Effect of Superoxide and Superoxide-Generating Systems on the Prooxidant Effect of Iron in Oil Emulsion and Raw Turkey Homogenates¹

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ABSTRACT  Mechanisms of superoxide-O₂⁻-generating systems on the pro-oxidant effect of iron from various sources were studied. Reaction mixtures were prepared with distilled water, oil emulsion, or meat homogenates. Free ionic iron (ferrous and ferric), ferritin and hemoglobin (Hb) were used as iron sources, and KO₂ and xanthine oxidase (XOD) systems were used to produce -O₂⁻. Thiobarbituric acid reactive substances (TBARS) values and iron contents of the reaction mixtures were determined.

Ferric iron and ferritin, in the presence or absence of superoxide-generating systems, had no catalytic effect on the oxidation of oil emulsion but became pro-oxidants when reducing agent (ascorbate) was present. Ferrous iron and Hb had strong catalytic effects on the oxidation of oil emulsion as shown by TBARS values. Superoxide and H₂O₂, generated from superoxide-generating systems, oxidized ferrous iron and ascorbate, and lowered the pro-oxidant effect of ferrous iron in oil emulsion. Addition of ferric or ferrous iron increased but Hb did not have any effect on the TBARS values of raw meat homogenates.

The reaction mechanisms of superoxide and the superoxide-generating systems on the prooxidant effect of various iron sources indicated that -O₂⁻ was a strong oxidizer rather than a reducing agent, and the antioxidant effect of XOD system in oil was caused by the oxidation of ferrous iron to the ferric form by -O₂⁻ and/or H₂O₂.

(Key words: lipid oxidation, superoxide, iron sources, oil emulsion, meat homogenates)

INTRODUCTION

The direct and indirect involvements of superoxide (-O₂⁻) and H₂O₂ on the catalysis of lipid peroxidation in vivo and in vitro have been reported by many researchers (Thomas et al., 1988; Suzuki and Ford, 1994; Stohs and Bagchi, 1995). Liochev and Fridovich (1994) suggested the traditional Fenton-type reaction to describe the formation of reactive oxygen species, the initiator of lipid oxidation. In the reaction, -O₂⁻ reduces Fe³⁺ to Fe²⁺, which react with H₂O₂ to yield Fe³⁺O or hydroxyl radicals (OH) in vitro. Miller et al. (1990) rejected the traditional Fenton-type reaction and suggested that an Fe(II):Fe(III) complex is the catalyst of lipid oxidation. Others (Kanner and Harel, 1985; Halliwell and Gutteridge, 1990; Shen et al., 1992) have also suggested that the initiator of lipid oxidation is not OH, but rather is ferryl or perferryl radicals.

Free ionic irons are considered to be active forms of iron in the generation of OH via the Haber-Weiss reaction, but other forms of iron such as iron-compounds or chelated iron may also be involved in the reaction (Halliwell, 1978; McCord and Day, 1978; Gutteridge et al., 1982). Heme pigments, ferritin, and transferrin are the predominant forms of iron-containing proteins in muscle tissues and act as powerful lipid oxidation catalysts when the heme pigments are activated by H₂O₂ or iron is released from the iron-containing molecules (Kanner and Harel, 1985; Kanner and Doll, 1991; Kanner et al., 1991). Superoxide is considered as the primary reductant in ascorbate-mediated iron release from ferritin (Biemand et al., 1984; Boyer and McCreary, 1987), but other reductants such as dithionite, thioglycollate, and ascorbate can also release ferrous iron from ferritin (Funk et al., 1985; Biemand et al., 1986; Decker and Welch, 1990).

Superoxide can be produced by KO₂ in vitro (Marklund, 1976), and by mitochondria, macrophages (Hal-
liwell and Gutteridge, 1990), and the xanthine oxidase (XOD) system (Granger et al., 1981; Sussman and Buckley, 1990) in vivo. Any system that can generate -O2− is known to produce H2O2. Superoxide can also be generated via the XOD system when hypoxic tissues are reoxygenated, and is responsible for the injuries in reoxygenated ischemic tissues (Grisham et al., 1986).

In postmortem carcasses, the amount of xanthine and hypoxanthine, the substrates from which XOD generates -O2− and H2O2, increases with time because of the degradation of adenosine triphosphate (ATP). The subsequent meat processing steps such as cutting and grinding could replenish oxygen and promote the generation of free radicals in meat by the xanthine-XOD reaction. This free radical generation is very similar to the conditions of reperfused ischemic tissues. If -OH is generated by the -O2−-dependent Fenton reaction and is responsible for the initiation of lipid oxidation (Halliwell and Gutteridge, 1990), the XOD system in raw meat during processing would be expected to promote the peroxidation of lipids. However, our previous study indicated that the XOD system added in raw turkey-meat homogenates did not increase, but significantly decreased lipid peroxidation (Ahn et al., 1993). It was presumed that the antioxidant effect of XOD system could be coming from the chelation of -OH and Fe2+ by urate, a product of the xanthine and XOD reaction, but this did not provide a clear answer to these unexpected results. The objective of the present study was to determine the mechanisms of -O2− and -O2−-generating systems on the prooxidant effect of various iron sources.

MATERIALS AND METHODS

Chemicals and Reagents

Ascorbate was purchased from Fisher Scientific3 and butylated hydroxyanisol (BHA), SOD, xanthine, XOD (2.26 U/mL), catalase, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), chelex-100 (50-100 mesh, sodium form), ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl sulfonic acid)-1,2,4-triazine), and neocuproine were obtained from Sigma.4 All chemicals used were reagent grade.

Ascorbate (5.68 mM), SOD (1,000 U/mL), xanthine (20 mM), potassium superoxide (KO2, 20 mM), hydrogen peroxide (20 mM), iron protein [50 ppm iron equivalent: hemoglobin (Hb), 15.6 mg/mL; ferritin, 250 µg/mL] solutions were prepared by dissolving an appropriate amount of each chemical directly in deionized distilled water (DDW). The ionic iron solutions were prepared by dissolving 178 mg FeCl2·4H2O or 242 mg FeCl3·6H2O in 1 L of 0.1 N HCl to make 50 µg Fe/mL solution. Trichloroacetic acid/thiobarbituric acid (TCA/TBA) stock solution was prepared by dissolving 15% TCA (wt/vol) and 20mM 2-thiobarbituric acid in DDW. Seventy-five mg ferrozone and 75 mg neocuproine were dissolved in 25 mL DDW to make ferroin color reagent (Carter, 1971). The BHA (72 mg/mL) was dissolved in 97% ethanol.

Sample Preparations

For the oil emulsion, 152 mL chelex-100-treated DDW, 8 mL 1 M maleate buffer, pH 6.5, and 0.25 mL oil (flax oil) were added in a glass jar and blended for 2 min by using a Waring blender5 (Model 7010). The oil emulsion prepared was used immediately for the subsequent study. The flax oil used in this study was composed of 5.4%, myristic, 2.7% palmitic, 12.1% oleic, 16.7% linoleic, and 63.2% linolenic acids.

Thigh meats were deboned from eight turkeys and skinned. The meats from two birds were pooled and ground twice through a 3-mm plate. The ground turkey thigh meat was used to prepare meat homogenates. A 5-g meat sample was placed in a 50-mL test tube and homogenized with 15 mL of DDW by using a Polytron6 (Type PT 10/35) for 15 s at speed 7 to 8. Iron from each of the various sources (0.1 mL) and 0.5 mL oil emulsion or meat homogenate were added to disposable test tubes (13 × 100 mm). The homogenates were then added with 0.1 to 0.4 mL DDW and other treatments. In ascorbate-containing homogenates, 0.1 mL ascorbate solution was added instead of DDW to give a total volume of 1 mL. One 5-g homogenate was used as one replication and four replications per treatment were prepared.

Lipid Peroxidation and Nonheme Iron Determinations

Lipid peroxidation was determined by the method of Buege and Aust (1978). Test tubes containing 1 mL sample mixture, prepared as above, were incubated for 1 h in a 37 °C water bath. Immediately after incubation, 25 µL 7.2% BHA and 2 mL TBA/TCA solution were added. The mixture was vortexed and then incubated in a boiling waterbath for 15 min to develop color. After color development, the samples were cooled in cold water for 10 min and then centrifuged for 15 min at 2,000 × g. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL DDW and 2 mL TBA/TCA solution. The TBARS numbers were expressed as milligrams malonaldehyde (MDA) per liter of incubation reaction mixtures. The ferrozone method of Carter (1971), modified for use in meat samples (Ahn et al., 1993), was used to analyze reduced iron and total iron when needed. Iron sources in the model system were filtered through a

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4Sigma Chemical Co., St. Louis, MO 63178-9916.
5Dynamic Corp., America, New Hartland, CN 06507.
6Brinkman Instruments Inc., Westbury, NY 11590-0207.
AHN AND KIM

TABLE 1. Effect of superoxide-generating systems¹ on the thiobarbituric acid reactive substances (TBARS) values of oil emulsion with ascorbate and various iron sources²,³

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>Fe³⁺</th>
<th>Fe²⁺</th>
<th>Hb</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg MDA/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ascorbate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.02</td>
<td>0.04</td>
<td>2.74a</td>
<td>3.16a</td>
<td>0.02</td>
</tr>
<tr>
<td>KO₂</td>
<td>0.01</td>
<td>0.06</td>
<td>0.41b</td>
<td>3.07a</td>
<td>0.03</td>
</tr>
<tr>
<td>XOD system</td>
<td>0.03</td>
<td>0.04</td>
<td>0.35b</td>
<td>1.88b</td>
<td>0.04</td>
</tr>
<tr>
<td>SEM</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>With ascorbate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.22a</td>
<td>2.00a</td>
<td>2.40a</td>
<td>3.05a</td>
<td>1.47a</td>
</tr>
<tr>
<td>KO₂</td>
<td>0.05b</td>
<td>0.11c</td>
<td>1.05b</td>
<td>2.89b</td>
<td>0.14c</td>
</tr>
<tr>
<td>XOD system</td>
<td>0.08b</td>
<td>0.52b</td>
<td>1.04b</td>
<td>1.37c</td>
<td>0.44b</td>
</tr>
<tr>
<td>SEM</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

¹Means within a column of same ascorbate treatment with no common superscript differ significantly (P < 0.05).
²KO₂ and XOD (xanthine oxidase + xanthine) systems.
³Values are means of four replications.
⁴Iron sources: 5 ppm iron equivalent. XOD, 0.113 U/mL; ascorbate, 0.568 mM; all the rest, 2 mM (final concentrations). Reaction mixtures were incubated for 60 min at 37 °C.

Centricon™ membrane filter⁷ (cut-off size, 10,000 kDa), centrifuged at 3,000 × g for 120 min. The filtrate was then used to determine the amount of free iron released from different iron sources under various conditions.

**Ascorbic Acid Analysis**

Changes in ascorbic acid were analyzed by the method of Sikic et al. (1977). Two milliliters of samples, prepared with 4% TCA solution, were added with 0.1 mL of 85% orthophosphoric acid, 0.1 mL of 8% α, α’-dipyridyl in ethanol, and 0.1 mL of 3% aqueous ferric chloride. The mixture was vortexed, allowed 60 min for the ferrous-dipyridyl chromophore to develop, and then read at 525 nm. Standard curves were also made using known amounts of ascorbic acid.

**Statistical Analysis**

The experiment was designed primarily to determine the role of -O₂⁻ and -O₂⁻ generating systems on the status of iron and iron-mediated lipid peroxidation in oil emulsion and meat homogenates. One-way ANOVA was conducted to test treatment effects within an iron source by SAS® software (SAS Institute, 1988). Four replications were performed from deboned meat from eight carcasses (two carcasses per replication). The mean values of each treatment were compared for statistical significance (P < 0.05) using a Student-Newman-Keuls multiple range test. Mean values and SEM were reported, and replications were used as the error terms for the calculations.

**RESULTS AND DISCUSSION**

**Superoxide-Generating Systems on the Oxidation of Oil Emulsion**

Data in Table 1 clearly showed that the status of iron is very important for the pro-oxidant effects of iron in oil emulsion, and the presence of ascorbate or the -O₂⁻- generating systems significantly influenced the catalytic effects of ionic iron and ferritin. Without ascorbate, treatments with no iron (none), ferric iron and ferritin had no effect on the oxidation of oil emulsion, and the addition of -O₂⁻-generating system (KO₂ or XOD system) to the oil emulsion did not increase the thiobarbituric acid reactive substances (TBARS) values for these treatments. Only ferrous iron and Hb had very strong pro-oxidant effects and produced large amount of lipid oxidation products in oil emulsion. Both KO₂ and XOD systems reduced (P < 0.05) the catalytic effect of ferrous iron; however, only the XOD system reduced the catalytic effect of Hb in oil emulsion but the effect of the XOD was stronger than that of the KO₂ system (Table 1). These results are similar to those observed by Ito et al. (1987), where -O₂⁻ produced from the KO₂ and XOD

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⁷Amicon Inc., Beverly, MA 01915.
systems have different inactivating actions on *Bacillus subtilis* DNA in vitro.

**Products of \( \cdot \text{O}_2^- \)-Generating Systems on the Oxidation of Oil Emulsion**

Superoxide and urate are considered to be end products of the XOD system; however, \( \text{H}_2\text{O}_2 \) also could be generated from the XOD system in aqueous conditions (Halliwell and Gutteridge, 1989). In the absence of ascorbate, the pro-oxidant effects of ferric iron and ferritin in oil emulsion were not influenced by the presence of the products of superoxide-generating systems – \( \cdot \text{O}_2^- \), \( \text{H}_2\text{O}_2 \), and urate (Table 2). This result indicates that none of the products of \( \cdot \text{O}_2^- \)-generating systems can reduce ferric iron to ferrous form. The catalytic effect of ferrous iron was not affected by the presence of urate but was significantly reduced by \( \text{H}_2\text{O}_2 \), \( \text{H}_2\text{O}_2 \) plus \( \cdot \text{O}_2^- \), and \( \cdot \text{O}_2^- \) plus urate. The pro-oxidant effect of Hb was also decreased with the presence of \( \text{H}_2\text{O}_2 \) or \( \text{H}_2\text{O}_2 \) plus \( \cdot \text{O}_2^- \) but the decrease was smaller than that of the ferrous iron. These results do not agree with those of Kanner and Harel (1985), who reported that the presence of \( \text{H}_2\text{O}_2 \) activated heme pigments and caused the oxidation of sarcosomal lipids prepared from muscle tissues. Ahn and Kim (1998) found that the pro-oxidant effects of Hb differ dramatically by the study systems, which most likely could be the major reason for the disagreement between the two studies. Urate is known to have iron-chelating capability; however, in this study, it had no effect on the TBARS value of oil emulsion containing ferrous iron probably because the iron chelating effect of urate is limited only to ferric iron.

In the presence of ascorbate, ferric iron and ferritin showed strong pro-oxidant effects in oil emulsion when no iron or urate was added. However, the pro-oxidant effect of ferric iron and ferritin in oil emulsion disappeared when \( \text{H}_2\text{O}_2 \), \( \cdot \text{O}_2^- \) plus \( \text{H}_2\text{O}_2 \), or \( \cdot \text{O}_2^- \) plus urate was added (Table 2). The catalytic effects of ferrous iron and Hb were not influenced by the presence of urate but were reduced significantly when \( \text{H}_2\text{O}_2 \) and/or \( \cdot \text{O}_2^- \) were present. The greatest decrease in catalytic effect of ferrous iron was observed when \( \text{H}_2\text{O}_2 \) was present. Hemoglobin itself—even without \( \cdot \text{O}_2^- \) or \( \text{H}_2\text{O}_2 \)—showed a strong pro-oxidant effect and the decomposition of \( \text{H}_2\text{O}_2 \) by the Fenton-like reaction seems to play a minor role on the pro-oxidant effect of Hb in oil emulsion (Table 2). Benatti *et al.* (1983) reported that ascorbate-mediated interconversion of metmyoglobin and oxymyoglobin produces \( \text{H}_2\text{O}_2 \), and eventually results in the production of \( \cdot \text{OH} \) by the Fenton-like reaction and promotes lipid oxidation. Our results, however, did not agree with the conclusion of Benatti *et al.* (1983) because no catalysis of lipid oxidation of \( \cdot \text{OH} \) (increase in TBARS values) was observed in oil emulsion samples containing Hb, ascorbate, and \( \text{H}_2\text{O}_2 \).

Tables 1 and 2 clearly indicate that there are certain relationships between the \( \cdot \text{O}_2^- \)-generating systems or their products and the changes in the amount of ascorbate and the status of iron, which would determine the pro-oxidant effect of iron. The relationships between \( \cdot \text{O}_2^- \)-generating systems and the status of ionic iron or the amount of ascorbate are shown in Figures 1 and 2. The amount of ferrous iron gradually decreased with the increasing amount of the KO2 or XOD system (Figure 1). The amount of ferrous iron gradually decreased with the increasing amount of added KO2, or the incubation time of samples containing the XOD system (Figure 2). These results indicate that the decreased pro-oxidant

### Table 2. Effect of the products superoxide-generating systems\(^1\) on the thiobarbituric and reactive substances (TBARS) values of oil emulsion with ascorbate and various iron sources

<table>
<thead>
<tr>
<th>Treatment</th>
<th>none+asc.</th>
<th>Fe(^{3+})</th>
<th>Fe(^{2+})</th>
<th>Hemoglobin</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg MDA/L reaction mixture)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No ascorbate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.02</td>
<td>0.04</td>
<td>2.74(^a)</td>
<td>3.16(^a)</td>
<td>0.02</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )</td>
<td>0.01</td>
<td>0.08</td>
<td>0.34(^b)</td>
<td>1.86(^b)</td>
<td>0.04</td>
</tr>
<tr>
<td>Urate</td>
<td>0.02</td>
<td>0.05</td>
<td>2.63(^a)</td>
<td>3.13(^a)</td>
<td>0.03</td>
</tr>
<tr>
<td>( \cdot \text{O}_2^- ) + ( \text{H}_2\text{O}_2 )</td>
<td>0.05</td>
<td>0.08</td>
<td>0.41(^b)</td>
<td>1.40(^c)</td>
<td>0.05</td>
</tr>
<tr>
<td>( \cdot \text{O}_2^- ) + urate</td>
<td>0.01</td>
<td>0.04</td>
<td>0.09(^c)</td>
<td>3.07(^a)</td>
<td>0.04</td>
</tr>
<tr>
<td>SEM</td>
<td>0.00</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>With ascorbate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.22(^a)</td>
<td>2.00(^a)</td>
<td>2.40(^a)</td>
<td>3.05(^a)</td>
<td>1.47(^a)</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )</td>
<td>0.09(^b)</td>
<td>0.16(^b)</td>
<td>0.42(^d)</td>
<td>2.44(^b)</td>
<td>0.14(^b)</td>
</tr>
<tr>
<td>Urate</td>
<td>0.02(^a)</td>
<td>1.27(^a)</td>
<td>2.37(^a)</td>
<td>2.97(^b)</td>
<td>1.45(^a)</td>
</tr>
<tr>
<td>( \cdot \text{O}_2^- ) + ( \text{H}_2\text{O}_2 )</td>
<td>0.23(^a)</td>
<td>0.26(^c)</td>
<td>1.02(^b)</td>
<td>2.61(^b)</td>
<td>0.17(^b)</td>
</tr>
<tr>
<td>( \cdot \text{O}_2^- ) + urate</td>
<td>0.02(^b)</td>
<td>0.10(^c)</td>
<td>0.80(^c)</td>
<td>1.98(^c)</td>
<td>0.15(^b)</td>
</tr>
<tr>
<td>SEM</td>
<td>0.00</td>
<td>0.02</td>
<td>0.02</td>
<td>0.08</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^{a-d}\)Means within a column of same ascorbate treatment with no common superscript differ significantly (\( P < 0.05 \)).

\(^1\)Values are means of four replications.

\(^3\)Iron sources: 5 ppm iron equivalent. Xanthine oxidase (XOD), 0.113 U/mL; superoxide dismutase (SOD), 100 U/mL; catalase, 500 U/mL; ascorbate, 0.568 mM; all the rest, 2 mM (final concentrations). KO2 was used to generate \( \cdot \text{O}_2^- \). TBARS: mg malonaldehyde (MDA)/L reaction mixture. XOD system: XOD + xanthine. Reaction mixtures were incubated for 60 min at 37 C.
effect of ferrous iron in oil emulsion with superoxide-generating systems (KO2 and XOD system) in Table 1 were caused by the oxidation of ferrous iron to ferric form by \( \cdot \text{O}_2^- \).

Figure 3 indicates that there is no detectable ferrous iron in the sample after the reaction of \( \cdot \text{O}_2^- \) with ferric iron but the addition of ascorbate reduced ferric iron to ferrous form. Superoxide is generally known as a reducing agent for iron. However, our results indicated that \( \cdot \text{O}_2^- \) is not a reducing agent but an oxidizer of ionic iron and ascorbate in aqueous solution or oil emulsion. Halliwell and Gutteridge (1989) also noted that superoxide in aqueous solution can be an oxidizing agent for ferrous iron and ascorbate. Because the ferrozine method has very low affinity for Fe\(^{3+}\) and detects only Fe\(^{2+}\) (Boyer et al., 1988), it was concluded that ferrous iron was not formed from the reaction of \( \cdot \text{O}_2^- \) and ferric iron, which confirms the role of \( \cdot \text{O}_2^- \) as an oxidizing agent for ionic iron rather than a reducing agent \textit{in vitro}.

Figure 4 shows the relationship between the changes of ferrous iron and TBARS values in oil emulsion during incubation. The amount of ferrous iron added in oil emulsion containing XOD system declined rapidly during the first minute of incubation and then leveled off after

![Figure 1](image1.png)

**FIGURE 1.** Effect of potassium superoxide (KO2) and the xanthine oxidase (XOD) system on the content of ferrous iron in 50 mM maleate buffer (pH 6.5) solution. Five parts per million of ferrous iron and appropriate amounts of KO2 or XOD system were added in 50 mM maleate buffer, pH 6.5 solution, and then incubated at 37 C for 30 min before iron (ferrous) analysis.

![Figure 2](image2.png)

**FIGURE 2.** Effect of potassium superoxide (KO2) and the xanthane oxidase (XOD) system on the content of residual ascorbate during incubation. A) KO2 was added in ascorbate solution (100 ppm) prepared with distilled water, and incubated at room temperature (23 C) for 30 min before analysis; B) XOD system was added in model system (50 mM maleate buffer, pH 6.5) or oil emulsion containing 63 ppm ascorbate, and then incubated at 37 C. ( model system; O, oil emulsion). XOD system contains 2 mM xanthine and 0.113 U XOD/mL (final concentration). Oil emulsion was prepared by emulsifying 0.25 mL oil in 160 mL maleate buffer (50 mM, pH 6.5).
that. The changes in TBARS values were the mirror image of ferrous iron change and increased dramatically during the first minute of incubation. These results indicate that lipid oxidation occurs simultaneously with the conversion of ferrous to ferric iron that generates \( \cdot \)OH in oil emulsion.

According to the \( \cdot \)O\(_2^\cdot\)-dependent Haber-Weiss reaction, \( \cdot \)O\(_2^\cdot\) reduces ferric iron to the ferrous form Reaction [1] and \( \text{H}_2\text{O}_2 \) is produced from \( \cdot \)O\(_2^\cdot\) by Reaction [2] with the help of ferrous iron generated from Reaction 1.

\[
\begin{align*}
\cdot \text{O}_2^\cdot + \text{Fe}^{3+} & \rightarrow \text{Fe}^{2+} + \text{O}_2 \quad [1] \\
\cdot \text{O}_2^\cdot + 2\text{H}^+ & \rightarrow \text{H}_2\text{O}_2 \quad [2] \\
\text{H}_2\text{O}_2 + \text{Fe}^{2+} & \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot \text{OH} \quad [3]
\end{align*}
\]

![Image](image.png)

**FIGURE 3.** Amount of reduced iron in the model system (\( \bullet \), control; \( \diamond \), ascorbate; \( \text{\textcircled{K}} \), \( \text{K} \text{O}_2 \); +, ascorbate + \( \text{K} \text{O}_2 \)). Iron content was determined after the reaction of ascorbate, superoxide, and ascorbate plus superoxide with ferric or ferrous iron. Samples were incubated at room temperature (22 °C) for 30 min, and then analyzed for ferrous iron by the ferrozine method.

**FIGURE 4.** Effect of xanthine oxidase (XOD) system on the thiobarbituric acid reactive substances (TBARS) values and ferrous iron content in oil emulsion during incubation. XOD system (2 mM xanthine and 0.113 U XOD/mL, final concentration) was added in oil emulsion prepared with 50 mM maleate buffer, pH 6.5 and then incubated at 37 °C (\( \bullet \), amount of ferrous iron; \( \text{\textcircled{K}} \), TBARS values).

Therefore, both the ferric and ferrous forms of iron added in the oil emulsion with \( \cdot \)O\(_2^\cdot\) or \( \text{H}_2\text{O}_2 \) should catalyze lipid oxidation via the action of \( \cdot \)OH. The results of the present study showed that only ferrous iron had a pro-oxidant effect in oil emulsion and the reduction of ferric iron by ascorbate catalyzed lipid oxidation and increased the TBARS values of oil emulsion. In the absence of ascorbate in the oil emulsion containing \( \text{H}_2\text{O}_2 \), the production of \( \cdot \)OH via the Fenton reaction and the catalysis of lipid oxidation lasted only for a short time. When ascorbate was added in oil emulsion, however, the added ascorbate reduced ferric iron to ferrous form even in \( \cdot \)O\(_2^\cdot\) and \( \text{H}_2\text{O}_2 \) treatments. Therefore, the cycling of ferrous iron \( \leftrightarrow \) ferric iron in oil emulsion with ascorbate, ferrous iron, and XOD system produced \( \cdot \)OH continu-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>( \text{Fe}^{3+} )</th>
<th>( \text{Fe}^{2+} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.22(^b)</td>
<td>1.07(^b)</td>
<td>1.34(^b)</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>0.35(^a)</td>
<td>1.17(^ab)</td>
<td>1.52(^a)</td>
</tr>
<tr>
<td>( \text{K} \text{O}_2 )</td>
<td>0.30(^a)</td>
<td>1.27(^a)</td>
<td>1.72(^b)</td>
</tr>
<tr>
<td>XOD system</td>
<td>0.12(^c)</td>
<td>0.60(^c)</td>
<td>0.69(^c)</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\(^a\)\(^c\)Means within a column with no common superscript differ significantly \( (P < 0.05) \).

\(^1\)\( \text{K} \text{O}_2 \) and xanthine oxidase (XOD) (XOD + xanthine) systems.

\(^2\)Values are means of four replications.

\(^3\)Iron sources: 5 ppm iron equivalent. XOD, 0.113 U/mL; ascorbate, 0.568 mM; all the rest, 2 mM (final concentrations). TBARS: mg malonaldehyde (MDA)/L reaction mixture. Reaction mixtures were incubated for 60 min at 37 °C.
the antioxidant effect of \( \cdot O_2^- \)-generating systems in oil emulsion was caused by the oxidation of ferrous iron to the ferric form by \( \cdot O_2^- \) and/or \( H_2O_2 \). 3) both ferrous iron and Hb had strong prooxidant effects but ferric iron had no pro-oxidant effect in oil emulsions, 4) the pro-oxidant effect of ferric iron in raw meat homogenates was caused by the conversion of ferric iron to ferrous form by reducing enzymes and agents, and 5) the status of heme iron and the released iron from Hb had minor effects on the catalytic effect of Hb in oil emulsion.

REFERENCES


**Table 4. Thiobarbituric acid reactive substances (TBARS) values of oil emulsion and turkey raw-meat homogenates with hemoglobin (Hb) treated with \( H_2O_2 \) or desferioxamine**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oil emulsion</th>
<th>Raw meat homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td>Hb</td>
<td>3.99</td>
<td>0.07</td>
</tr>
<tr>
<td>Hb + ( H_2O_2 )</td>
<td>3.21</td>
<td>0.08</td>
</tr>
<tr>
<td>Hb + desferioxamine</td>
<td>3.17</td>
<td>0.06</td>
</tr>
<tr>
<td>SEM</td>
<td>0.04</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 4 indicated that Hb had a very strong pro-oxidant effect in oil emulsion but had no catalytic effect on the oxidation of lipids in raw meat homogenates. Hemoglobin catalyzed lipid oxidation in oil emulsion with or without \( H_2O_2 \) but Hb could not catalyze lipid oxidation in raw-meat homogenates even with the presence of \( H_2O_2 \).}

The oxidation of lipids continued while ascorbate was available (Tables 1 and 2).

**Superoxide-Generating Systems on the Oxidation of Meat Homogenates**

The addition of either ferrous or ferric iron increased the TBARS values of raw meat homogenates by three- to five-fold over the control. However, the pro-oxidant effects of iron in raw meat homogenates by the addition of ascorbate or \( \cdot O_2^- \)-generating systems were smaller than those in oil emulsion (Table 3). The KO\(_2\) system increased the pro-oxidant effect of ferric iron but the XOD system decreased the catalytic effect of ferric and ferrous iron in raw meat homogenates. It was presumed that the strong pro-oxidant effect of ferric iron in raw meat homogenates was caused by reducing enzymes and agents (e.g., ascorbate, glutathione) naturally present in meat. The amount of ascorbate in raw turkey leg meat was approximately 50 ppm and should have played an important role on the conversion of ferric iron to ferrous form in raw meat homogenates.

The results shown in this study underscore a few important points: 1) \( \cdot O_2^- \) was a strong oxidizer rather than a reducing agent of ferrous iron and ascorbate in vitro, 2)


