The data further confirm that storage of comminuted high-nitrate fresh spinach and beets will eventually result in accumulation of large amounts of nitrite at the expense of nitrate. Since the six nitrosamines sought by highly specific and sensitive methods were not found, even in materials stored beyond the limits of edibility, the principal public health concern with these high-nitrite vegetables remains the relation of nitrite to methemoglobinemia in infants.

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Metmyoglobin and Nonheme Iron as Prooxidants in Cooked Meat

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The role of metmyoglobin (MetMb) and nonheme iron in accelerating lipid oxidation in cooked meat was studied using a model system containing water-extracted muscle residue. The effects of various components added to the system prior to heating and storage upon oxidation were determined by the TBA method. Comparison of the prooxidant activity of the aqueous extract from muscle and its nondialyzable (dialyzate) and dialyzable (diffusate) fractions suggested that the prooxidant activity was located in the low molecular weight fraction of the extract. Addition of purified MetMb and Fe^{2+} to the system showed that Fe^{2+} was effective as a prooxidant in cooked meat, whereas MetMb at concentrations from 1 to 10 mg/g of meat failed to catalyze the oxidation of lipids. Low levels of ascorbic acid enhanced the prooxidant activity of Fe^{2+}. Results indicated that nonheme iron acts as a prooxidant in cooked meat, while MetMb has little or no prooxidant activity.

Flavor deterioration resulting from the oxidation of phospholipids is a problem in a variety of food products, including cooked meats (Keller and Kinsella, 1973; Sato and Hegarty, 1971; Younathan and Watts, 1960). In contrast to the traditional view that heme pigments are the major catalysts of lipid oxidation in meat, Sato and Hegarty (1971) have proposed that nonheme iron plays a major role in accelerating lipid oxidation in cooked meat. These investigators also reported that hemoglobin and myoglobin have no prooxidant activity in cooked meat systems; however, they utilized only one level of added myoglobin in their studies. On the other hand, several researchers, including Hirano and Olcott (1971) and Kendrick and Watts (1969), have reported that heme compounds may act as either accelerators or inhibitors of lipid oxidation with their action depending on the ratio of heme to unsaturated fatty acid.

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The present investigation was undertaken to clarify the effects of variable concentrations of metmyoglobin (MetMb) on lipid oxidation in a cooked meat model system. The influence of ferrous iron (Fe^{2+}) and ascorbic acid was also investigated.

MATERIALS AND METHODS

Extraction of Muscle Tissue. After removing all visible fat and connective tissue, beef round (semitendinosus) or pork loin (longissimus) muscle was ground through a 1/8-in. plate of an electric grinder. Weighed samples of the ground muscle were then extracted with distilled, deionized water at 4°. The slurry was filtered through cheesecloth, and the muscle residue was reextracted until it appeared to be devoid of heme pigment. Extracted muscle (10-g aliquots) was placed in Kapak bags, flushed with nitrogen, sealed, and stored frozen at −25°. This was the source of extracted muscle in the model meat systems described below.

Concentration and Fractionation of the Water Extract. The aqueous extracts obtained from muscle tissue were combined and concentrated by freeze-drying. Concentrated extracts were placed in cellulose dialysis tubing (pore diameter = 48 Å; Arthur H. Thomas Co., Philadel-

Table I. TBA Values Obtained following the Addition of Various Fractions from an Aqueous Muscle Extract to Water Extracted Beef Muscle Residue followed by Heating and Storage at 4° for 48 hr

Substrate and treatment	TBA no.'s[a]
Muscle residue and water (control)	1.23
Muscle residue and undialyzed aqueous muscle extract	5.21
Muscle residue and dialyzate of aqueous muscle extract	1.66
Muscle residue and diffusate of aqueous muscle extract	2.88

[a] TBA numbers = mg of malonaldehyde produced per 1000 g of meat.

Table II. Effects of Various Concentrations of MetMb and Fe^{2+} on TBA Numbers of Beef Muscle Residue[a]

MetMb		Fe^{2+}	
Concn, mg/g	TBA no.'s[b]	Concn, ppm	TBA no.'s[b]
0	1.4	0	1.0
1.0	1.4	1.0	1.5
2.5	1.4	2.0	3.6
5.0	1.4	3.0	4.1
10.0	1.4	4.0	5.4

[a] The reactants were mixed, heated, and stored for 48 hr at 4° before measuring the TBA values. [b] TBA numbers = mg of malonaldehyde produced per 1000 g of meat.

phia, Pa.) and dialyzed against distilled, deionized water at 4°, using several changes of water. Both the diffusate and dialyzate were saved and concentrated for use in the model meat systems. The muscle extracts were also concentrated by ultrafiltration at 4° using an Amicon Model 402 ultrafiltration cell (Amicon Corp., Lexington, Mass.) with a UM10 membrane, which retains substances with a molecular weight greater than 10,000.

Preparation of Model Systems. The frozen extracted muscle residue (10-g aliquots) was thawed and used in all model systems. Concentrates of either the undialyzed, the dialyzate, or the diffusate fractions from aqueous muscle extracts were added to aliquots of the extracted muscle residue. Water was also added to aliquots of the extracted muscle residue and served as control samples. Skeletal muscle whale MetMb (A grade; Calbiochem, Los Angeles, Calif.) and ferrous sulfate were utilized as the sources of MetMb and Fe^{2+}, respectively, and were blended in the model systems. Distilled deionized water was used in preparing all samples and solutions, and all chemicals used were of reagent grade.

TBA Analysis. The distillation method of Tarladgis *et al.* (1960) was used to analyze for thiobartituric acid reactive material. EDTA was added at a level of 0.2% prior to blending the meat sample in order to minimize iron-catalyzed lipid oxidation during blending and distillation. Absorbance was read at 538 nm with a Beckman DU-2 spectrophotometer. Results are expressed as milligrams of malonaldehyde per 1000 g of meat.

RESULTS AND DISCUSSION

TBA values obtained upon adding various fractions of the aqueous muscle extract back to the extracted muscle residue followed by heating and then by storing for 48 hr at 0° are shown in Table I. The TBA value for the control sample was 1.23, which is near the threshold TBA value of 1-2 at which off-odors become apparent (Watts, 1962). Addition of undialyzed aqueous extract to the extracted muscle residue caused a marked increase in TBA number (Table I), which indicated it contained prooxidant substances. TBA numbers following the addition of either the nondialyzable or diffusate fractions (Table I) showed part of the prooxidant activity was present in each fraction, although addition of the diffusate gave twofold higher TBA values than the dialyzate. As the amount of undialyzed muscle extract, dialyzate, or diffusate added to the muscle residue was increased fivefold, there was a further increase in TBA values. The increase in oxidation as the volume of the various muscle extracts was added to the meat model systems indicated the importance of the concentration of prooxidants.

Lewis and Wills (1963) reported that heme compounds present in tissue homogenates catalyzed oxidation of unsaturated fatty acids in dilute (1% w/v) suspensions, but at higher (5% w/v) concentrations acted as inhibitors. Other researchers (Hirano and Olcott, 1971; Nakamura and Nishida, 1971; Kendrick and Watts, 1969; Banks *et al.*, 1961) have discussed the ability of hemoproteins to act as either pro- or antioxidants, depending on the ratio of heme to unsaturated fatty acid. If hemoproteins served as active prooxidants in the muscle extract or dialyzate in the current study, no inhibitory effect was apparent. Either an inhibitory concentration of hemoproteins was not reached or else the heme compounds did not influence the extent of oxidation. These results confirm the observations of Sato and Hegarty (1971), suggesting that the major prooxidant in cooked meat is water soluble and dialyzable, and thus, is not a heme protein. Therefore, nonheme iron may be a more important catalyst of lipid oxidation in cooked meat than denatured muscle pigments. In order to test this hypothesis, Fe^{2+} and purified MetMb were added to the water-extracted muscle residue and were then heated to 70° and stored at 4° for 24 hr prior to TBA analysis. The added levels of MetMb and Fe^{2+} approximated the concentration of heme proteins and nonheme iron reported to be present in beef muscle (Sato and Hegarty, 1971; Craig *et al.*, 1966).

Liu and Watts (1970) indicated that a rough calculation of the ratio of polyunsaturated fatty acids to myoglobin in meat suggests that myoglobin in meat is present at a concentration that would cause strong prooxidant activity. However, they also pointed out that myoglobin could be present in inhibitory concentrations in localized areas of the cell and out of contact with unsaturated lipids in other areas. Thus, MetMb concentration was varied over a tenfold range.

TBA values for samples containing 1.0-10.0 mg of MetMb/g of muscle were identical with the control, indicating that MetMb was not acting as either a pro- or an antioxidant (Table II). In contrast, levels of Fe^{2+} as low as 1.0 ppm resulted in some increase in production of TBA reactive material (Table II). These results confirm the observations of Sato and Hegarty (1971) suggesting that nonheme Fe^{2+} is the major prooxidant in cooked meat.

Since myoglobin may exert a prooxidant effect over longer periods of time, TBA values were measured over a 72-hr period (Table III). At levels of 5 mg/g of meat, MetMb did not cause increased oxidation in cooked meat over a 72-hr period. In contrast, low levels of Fe^{2+} (1 ppm) increased TBA values during storage. Addition of 5 ppm of ascorbic acid increased the prooxidant activity of Fe^{2+}, although ascorbic acid alone at this level was not a prooxidant. These results are in agreement with Sato and Hegarty (1971) who have reported that low levels of ascorbic acid stimulated Fe^{2+} catalysis in cooked meat, while high levels were inhibitory.

Table III. Effect of Addition of Fe^{2+}, MetMb, and Ascorbic Acid to Pork Muscle Residue and Length of Storage at 4° upon TBA Numbers[a]

Sample	TBA no.'s[b] 0 hr	24 hr	48 hr	72 hr
Control, muscle residue and water	0.30	0.40	0.60	0.70
Muscle residue and Fe^{2+} [c]	0.35	0.65	1.05	1.35
Muscle residue and Fe^{2+} and ascorbic acid[c]	0.35	1.55	1.85	2.35
Muscle residue and MetMb[d]	0.20	0.30	0.40	0.60

[a] The reactants were mixed and heated and TBA numbers were measured at the times specified. [b] TBA numbers = mg of malonaldehyde produced per 1000 g of meat. [c] Fe^{2+} = 1 ppm; ascorbic acid = 5 ppm. [d] Concentration of MetMb = 5 mg/g.

Liu and Watts (1970) have reported that both heme and nonheme iron were capable of accelerating oxidation of lipids in cooked meat. Rather than attempting to remove prooxidant substances and add back heme and nonheme iron, as was done in the present investigation, Liu and Watts (1970) treated meat with H_2O_2 to destroy the heme compounds. Since the H_2O_2-treated samples had lower TBA values, they concluded that the myoglobin also served as a prooxidant in cooked meat. Sato and Hegarty (1971) concluded that the inhibiting activity of H_2O_2 is associated with its activity as an oxidizing agent. Presumably, the oxidation of ascorbic acid, which enhances the effect of Fe^{2+}, accounts for the inhibition of lipid oxidation in H_2O_2-treated samples.

There is indirect evidence that nonheme iron plays a major role in accelerating lipid oxidation in muscle tissue, since lipid oxidation in meat has been observed to proceed more rapidly at lower pH values (Keskinel *et al.*, 1964). Liu (1970) pointed out that the prooxidant activity of Fe^{2+} is at a maximum in the range of pH 5.0-5.5. The effectiveness of EDTA and polyphosphates as inhibitors of lipid oxidation in meat (Sato and Hegarty, 1971; Timms and Watts, 1958) can be easily explained if nonheme iron is a catalyst of lipid oxidation.

In studies on cooked meat pigments, Ledward (1971) reported that porphyrins in the denatured heme compounds may possess some low-spin characteristics. Low-spin compounds are known to be less effective as catalysts of lipid oxidation. These observations may help explain why MetMb did not act as a prooxidant in cooked meat systems.

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Determination of Lead in Organic Coloring Dyes by Atomic Absorption Spectroscopy

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A fairly rapid method has been developed for determining Pb contaminants in food coloring dyes. The sample is digested in HNO_3-$HClO_4$ and taken to dryness, the salts are dissolved, and a Pb-diethyldithiocarbamate chelate is formed and extracted with xylene. The lead content is determined by atomic absorption spectroscopy. The detection limit with respect to the dyes is about 0.4 ppm on the average.

The lead content of food coloring dyes should not exceed 10 ppm as required by the CODE of Federal Regulations-Title 21. A faster method than the Official Methods of Analysis of the AOAC (1970a-c) is desired. These methods, while sensitive enough, require 1-2 days for the dry ashing and the multiple extractions required to remove the interferences. The proposed procedure can be accomplished in 1-2 hr.

EXPERIMENTAL SECTION

Equipment. Perkin-Elmer Models 290 and 303 atomic absorption spectrophotometers with a Boling burner and a Jarrell-Ash unit consisting of a 0.5-m monochromator mounted on an optical rail and a Beckman total consumption burner were used. The lamp was a Westinghouse high spectral output lamp, type pF 290 with an 80-μF damping capacitor.

Chemicals. Food, Drug, and Cosmetic (1960) food coloring dyes used were: FD&C Red 2 (trisodium salt of 1-

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