Chemical, Physical, and Functional Properties of Oxidized Turkey White Muscle Myofibrillar Proteins†

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Oxidative damage to turkey white muscle myofibrillar proteins (MP) was investigated by measuring changes in chemical, physical, and functional properties after exposure to iron or copper (25 μM) and ascorbate. Both iron- and copper-oxidized MP exhibited increased absorbance at 265 nm and carbonyl content compared to controls. Increasing the ascorbate concentration from 0 to 25 mM increased the absorbance 6.8- and 4.0-fold and the carbonyl content 2.6- and 1.9-fold for MP in the presence of iron and copper, respectively. The iron- and copper-oxidized MP showed lower solubility, gel strength, and gel water-holding capacity than controls. SDS–polyacrylamide gel electrophoresis demonstrated that both iron- and copper-catalyzed oxidation resulted in a major loss of myosin and actin with concomitant formation of protein polymers. These data suggest that the decreased functionality of proteins in muscle foods exposed to oxidative environments could be due to chemical and physical changes resulting from oxidative reactions.

INTRODUCTION

Oxidative processes in muscle foods result in decreased quality by causing changes in flavor, color, nutritional composition, and texture. While oxidatively induced off-flavors have been extensively studied, the effect of oxidation on the chemical and physical properties of muscle proteins is not fully understood. Smith (1987) found that both hand and mechanically deboned chicken exhibited loss in protein solubility, Ca^{2+}-ATPase activity, and cooking yields during storage. These changes in chemical and functional properties were reduced by addition of antioxidants, suggesting that oxidative reactions were involved in the observed protein deterioration.

Oxidation of proteins can occur in the presence or absence of lipids. Site-specific metal-catalyzed protein oxidation has been proposed to occur via hydroxyl free radicals which are produced from hydrogen peroxide at specific iron-binding sites on proteins (Stadtman and Oliver, 1991). Site-specific metal-catalyzed oxidation results in loss of enzyme activity and solubility, increases in carbonyl groups and fluorescence, changes in amino acid composition, and increased proteolytic susceptibility (Levine et al., 1990; Meucci et al., 1991). Protein oxidation can also couple with lipid oxidation, resulting in loss of enzyme activity and solubility and formation of protein complexes and nonenzymic browning products (Kanner and Karel, 1976; Funes et al., 1982; Pokorny et al., 1990).

Muscle foods are susceptible to oxidative reactions due to their high concentrations of oxidation catalysts (iron and myoglobin) and lipids (Asghar et al., 1988). Processes such as mincing and grinding increase oxidative reactions in muscle foods by mixing oxidation catalysts with lipids and oxygen. Muscle foods subjected to comminution processes, such as mechanical deboning, show decreased protein functionality which could be due, in part, to the oxidation of proteins (Smith, 1987). Therefore, characterization of oxidized proteins would help identify factors that affect protein functionality and the quality of finished products. Furthermore, protein oxidation studies could help meat processors and researchers to develop strategies to minimize these oxidation reactions in muscle foods, especially for those low-value, underutilized meat products or parts.

The objectives of this research were to determine changes in the physicochemical properties of turkey myofibrillar proteins subjected to iron and copper oxidation systems. Functional properties of oxidized myofibrillar proteins were also evaluated to examine how these changes correlated to the chemical deterioration of myofibrillar proteins.

MATERIALS AND METHODS

Protein Sample Preparation. Turkey breasts were obtained from a local retailer and were utilized within 4 days post-mortem. Myofibrils were isolated from breast muscle, and salt-soluble protein (SSP) was extracted from the myofibril pellet in 0.6 M NaCl and 25 mM KH_2PO_4/K_2HPO_4 at pH 6.0 (Xiong and Brekke, 1989). Protein concentration was determined according to the biuret method (Torten and Whitaker, 1963).

Model Oxidation System. Protein oxidation studies were performed in a model oxidation system adapted from that of Levine (1984) using myofibrils and SSP. The oxidized protein samples used for measurement of carbonyls and absorbance changes were prepared as described below. SSP (6 mg/mL) was suspended in buffer (0.12 M KCl, 25 mM KH_2PO_4/K_2HPO_4, pH 6.0) containing 25 μM $FeCl_3$ or $CuCl_2$ and varying concentrations of ascorbate (0–25 mM). The mixture was incubated for 6 h in a shaking water bath at 23 °C. Controls contained protein and buffer only. Following oxidation, samples were dialyzed at 4 °C for 24 h in 100 volumes of a buffer containing 0.6 M NaCl and 25 mM KH_2PO_4/K_2HPO_4 at pH 6.0 using 3500 molecular weight cutoff dialysis tubing to remove metals and ascorbate. Dialysis buffer was changed after 4 and 8 h. The effect of oxidation on protein solubility, gelation, and electrophoretic migration patterns was examined using isolated myofibrils (20 mg/mL). Oxidation was performed as described above except that incubation was carried out overnight (16 h) at 4 °C. Oxidized myofibrils were analyzed immediately after incubation.

Measurements of the chemical, physical, and functional characteristics of oxidized proteins are outlined below.

Carbonyls. Carbonyls were measured according to the method outlined by Levine (1984). Oxidized proteins (6 mg/

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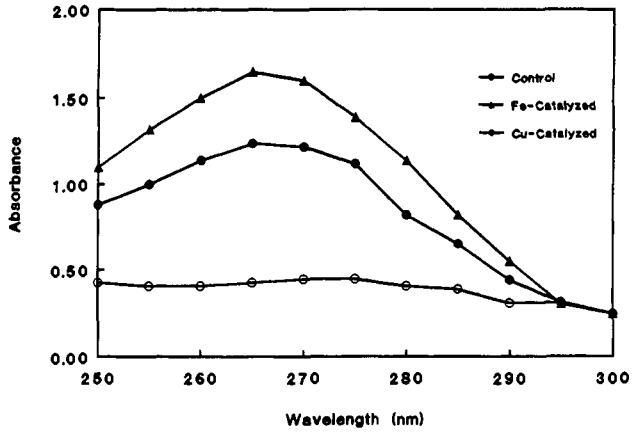

Figure 1. Iron- and copper-induced absorbance changes of salt-soluble protein (0.5 mg/mL) isolated from turkey breast muscle. The oxidation systems (6.0 mg/mL protein, 25 μM copper or iron, and 10 mM ascorbate) were incubated at 23 °C for 6 h.

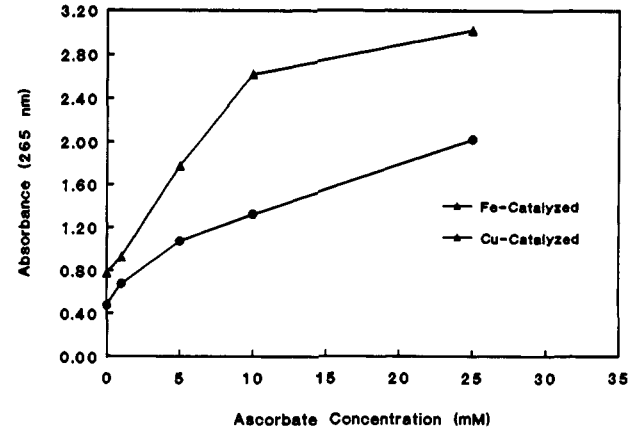

Figure 2. Ascorbate concentration-dependent absorbance changes of salt-soluble protein (0.5 mg/mL) in the presence of iron and copper (25 μM). Oxidation was performed at 23 °C for 6 h. Absorbance of control samples (protein and buffer only) was 0.425.

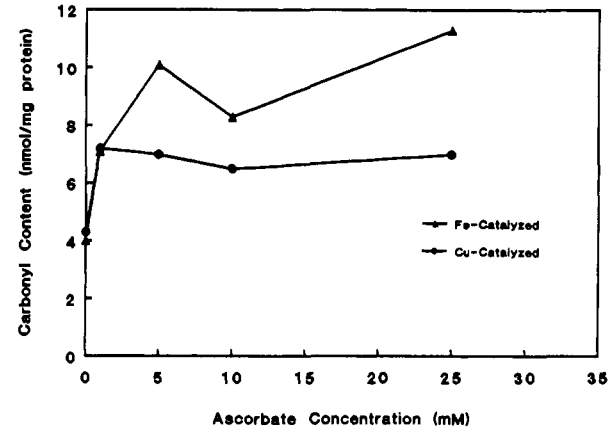

Figure 3. Effect of ascorbate concentration on the formation of carbonyls from salt-soluble protein (6 mg/mL) as catalyzed by iron and copper (25 μM). Oxidation was performed at 23 °C for 6 h. The carbonyl content of control samples (protein and buffer only) was 3.8 nmol/mg of protein.

mL) were incubated with dinitrophenylhydrazine (DNPH) reagent for 30 min at room temperature (23 °C). Absorbances at 387 and 400 nm were measured with a Milton Roy spectrophotometer. Carbonyl content (nanomoles per milligram of protein) was calculated from the delta absorptivity ($Abs_{387} - Abs_{400}$) using 6.9×10^2 M^{-1} cm^{-1} as the extinction coefficient (Levine, 1984).

Absorbance Scan. Absorbance spectra (250–310 nm) were produced from samples (0.5 mg of protein/mL) using a Gilson spectrophotometer. Preliminary results showed that maximal absorption of oxidized protein occurred at 265 nm (Figure 1); therefore, absorbance readings at this wavelength were used in other experiments to determine conformational changes resulting from protein oxidation.

Protein Solubility. Oxidized protein (20 mg/mL) samples were diluted to 5 mg/mL in 0.6 M NaCl and 25 mM KH_2PO_4/K_2HPO_4 (pH 6.0) and then centrifuged at 5000*g* for 15 min. Protein solubility was calculated using the formula

% solubility =

(protein concn in supernatant/original protein concn) × 100

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to monitor oxidation-induced polymerization or fragmentation of the oxidized proteins. SDS-PAGE was performed in 10% acrylamide resolving gel (Laemmli, 1970) using an SE 250 Mighty Small II slab gel electrophoresis apparatus (Hoefer Scientific Instruments, San Francisco, CA). A stacking gel containing 3% acrylamide was also used.

Gelation and Gel Strength. Protein gels were prepared from 20 mg/mL (total of 5 mL) myofibrils suspended in 0.6 M NaCl and 50 mM KH_2PO_4/K_2HPO_4 (pH 6.0) in 16.5 mm (diameter) × 80 mm (length) vials by heating from 20 to 70 °C at 0.8 °C/min and subsequently cooling to 4 °C. This heating rate resulted in maximal gel strength as determined by preliminary experiments.

Gels were equilibrated at 23 °C for 30 min and then penetrated at 20 mm/min using a 12.5 mm diameter stainless steel probe attached to a Model 1122 Instron universal testing instrument (Instron Corp., Canton, MA). The force required to disrupt gels (force at the first peak) was used to represent gel strength.

Water-Holding Capacity (WHC). Myofibril gels (ca. 5 g) were placed in a cone made from two layers of Whatman No. 4 filter paper and then centrifuged at 10000*g* for 15 min. WHC was defined as the pellet weight (grams) divided by the original weight (grams) of the gel times 100%.

RESULTS

Figure 1 shows changes in the absorbance spectra of salt-soluble myofibrillar proteins (SSP; 0.5 mg/mL) oxidized by iron or copper (25 μM) and 10 mM ascorbate. Maximal absorbance increase was observed at approximately 265 nm, where the absorbance increased over 3- and 4-fold for copper and iron oxidation systems, respectively. Absorbance (265 nm) of oxidized SSP increased with increasing ascorbate concentration up to 25 mM (Figure 2). Meucci et al. (1991) also reported increased absorbance of human serum albumin oxidized by 100 mM ascorbate and trace metals. Oxidation-induced absorbance increase has been postulated to result from the disappearance of ordered protein structure (Meucci et al., 1991).

Metal-catalyzed formation of active oxygen species such as the hydroxyl radical can lead to the conversion of some amino acid residues to carbonyl derivatives (Levine et al., 1990). Carbonyl concentrations in oxidized proteins can be measured spectrophotometrically by forming 2,4-dinitrophenylhydrazine (DNPH) adjuncts which absorb at 360–390 nm (Levine et al., 1990). Oxidized proteins were dialyzed prior to analysis since oxidation of ascorbate can result in the formation of carbonyls which react with DNPH (Levine et al., 1990). Both iron- and copper-catalyzed oxidation of SSP resulted in increased carbonyl concentrations in the presence of 1–25 mM ascorbate. Copper increased the carbonyl concentrations of SSP 1.9-fold at an ascorbate concentration of 1 mM (Figure 3). Increasing ascorbate concentrations from 1 to 25 mM did not further increase copper-catalyzed formation of carbonyl groups. Iron-catalyzed oxidation of SSP resulted in 1.9- and 2.6-fold increases in carbonyl content in the presence of 1 and 5 mM ascorbate, respectively (Figure 3).

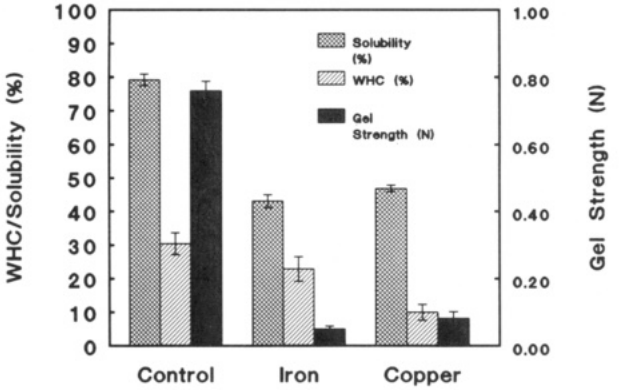

Figure 4. Changes in the functional properties (protein solubility, gel strength, gel water-holding capacity) of myofibrillar proteins resulting from ascorbate (10 mM)/metal (25 μM)-catalyzed oxidation. Oxidation was performed at 4 °C for 16 h. Gels contained 20 mg/mL protein.

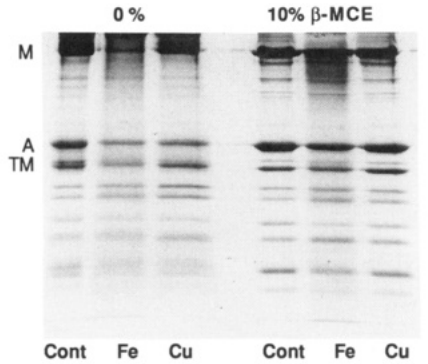

Figure 5. Gel electrophoretic patterns of myofibrillar proteins subjected to iron and copper (25 μM) oxidation in the presence of 10 mM ascorbate. Oxidation was performed at 4 °C for 16 h. M, myosin heavy chain; A, actin; TM, tropomyosin; β-MCE, β-mercaptoethanol. Each lane contained 15 μg of protein.

Increasing ascorbate from 5 to 25 mM did not increase the ability of iron to increase the carbonyl content of SSP.

Figure 4 shows the solubility, gel strength, and gel water-holding capacity of myofibrillar proteins oxidized by 25 μM copper or iron and 10 mM ascorbate. Protein solubility decreased 32.3 and 36.1% for copper- and iron-catalyzed oxidation, respectively, compared to the control. Oxidation reactions catalyzed by both metals severely decreased the gel-forming ability of the myofibrils as gel strength decreased from 0.76 to 0.08 and 0.05 N in the presence of copper and iron, respectively. The decreased gelling ability of the oxidized myofibrils was further manifested by 10.0 and 22.9% reductions in the WHC of gels oxidized by copper and iron, respectively.

SDS–PAGE migration patterns [without β-mercaptoethanol (β-MCE)] of oxidized myofibrils revealed a large decrease in the actin and myosin content and a small decrease in the concentration of tropomyosin (Figure 5). Polymers were formed in oxidized proteins as evidenced by dark bands which were unable to migrate far into the 10% acrylamide gel. Addition of β-MCE to the oxidized proteins substantially decreased the amount of these polymers and increased the concentrations of actin, myosin, and tropomyosin. However, β-MCE was unable to completely dissociate the polymers.

Myofibril suspensions were centrifuged, and SDS–PAGE of the supernatants (SSP) was performed to examine the composition of the soluble fraction of the oxidized myofibrils. In the absence of β-MCE, the supernatants contained very little myosin, actin, and tropomyosin (iron only) compared to the control, indicating that oxidation decreased the solubility of these proteins.

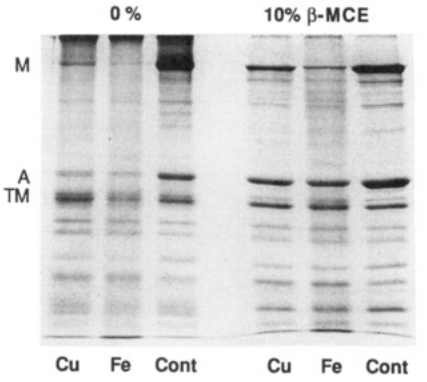

Figure 6. Gel electrophoretic patterns of the soluble fraction of the myofibrillar proteins subjected to iron and copper (25 μM) oxidation in the presence of 10 mM ascorbate. Oxidation was performed at 4 °C for 16 h. M, myosin heavy chain; A, actin; TM, tropomyosin; β-MCE, β-mercaptoethanol. Each lane contained 15 μg of protein.

DISCUSSION

Oxidative processes in muscle foods have been suggested to affect protein functionality. However, very little information is available on the physical and chemical properties of oxidized myofibrillar proteins. Both iron and copper, in the presence of ascorbate, were capable of oxidizing muscle proteins and decreasing the protein functionality as manifested by the following changes: alteration in protein conformation (Figures 1 and 2); increase in carbonyl content (Figure 3); loss in protein solubility, reduction in myofibril gel strength and gel water-holding capacity (Figure 4); and formation of protein polymers (Figures 5 and 6).

Only small increases in carbonyl content and absorbance (Figures 2 and 3) were observed in the absence of ascorbate and the presence of metals, suggesting that the oxidation was due to ascorbate-driven redox cycling of the metals (Kanner et al., 1977). Ascorbate is believed to promote metal-catalyzed lipid oxidation in muscle tissue from mackerel (Decker and Hultin, 1990), turkey (Kanner et al., 1991), and beef (Seman et al., 1991). The exact origin of the free radicals in the model system is not known; however, Levine (1984) has reported that metals can produce hydroxyl radicals from endogenous hydrogen peroxide (as evidenced by inhibition of the reaction by catalase), resulting in the oxidation of proteins such as glutamine synthetase, 3-phosphoglycerate kinase, and yeast enolase. It is also possible that free radicals generated via lipid oxidation were involved in the oxidation of myofibrillar proteins since myofibrillar protein preparations would contain a residual amount of membrane lipid.

SDS–PAGE revealed that both iron- and copper-catalyzed protein oxidation caused dramatic changes in protein migration patterns. That the high molecular weight polymers (which barely entered the polyacrylamide gel) formed during oxidation were largely dissociated to myosin and actin by the reducing agent suggests that the polymers were produced via disulfide linkages and were mainly derived from myosin and actin. However, polymerization also occurred via other covalent bonds because not all of the polymers were dissociable in the presence of β-MCE. It is interesting to note that the soluble fraction of the oxidized myofibrillar suspensions also contained similar high molecular weight polymers that were not removed by centrifugation (Figure 6). Presumably, these oxidation-generated polymers behaved as nonsediment-

able hydrocolloids. Furthermore, significant dark areas were observed immediately below the myosin heavy chain in iron-oxidized myofibrillar proteins even in the presence of β-MCE (Figure 5). No discrete protein bands could be identified, suggesting that nonspecific protein fragmentation or polymerization of low molecular weight proteins was occurring in iron-oxidized proteins.

Iron caused greater changes in protein conformation (Figure 2), carbonyl content (Figure 3), formation of protein polymers, and loss of myosin, actin, and tropomyosin (Figures 5 and 6) than equal molar concentrations of copper. Increased iron-catalyzed protein oxidation could be due to iron being more effective at catalyzing the formation of hydroxyl radicals than copper (Rowley and Halliwell, 1983). Increased iron-catalyzed oxidation of the myofibrillar proteins could also be the result of specific iron binding sites on the proteins which allow more efficient transfer of metal-generated free radicals to the protein. Since iron was more effective at oxidizing myofibrillar proteins and since iron concentrations are generally higher than copper in muscle tissue, iron would be suggested to be a more important catalyst of protein oxidation in muscle foods.

The results clearly show that the reduced protein functionality can be related to the changes in the chemical and physical properties of oxidized proteins. Therefore, to maximize the functionality of muscle proteins during meat processing and to enhance the utilization of low-value meat byproducts (which are often susceptible to oxidation), it is important to design processing procedures and formulations that would minimize protein oxidation.

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