 1098–1102.
Satoh, Y.; Shikama, K. *J. Biol. Chem.* **1981**, *256*, 10272–10275.
Schricker, B. R.; Miller, D. D. *J. Food Sci.* **1983**, *48*, 1340–1343, 1349.
Schricker, B. R.; Miller, D. D.; Stouffer, J. R. *J. Food Sci.* **1982**, *47*, 740–743.
Tappel, A. L. *Food Res.* **1952**, *17*, 550–559.
Tappel, A. L. *Food Res.* **1953**, *18*, 104–108.
Tarladgis, B. G.; Watts, B. M.; Younathan, M. T.; Dugan, L. R. *J. Am. Oil Chem. Soc.* **1960**, *37*, 44–48.
Tarladgis, B. G.; Pearson, A. M.; Dugan, L. R. *J. Sci. Food Agric.* **1964**, *15*, 602–607.
Tichivangana, J. Z.; Morrissey, P. A. *Meat Sci.* **1985**, *15*, 107–116.
Verma, M. M.; Ledward, G. A.; Lawrie, R. A. *Meat Sci.* **1984**, *11*, 171–189.
Wallace, W. J.; Hautcheus, R. A.; Maxwell, J. C.; Caughey, W. S. *J. Biol. Chem.* **1982**, *257*, 4966–4977.
Younathan, M. T.; Watts, B. M. *Food Res.* **1959**, *24*, 728–734.

Received for review September 29, 1986. Revised manuscript received April 20, 1987. Accepted June 5, 1987.

Carotenes in Typical and Dark Orange Carrots

Philipp W. Simon* and Xenia Y. Wolff

Carotenes from a genetically diverse collection of carrots were separated and quantified with reversed-phase high-performance liquid chromatography and their identities verified with column chromatography and thin-layer chromatography. Extraction of carotenes from lyophilized samples stored less than 1 month was comparable to that from raw or frozen samples, and saponification was not necessary. Six carotenes (α-, β-, γ-, ζ-carotene; β-zeacarotene; lycopene) were routinely detected. β-Carotene accounted for 44–79% of the total carotenes whereas β-, α-, and ζ-carotene accounted for 94–97% of the total carotenes. Total carotene content ranged from 63 to 548 ppm over lines and location/years. The very dark orange line HCM contained more than twice as much total carotene as any other line tested in all location/years.

Much of the dietary vitamin A, especially in developing countries, is derived from carotenes in vegetables and fruits (Simpson, 1983). Carotenes have also been implicated as anticancer compounds in numerous studies (Moon and Itri, 1984). Carrots are the major single source of provitamin A carotenoids in the American diet, contributing 14% of the total vitamin A consumption (Senti and Rizek, 1975). Although an "average" carrot in the United States contains 66 ppm total carotenes (Adams, 1975), carrots from different genetic sources have been reported to contain 0–370 ppm carotenes, with β-carotene usually accounting for approximately half of this total (Umiel and Gabelman, 1971).

With the potential for genetically increasing the carotene content of carrots, selection for dark orange, high provitamin A carrot roots was initiated in 1977 (Simon et al., 1985). To increase carotene concentration over successive generations, large populations must be analyzed. The evaluation of large numbers of carrot roots for carotene concentration is time consuming and made difficult by analytical methods that can result in carotene breakdown or isomerization. Important factors to consider in carotene analysis include method of sample preparation, extraction conditions, saponification, and method of chromatographic separation and quantification (Davies, 1976; DeRitter and Purcell, 1981).

Carotenes from carrots of diverse genetic background have been quantified with thin-layer chromatography (Umiel and Gabelman, 1971; Buishand and Gabelman, 1979). Six colored pigments (α-, β-, γ-, ζ-carotene; lycopene; one unknown) were able to be separated with that system. Reversed-phase, high-performance liquid chromatographic determination of carotenes lends itself to a more rapid, quantitative analysis of carotenes, and systems for measuring lycopene and α- and β-carotene in higher plants, including carrots, are reported (Zakaria et al., 1979; Bushway and Wilson, 1982).

This paper details a method to analyze carotenes in typical and dark orange carrots. The method utilizes lyophilized samples, direct extraction of carotenes into hexane, and quantification with a reversed-phase HPLC solvent system modified from that developed by Nells and DeLeenheer (1983) and used by Bieri et al. (1985) for analysis of human plasma carotenoids. Time needed for this method is less than that for other methods used with plant samples, carotene breakdown is minimal, and six carotenes (α-, β-, γ-, ζ-carotene; β-zeacarotene; lycopene)

U.S. Department of Agriculture—Agricultural Research Service, Department of Horticulture, University of Wisconsin, Madison, Wisconsin 53706.