Influence of early postmortem protein oxidation on beef quality

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Abstract
The objective of this study was to examine the effect of early postmortem protein oxidation on the color and tenderness of beef steaks. To obtain a range of oxidation levels, the longissimus lumborum muscles (LM) from both strip loins of 20 steers fed either a finishing diet with vitamin E (1,000 IU per steer daily, minimum of 126 d [VITE]; n = 10 steers) or fed the same finishing diet without vitamin E (CON; n = 10 steers) were used. Within 24 h after slaughter, the LM muscle from each carcass was cut into 2.54-cm-thick steaks and individually vacuum packaged. Steaks from each steer were assigned to a nonirradiated group or an irradiated group. Steaks were irradiated within 26 h postmortem, and were aged at 4°C for 0, 1, 3, 7, and 14 d after irradiation. Steaks from each diet/irradiation/aging time treatment were used to determine color, shear force, and degree of protein oxidation (carbonyl content). Steaks from steers fed the VITE diet had higher (P < 0.01) a-tocopherol contents than steaks from steers fed the CON diet. Immediately following irradiation, steaks that had been irradiated had lower (P < 0.05) L* values regardless of diet. Irradiated steaks, regardless of diet, had lower a* (P < 0.05) and b* (P < 0.01) values than nonirradiated steaks at all aging times. Carbonyl concentration was higher (P < 0.05) in proteins from irradiated steaks compared to nonirradiated steaks at 0, 1, 3, and 7 d postirradiation. Immunoblot analysis showed that vitamin E supplementation decreased the number and extent of oxidized sarcoplasmic proteins. Protein carbonyl content was positively correlated with Warner-Bratzler shear force values. These results indicate that increased oxidation of muscle proteins early postmortem could have negative effects on fresh meat color and tenderness.

Keywords
beef, irradiation, oxidation, protein, quality, tenderness

Disciplines
Agriculture | Animal Sciences | Meat Science

Comments
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Influence of early postmortem protein oxidation on beef quality1

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ABSTRACT: The objective of this study was to examine the effect of early postmortem protein oxidation on the color and tenderness of beef steaks. To obtain a range of oxidation levels, the longissimus lumborum muscles (LM) from both strip loins of 20 steers fed either a finishing diet with vitamin E (1,000 IU per steer daily, minimum of 126 d [VITE]; n = 10 steers) or fed the same finishing diet without vitamin E (CON; n = 10 steers) were used. Within 24 h after slaughter, the LM muscle from each carcass was cut into 2.54-cm-thick steaks and individually vacuum packaged. Steaks from each steer were assigned to a nonirradiated group or an irradiated group. Steaks were irradiated within 26 h postmortem, and were aged at 4°C for 0, 1, 3, 7, and 14 d after irradiation. Steaks from each diet/irradiation/aging time treatment were used to determine color, shear force, and degree of protein oxidation (carbonyl content). Steaks from steers fed the VITE diet had higher (P<0.01) \( \alpha \)-tocopherol contents than steaks from steers fed the CON diet. Immediately following irradiation, steaks that had been irradiated had lower (P < 0.05) \( L^* \) values regardless of diet. Irradiated steaks, regardless of diet, had lower a* (P < 0.05) and b* (P < 0.01) values than nonirradiated steaks at all aging times. Carbonyl concentration was higher (P < 0.05) in proteins from irradiated steaks compared to nonirradiated steaks at 0, 1, 3, and 7 d postirradiation. Immunoblot analysis showed that vitamin E supplementation decreased the number and extent of oxidized sarcoplasmic proteins. Protein carbonyl content was positively correlated with Warner-Bratzler shear force values. These results indicate that increased oxidation of muscle proteins early postmortem could have negative effects on fresh meat color and tenderness.

Key Words: Beef, Irradiation, Oxidation, Protein, Quality, Tenderness

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Introduction

Metabolic and other processes occurring in muscle tissue give rise to formation of reactive oxygen species and other oxidative compounds. These oxidative species include hydroxyl radicals, peroxyl radicals, superoxide anions, hydrogen peroxide, and nitric oxide (Butterfield et al., 1998; Burton and Traber, 1990). Reactive oxygen species can interact with both lipids and proteins. In postmortem muscle tissue, when proteins are targeted by reactive oxygen species, the result of this interaction is often carbonyl formation and decreased sulphydryl content of the protein (Hoffman and Hamm, 1978; Martinaud et al., 1997; Xiong, 2000). These modifications can significantly alter the properties of meat proteins and may ultimately influence the quality of meat products (Xiong, 2000).

Several processes influence oxidation of fresh meat. High levels of vitamin E in muscle results in meat with decreased rate of lipid oxidation, delayed metmyoglobin formation (Arnold et al., 1992, 1993; Liu et al., 1996), and increased rate of tenderization (Harris et al., 2001). Irradiation, while a powerful food safety tool, has been implicated in accelerating oxidation and producing free radicals, thereby changing the oxidative potential of meat products (Jo and Ahn, 2000). Irradiation can influence the color of fresh meat (Nanke et al., 1998), yet reports of its effects on tenderness have been mixed (Heath et al., 1990; Yoon, 2003). However, few studies have been performed on early postmortem product. A recent report has shown that low-dose irradiation of chicken breasts within 24 h of slaughter resulted in product with significantly higher shear force values than nonirradiated controls (Yoon, 2003).
Currently, little is known about the effects of protein oxidation during the first 24 to 48 h after exsanguination on beef quality. Therefore, the objective of this study was to examine the impact of early postmortem protein oxidation on the color and tenderness of beef steaks.

Materials and Methods

Animals

Twenty beef steers of similar age and genetics were used in this study. To obtain a range of oxidative conditions, 10 steers were group fed a normal finishing diet (CON), whereas another 10 steers were group fed the same finishing diet that included 1,000 IU per head per day of vitamin E (VITE; Roche Vitamins, Inc., Parsippany, NJ) for at least the last 126 d before slaughter. The CON diet contained the following ingredients (DM basis): dry rolled corn (59%), chopped grass hay (8%), cane molasses (0.40%), wet corn gluten feed (30%), urea (0.15%), ground limestone (1.40%), trace mineral premix (0.024%); premix contained 13.2% Ca++, 0.10% Co, 1.5% Cu++, 10.0% Fe++, 0.44% Fe+++, 0.2% I, 8% Mn++, 5.0% S, and 12.0% Zn), salt (NaCl; 0.30%), Rumensin premix (0.0195%); Rumensin premix provided 34.4 mg of monensin sodium/kg feed on a DM basis), and vitamin A premix (0.08% on a dry matter basis; vitamin A premix provided 3,084 IU of vitamin A activity per kilogram of feed on a DM basis). Steers were approximately 9 mo old and weighed an average of 396 kg at the start of the feeding trial. Steers were weighed at 28-d intervals during the feeding trial to calculate ADG. Steers were slaughtered at an average weight of 634 kg using approved humane procedures at Iowa State University Meat Laboratory. At each slaughter time, four steers (two from each dietary treatment), were slaughtered, and the carcasses were conventionally chilled at −5°C for 24 h.

At 2, 4, 6, 8, and 24 h postmortem, temperature was measured using an Electrotherm digital probe (model No. TM99A; Cooper Instrument Corp, Middlefield, CT), and pH measurements were taken using a glass body insertion electrode (pH-Star S, SFK Technologies, Herlev, Denmark). Measurements were taken in the longissimus thoracis at the 12th rib on both sides of the carcass.

Collection of Steaks

Strip loins were removed from both sides of each carcass between 21 to 24 h postexsanguination, and 2.54-cm-thick longissimus lumborum (LM) steaks were cut from each strip loin and immediately vacuum packaged. All LM steaks (10 steaks/strip loin) from one loin from each carcass were assigned as a nonirradiated control, whereas all LM steaks from opposite-side strip loin of each carcass were assigned to be irradiated. Two adjacent steaks from each strip loin were assigned to an aging period of 0, 1, 3, 7, or 14 d postirradiation (1, 2, 4, 8, or 15 d postmortem) at 4°C. One steak from each aging period was designated for color, carbonyl, and sulfhydryl analysis, and one steak was designated for Warner-Bratzler shear force determination. In order to determine whether dietary treatment increased the vitamin E content of the steaks prior to irradiation, an additional steak was taken from each carcass at the posterior end of the strip loin, vacuum-packaged, frozen at −20°C, and sent to the University of Wisconsin Soil and Plant Analysis Laboratory (Madison, WI) for analysis of α-tocopherol content. α-Tocopherol content was determined according to the procedures of Liu et al. (1996).

Irradiation of Steaks

Irradiation was conducted at the Linear Accelerator Facility (LAF) in the Iowa State University Meat Laboratory. At 24 to 26 h postexsanguination, vacuum-packaged steaks from one side of each animal were irradiated (average dose = 6.4 kGy). Steaks from the opposite-side strip loins were not irradiated but were held at the same temperature (approximately 20°C) for the same length of time (approximately 10 min) as were the irradiated steaks. Samples were irradiated by a CIRCE IIR Electron Beam irradiator (Thomson-CSF Linac, St. Aubin, France) with an energy level of 10 MeV, a power level of 10 kW, and a conveyor speed of 0.232 m/min. After irradiation, all steaks (irradiated and nonirradiated) were held at 4°C for 0, 1, 3, 7, or 14 d postirradiation (1, 2, 4, 8, and 15 d postmortem). At the completion of each aging period, steaks designated for Warner-Bratzler shear force were frozen until subsequent analysis. Steaks designated for laboratory analysis were used immediately for color measurements and biochemical analysis.

Warner-Bratzler Shear Force

All procedures were done in accordance to AMSA (1995) guidelines. Frozen 2.54-cm-thick steaks were thawed at 2°C and used for Warner-Bratzler shear force (WBSF) determination. Steaks were broiled in an electric broiler (General Electric, Model 6850; Chicago Heights, IL) 15 cm away from the heat source. Steaks were broiled to an internal temperature of 30°C and then turned and broiled to a final temperature of 70°C. Temperature was monitored using an Electrotherm digital probe (model No. TM99A; Cooper Instrument Corp.) Steaks were covered with Saran wrap and allowed to chill overnight at 4°C. Steaks were equilibrated to room temperature (approximately 1 to 2 h), and six 1-cm-diameter cores were removed parallel to the muscle fibers. Each core was sheared perpendicular to the fiber direction using a TA XT2 Texture Analyzer with a 5-kg load cell (Texture Technologies Corp., Scarsdale, NY). All tests were performed using the Warner-Bratzler probe and guillotine set (TA-7B USDA, Tex-
Table 1. Carcass characteristics of steers fed control and vitamin E-supplemented diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Vitamin E</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of carcasses</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Hot carcass weight, kg</td>
<td>413.4</td>
<td>410.2</td>
<td>4.11</td>
</tr>
<tr>
<td>Marblinga</td>
<td>241.0</td>
<td>273.0</td>
<td>16.74</td>
</tr>
<tr>
<td>Longissimus muscle area, cm²</td>
<td>88.5</td>
<td>89.6</td>
<td>1.73</td>
</tr>
<tr>
<td>12th-rib fat, cm</td>
<td>1.1</td>
<td>1.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Kidney, pelvic, and heart fat, %</td>
<td>1.7</td>
<td>1.5</td>
<td>0.12</td>
</tr>
<tr>
<td>α-Tocopherol, μg/g (fresh weight)</td>
<td>1.22b</td>
<td>4.19g</td>
<td>0.163</td>
</tr>
<tr>
<td>pH 2 h</td>
<td>6.31</td>
<td>6.20</td>
<td>0.074</td>
</tr>
<tr>
<td>pH 4 h</td>
<td>5.87</td>
<td>5.86</td>
<td>0.062</td>
</tr>
<tr>
<td>pH 6 h</td>
<td>5.64</td>
<td>5.58</td>
<td>0.060</td>
</tr>
<tr>
<td>pH 8 h</td>
<td>5.43</td>
<td>5.44</td>
<td>0.044</td>
</tr>
<tr>
<td>pH 24 h</td>
<td>5.44</td>
<td>5.45</td>
<td>0.021</td>
</tr>
<tr>
<td>Temperature at 2 h, °C</td>
<td>38.1d</td>
<td>38.6e</td>
<td>0.10</td>
</tr>
<tr>
<td>Temperature at 4 h, °C</td>
<td>29.2d</td>
<td>30.5e</td>
<td>0.43</td>
</tr>
<tr>
<td>Temperature at 6 h, °C</td>
<td>21.0</td>
<td>22.0</td>
<td>0.51</td>
</tr>
<tr>
<td>Temperature at 8 h, °C</td>
<td>15.9</td>
<td>16.6</td>
<td>0.403</td>
</tr>
<tr>
<td>Temperature at 24 h, °C</td>
<td>0.9</td>
<td>1.0</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*100 = traces00, 200 = slight00, 300 = small00, 400 = modest00.

Within a row, means without a common superscript letter differ (P < 0.01).

Color Measurements

Color was measured after each aging period (0, 1, 3, 7, and 14 d after irradiation). Fresh steaks were removed from the vacuum packages and allowed to bloom for 15 min at 4°C. A Hunter Lab Mini Scan XE Plus (Hunter Associates Laboratories, Inc., Reston, VA) was used for measurement of L*, a*, and b* values. Illuminate D65 was used, and the instrument had a 10° observer and a port diameter of 25 mm. Three readings per steak were taken and averaged for statistical analysis.

Sarcoplastic Protein Extraction

Sarcoplastic proteins were extracted according to Shackelford et al. (1994) with modifications. At 0, 1, 3, 7, and 14 d postirradiation, 10 g of finely diced fresh meat were homogenized in 3 vol of ice-cold extraction buffer (10 mM EDTA, 2 μM E-64, 100 mg/L trypsin inhibitor, and 2 mM phenylmethylsulfonylfluoride

Table 2. L*, a*, b* values of strip loin steaks at each aging time point

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>L* values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON/ No Irrad</td>
<td>32.6d± 0.45</td>
<td>33.4± 0.82</td>
<td>34.0± 0.52</td>
<td>34.5± 0.56</td>
<td>37.4d± 0.47</td>
</tr>
<tr>
<td>VITE/ No Irrad</td>
<td>33.1f± 0.81</td>
<td>34.3± 0.79</td>
<td>35.5± 0.76</td>
<td>35.6± 0.78</td>
<td>38.1d± 0.47</td>
</tr>
<tr>
<td>CON/ Irrad</td>
<td>29.7f± 0.39</td>
<td>31.9± 0.43</td>
<td>34.3± 0.56</td>
<td>34.3± 0.70</td>
<td>35.6d± 0.51</td>
</tr>
<tr>
<td>VITE/ Irrad</td>
<td>31.0f± 0.63</td>
<td>33.6± 0.81</td>
<td>35.1± 0.78</td>
<td>34.7± 1.02</td>
<td>37.1e± 0.57</td>
</tr>
<tr>
<td>a* values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON/ No Irrad</td>
<td>19.4d± 0.41</td>
<td>19.4d± 0.42</td>
<td>19.9d± 0.29</td>
<td>20.6d± 0.52</td>
<td>19.8d± 0.32</td>
</tr>
<tr>
<td>VITE/ No Irrad</td>
<td>19.2d± 0.44</td>
<td>19.7d± 0.31</td>
<td>19.6d± 0.27</td>
<td>20.0d± 0.31</td>
<td>19.0d± 0.26</td>
</tr>
<tr>
<td>CON/ Irrad</td>
<td>10.8d± 0.67</td>
<td>12.7d± 0.64</td>
<td>13.4d± 0.41</td>
<td>13.4d± 0.59</td>
<td>14.4d± 0.25</td>
</tr>
<tr>
<td>VITE/ Irrad</td>
<td>9.9d± 0.72</td>
<td>10.6d± 0.68</td>
<td>11.8d± 0.56</td>
<td>12.4d± 0.51</td>
<td>12.8d± 0.43</td>
</tr>
<tr>
<td>b* values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON/ No Irrad</td>
<td>17.4d± 0.31</td>
<td>17.7d± 0.40</td>
<td>18.5d± 0.30</td>
<td>18.9d± 0.47</td>
<td>18.9d± 0.32</td>
</tr>
<tr>
<td>VITE/ No Irrad</td>
<td>17.3d± 0.36</td>
<td>18.3d± 0.26</td>
<td>18.4d± 0.29</td>
<td>18.8± 0.31</td>
<td>19.0d± 0.24</td>
</tr>
<tr>
<td>CON/ Irrad</td>
<td>13.3d± 0.33</td>
<td>15.3d± 0.39</td>
<td>15.7d± 0.11</td>
<td>15.8d± 0.40</td>
<td>16.9d± 0.23</td>
</tr>
<tr>
<td>VITE/ Irrad</td>
<td>13.2d± 0.39</td>
<td>14.4d± 0.39</td>
<td>15.2d± 0.23</td>
<td>15.9d± 0.38</td>
<td>16.3d± 0.14</td>
</tr>
</tbody>
</table>

aSteaks from steers not supplemented with vitamin E and not irradiated (n = 10).
bSteaks from steers supplemented with vitamin E and not irradiated (n = 10).
cSteaks from steers not supplemented with vitamin E and irradiated (n = 10).
dSteaks from steers supplemented with vitamin E and irradiated (n = 10).
e,f,gWithin L*, a*, or b* values in a column, means without a common superscript letter differ (P < 0.05).
hWithin b* values in a column, means without a common superscript letter differ (P < 0.01).
Figure 1. Solubility of sarcoplasmic proteins (mg protein extracted/g of tissue homogenized). Within a single time point, an asterisk (*) indicates a difference ($P < 0.05$) between irradiated (n = 20) and nonirradiated steaks (n = 20).

[FIGURE 1]

PMSF, 100 mM Tris-HCl, pH 8.3) using a polytron PT 3100 (Kinmetaica AG, Littau, Switzerland) set at 22,000 rpm. Samples were centrifuged (27,000 $\times$ g) for 30 min at 4°C. The supernatants were filtered through cheesecloth and sample volume was recorded. Protein concentration of each sample was determined using a Bradford assay (BioRad Protein Assay Kit; BioRad Laboratories, Hercules, CA; Bradford, 1976). The pellet fraction was used immediately for purification of myofibrils.

Myofibril Purification

Four grams of pellet from each sarcoplasmic protein extraction were weighed and homogenized in 10 vol of standard salt solution (100 mM KCl, 2 mM MgCl$_2$, 1 mM EGTA, 1 mM NaN$_3$, 20 mM K$_2$HPO$_4$, pH 7.0). Myofibrils were further purified by differential centrifugation (Huff-Lonergan et al., 1995), and protein concentration was determined using the Biuret method as modified by Robson et al. (1968).

Measurement of Protein Oxidation

Carbonyl Assay. Samples from each fraction (sarcoplasmic and myofibrillar) were diluted to 6 mg/mL using 1 mM EDTA, 50 mM NaHPO$_4$ (pH 7.4). Carbonyl content of both the sarcoplasmic proteins and highly purified myofibrils was assayed by reactivity with 2,4-dinitrophenylhydrazine (DNPH; Reznick and Packer, 1994). The carbonyl content was expressed as nanomoles of DNPH fixed per milligram of protein using an absorption coefficient of 21,000 M$^{-1}$cm$^{-1}$.

Sarcoplasmic Protein Gel Sample Preparation for Immunodetection of Carbonyls. Both DNPH-derivatized and their control samples from the carbonyl assay described previously were vortexed, and duplicate 200-μL aliquots were removed and placed in microcentrifuge tubes for sample preparation. Samples were concentrated by addition of an equal volume of ice-cold acetone, vortexed, and centrifuged at 21,000 $\times$ g for 10 min at 4°C. Supernatants were discarded, and the pellet was dissolved in 50 μL of 8 M urea and 40 mM Tris-HCl (pH 6.8). Samples were vortexed and heated at 37°C for 10 min to ensure solubilization. Protein concentra-
Oxidation of meat proteins

**Figure 4.** Western blot of oxidized sarcoplasmic proteins. Each lane was loaded with 4 μg of protein. Lane 1 is an oxidized positive control sample. Lane 2 was loaded with prestained molecular weight markers. Lanes 3 through 6 depict one representative animal that was fed the control diet and its respective irradiated and nonirradiated (Nonirr) steaks. Lanes 7 through 10 depict one representative animal that was fed the diet supplemented with vitamin E and its respective irradiated and nonirradiated steaks. Lanes 3, 5, 7, and 9 are from samples that were derivatized with 10 mM DNPH in 2.5 M HCl. Lanes 4, 6, 8, and 10 are from the same samples as Lanes 3, 5, 7, and 9 but were incubated with only 2.5 M HCl (no DNPH) and are negative controls. Bands indicate oxidized proteins (proteins with increased carbonyl content).

**Figure 5.** Western blot of oxidized myofibrillar proteins. All samples were made from purified myofibrils and are loaded with 15 μg of protein. Lane 1 is an oxidized positive control sample. Lane 2 was loaded with prestained molecular weight markers. Lanes 3 through 6 depict one representative animal that was fed the control diet and its respective irradiated and nonirradiated (Nonirr) steaks. Lanes 7 through 10 depict one representative animal that was fed the diet supplemented with vitamin E and its respective irradiated and nonirradiated steaks. Lanes 3, 5, 7, and 9 are from samples that were derivatized with DNPH. Lanes 4, 6, 8, and 10 are from the same samples as Lanes 3, 5, 7, and 9 but were not derivatized with DNPH and serve as negative controls. Bands indicate oxidized proteins (proteins with increased carbonyl content).

62.5 mM Tris-HCl, pH 6.8). Samples were stored at −80°C until SDS-PAGE and immunoblotting.

**Immunodetection of Oxidized Proteins.** Derivatized and control myofibril and sarcoplasmic samples were run on 10 cm (wide) × 12 cm (tall) × 1.5 mm (thick) 12% discontinuous polyacrylamide gels (Huff-Lonergan et al., 1996). After electrophoresis, samples were transferred onto Poly Screen polyvinylidene difluoride (PVDF) transfer membrane (NEN Life Science Products, Inc., Boston, MA). Transfer was done for 90 min at a constant 90 V in a TE-22 transfer tank (Amersham Biosciences, Piscataway, NJ) at refrigerated temperatures in 25 mM Tris, 192 mM glycine, and 15% methanol (vol/vol). Membranes were then blocked in PBS-Tween (80 mM disodium hydrogen orthophosphate, an-
Table 3. Correlations between carbonyl content of sarcoplasmic proteins and Warner-Bratzler Shear force (WBSF) measurement

<table>
<thead>
<tr>
<th>WBSF values</th>
<th>d 0</th>
<th>d 1</th>
<th>d 3</th>
<th>d 7</th>
<th>d 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 0</td>
<td>0.133</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1</td>
<td>0.134</td>
<td>0.195</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>d 3</td>
<td>0.205</td>
<td>0.224</td>
<td>0.558</td>
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<tr>
<td>d 7</td>
<td>0.031</td>
<td>0.347</td>
<td>0.406</td>
<td>0.386</td>
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</tr>
<tr>
<td>d 14</td>
<td>0.154</td>
<td>0.424</td>
<td>0.420</td>
<td>0.454</td>
<td>-0.266</td>
</tr>
</tbody>
</table>

*Pearson correlations in bold are significant (P < 0.05; n = 40/time point).

Table 4. Correlations between carbonyl content of myofibrillar proteins and Warner-Bratzler Shear force (WBSF) measurements

<table>
<thead>
<tr>
<th>WBSF values</th>
<th>d 0</th>
<th>d 1</th>
<th>d 3</th>
<th>d 7</th>
<th>d 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 0</td>
<td>0.272</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1</td>
<td>0.411</td>
<td>0.360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 3</td>
<td>0.453</td>
<td>0.487</td>
<td>0.364</td>
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</tr>
<tr>
<td>d 7</td>
<td>0.486</td>
<td>0.412</td>
<td>0.479</td>
<td>0.424</td>
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</tr>
<tr>
<td>d 14</td>
<td>0.633</td>
<td>0.530</td>
<td>0.425</td>
<td>0.530</td>
<td>0.167</td>
</tr>
</tbody>
</table>

*Pearson correlations in bold are significant (P < 0.05; n = 40/time point).
Color Analysis

Immediately after irradiation (d 0), instrumental color analysis showed that, within a diet group, irradiation resulted in lower \( P < 0.05 \) \( L^* \) values (Table 2). Nanke et al. (1998) reported similar values on vacuum-packaged beef strip loin steaks that were irradiated at specified doses. In the current study, saturation index and hue angle were calculated to determine the amount of discoloration incurred in the product (Little, 1975). Diet had no effect \( P > 0.05 \) on saturation index or hue angle; however, saturation index was lower \( P < 0.05 \) in the irradiated samples (CON/irradiated = 17.2, CON/ nonirradiated = 26.0, VITE/irradiated = 16.5, VITE/ nonirradiated = 25.9; data not shown). Irradiated beef had greater \( P < 0.05 \) hue angle irrespective of diet (CON/irradiated = 61.3, CON/ nonirradiated = 50.8, VITE/irradiated = 62.9, VITE/ nonirradiated = 50.9; data not shown). These results indicate that, shortly after processing, irradiation resulted in less intense color and greater discoloration of the steak surface regardless of diet.

At 1, 3, and 7 d postirradiation, no differences \( P > 0.05 \) were found among any of the treatment combinations for \( L^* \) value (Table 2). However, after 14 d of storage, irradiated steaks from animals fed the control diet (CON/irradiated) were darker (lower \( L^* \) values; \( P < 0.05 \)) than their nonirradiated counterparts (CON/ nonirradiated). In addition, when comparing across diets, the irradiated steaks from steers fed the control diet (CON/irradiated) were also darker (lower \( L^* \) values, \( P < 0.05 \)) than the other treatment groups.

At all aging times, nonirradiated steaks had higher \( P < 0.05 \) \( a^* \) values (indicating a redder color) than irradiated steaks, which is consistent with the results of Nanke et al. (1998). Further analysis at 1, 3, and 14 d postirradiation revealed that irradiated steaks from steers in the vitamin E supplementation group were less red in color \( P < 0.05 \) compared to irradiated steaks not supplemented with vitamin E (Table 2).

Analysis of \( b^* \) values revealed irradiated steaks had lower \( P < 0.01 \) \( b^* \) values at all days postirradiation studied (Table 2). However, the addition of vitamin E to the finishing diet had no \( P > 0.01 \) effect on \( b^* \). These results indicate that, after irradiation, the surfaces of steaks appeared less yellow in color compared to nonirradiated steaks. These results conflict with those reported by Nanke et al. (1998), who found no differences in \( b^* \) values at irradiation doses up to 7.5 kGy.

Collectively, these results indicate that irradiation has an immediate negative influence on the color of fresh, vacuum-packaged beef steaks. It has been hypothesized that the primary reason for the color change noted in irradiated beef is due to oxidation of myoglobin. Incorporation of vitamin E into the product by including it in the diet of steers prior to slaughter does not provide sufficient protection against discoloration when the product is irradiated at moderate doses.

Protein Solubility and Oxidation

The amount of soluble protein that can be extracted from the meat can give an indication of the relative level of denaturation that may have occurred. In this study, solubility of the sarcoplasmic proteins (on a milligram of protein extracted per gram of tissue) was evaluated 0, 3, and 14 d after irradiation. Whereas there was no difference \( P > 0.05 \) due to diet (VITE vs. CON), there was a difference \( P < 0.05 \) due to irradiation (Figure 1). No difference \( P > 0.05 \) was found when protein extractability was measured the day on which samples were irradiated (d 0); however, after 3 d of storage, the irradiated samples had less \( P < 0.05 \) extractable protein. After 14 d of storage, the irradiated samples still tended to have less \( P = 0.07 \) extractable protein than their nonirradiated counterparts (Figure 1), indicating that irradiation may influence the solubility of the sarcoplasmic protein.

In the current study, total carbonyl content was higher \( P < 0.05 \) in the sarcoplasmic proteins isolated from irradiated meat on 0, 1, 3, and 7 d after irradiation than sarcoplasmic proteins isolated from nonirradiated meat (Figure 2). Total carbonyl content was higher \( P < 0.05 \) in myofibrillar proteins isolated from irradiated steaks than in myofibrillar proteins isolated from nonirradiated steaks at all time points (0, 1, 3, 7, and 14 d postirradiation; Figure 3). However, diet had no \( P > 0.05 \) effect on total carbonyl content of either sarcoplasmic or myofibrillar protein.

Several proteins contain amino acids that are very susceptible to oxidation. Some of these amino acids include cysteine, histidine, methionine, lysine, and tryptophan (Xiong, 2000). Oxidative reactions involving the side chains of amino acids can lead to the formation of carbonyl groups. This conversion may ultimately result in a loss of catalytic activity and increased susceptibility to protein degradation (Stadtman, 1990) or protein aggregation and loss of solubility. Formation of carbonyls in meat can be caused by several oxidative treatments and has even been shown to occur in beef myofibrils during postmortem aging (Martinaud et al., 1997).

Western Blotting of Oxidized Sarcoplasmic Proteins

Highly sensitive Western blotting of derivatized sarcoplasmic samples revealed the extent to which specific sarcoplasmic proteins were oxidized by the irradiation treatment and/or protected by supplementation with the antioxidant (vitamin E; Figure 4). Western blots showed that irradiation increased the number of oxidized proteins compared to steaks that were not irradiated, as indicated by the appearance of more bands (immunologically detectable DNP residues) in irradiated steaks (Figure 4). Differences due to diet were detected in sarcoplasmic proteins from irradiated steaks. Steaks from steers supplemented with vitamin E and irradiated at 24 h postmortem contained fewer oxidized sarcoplasmic proteins compared to steaks from...
steers not supplemented with vitamin E and irradiated. However, the differences in nonirradiated steaks were not as striking. Steaks that were not irradiated and were from steers supplemented with vitamin E had only slightly less total oxidized proteins than nonirradiated un supplemented steaks (Figure 4). From these results, it is apparent that vitamin E supplementation may help to protect some sarcoplasmic proteins from becoming oxidized when the tissue is exposed to highly oxidizing conditions (like irradiation) early postmortem.

**Western Blotting of Oxidized Myofibrillar Proteins**

Western blotting results of oxidized myofibrils revealed, again, that irradiation increased the number of oxidized proteins compared to samples that were not irradiated (as indicated by the appearance of more bands in irradiated samples; Figure 5). Unlike the sarcoplasmic proteins, however, there were no observable differences in carbonyl content between diets in the myofibrillar samples. This might be partially explained by the fact that vitamin E is lipid soluble and, therefore, can be concentrated in lipid bilayers or membranes (Burton and Traber, 1990). In general, it appears that vitamin E has a greater impact on sarcoplasmic proteins than myofibrillar proteins in this study.

**Correlations Between Carbonyl Content**

In the current study, it was observed that there were significant positive correlations between the carbonyl content of both the sarcoplasmic and the myofibrillar proteins and WBSF, indicating that higher carbonyl content of the each protein fraction was associated with higher shear force (Tables 3 and 4). It is important to note that the carbonyl content of both the sarcoplasmic fraction and the highly purified myofibrils measured 1 d after irradiation (2 d postmortem) were correlated ($P < 0.05$) with the shear force after 14 d of aging ($r = 0.424$ and 0.530 for sarcoplasmic protein and myofibrillar protein, respectively). Thus, increased early postmortem protein oxidation (as measured by carbonyl content) in both the sarcoplasmic and myofibrillar fractions of the tissue is associated with increased shear force values at later times postmortem. It is possible that aggregation and denaturation of myofibrillar proteins, and/or inactivation of some proteolytic enzymes compromised the ability of beef to tenderize during aging.

Very few studies have examined the effects of early postmortem protein oxidation on the tenderness of meat at later times postmortem. There are several ways that protein oxidation could influence meat tenderness, and they include 1) alterations of the function/structure of specific proteins that are oxidized, 2) the extent of oxidation, and 3) the timing of the oxidative modification. In some cases, mild oxidation may render certain proteins more susceptible to degradation (Stadtman, 1990). Extensive oxidation of proteins may lead to denaturation and aggregation (Decker et al., 1993). Oxidation of one of the most abundant myofibrillar proteins, myosin, can cause the formation of large, insoluble aggregates (Xiong, 2000) that may be more resistant to degradation. In addition, the formation of carbonyl groups in some enzymes can result in a lack of catalytic activity (Stadtman, 1990). For example, histidine residues are among the many amino acids that are susceptible to carbonyl formation. Some proteases depend on a histidine residue in their active site for their activity. One of the enzyme systems thought to be responsible for postmortem protein degradation and early increases in tenderness is the calpain system. Hydrolysis of peptide bonds by calpains requires a transfer of electrons between the active site histidine and cysteine residues (Mehdi, 1991). Therefore, in order to function in postmortem muscle, the calpains (and other cysteine proteases) need to be maintained in a reduced form. If either of these processes (aggregation/denaturation of structural proteins or oxidation and inactivation of key enzymes) occurs in meat before tenderization is complete, it is possible that subsequent tenderization could be compromised. Because a significant amount of proteolysis does occur early postmortem (Melody et al., 2004), it is important to know whether oxidation of proteins occurring before aging is complete influences tenderness.

**Implications**

Irradiation is a very useful tool to improve food safety; however, because of its potential to induce protein oxidation (as well as lipid oxidation), the effects of irradiation on fresh meat quality need continued study to ensure consumer acceptance. Results of this study indicate that early postmortem irradiation of fresh beef steaks increases oxidation of both sarcoplasmic and myofibrillar proteins. More importantly, increased protein oxidation during the first 24 h postmortem (as measured by carbonyl content) can substantially decrease beef tenderness even in steaks aged 14 d.

**Literature Cited**


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