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# Oxidative modification of amino acids in porcine myofibrillar protein isolates exposed to three oxidizing systems ☆

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**Abstract**

Susceptibility of amino acids in myofibrillar protein isolate (MPI) exposed to three oxidizing matrixes commonly encountered in muscle foods was compared. MPI suspensions (20 mg protein/mL) in 15 mM piperazine-*N,N* bis(2-ethane sulphonic acid) buffer (pH 6.0) were oxidized with an iron-catalyzed oxidizing system (IOS, 0.01 mM $FeCl_3$, 0.1 mM ascorbic acid, 0.0–10.0 mM $H_2O_2$), a lipid-oxidizing system (LOS, 0.0–10.0 mM linoleic acid, 3750 units of lipoxidase/mL), or a metmyoglobin (MetMb) oxidizing system (MOS, 0.0–0.5 mM $H_2O_2$/MetMb) for 24 h at 4 °C. Changes were quantitatively analyzed by determining amino acids on a reverse-phase liquid chromatographic (LC) system. In IOS, the amount of cysteine, methionine and tyrosine decreased ($P < 0.05$) with increasing [$H_2O_2$]. In LOS, only cysteine and methionine were lowered at increasing linoleic acid concentrations. In MOS, the quantity of alanine, cysteine, glycine, histidine, leucine and lysine, as well as the total amount of amino acids were significantly reduced at high concentrations of MetMb/$H_2O_2$. The results suggest that under typical meat processing conditions, iron- and metmyoglobin-catalyzed reactions play a major role in the oxidation of amino acids in muscle proteins.


*Keywords:* Protein oxidation; Hydroxyl radicals; Metmyoglobin; Amino acids

## 1. Introduction

Meats and meat products are prone to oxidative attack during processing and storage, which stems from the presence of high concentrations of unsaturated lipids, heme pigments, metal catalysts, and various other oxidizing agents (Johns, Birkinshaw, & Ledward, 1989). Reactive oxygen species (ROS), such as superoxide radical, hydroxyl radical, ferryl radical, hydroperoxy radical, perferryl radical and porphyrin cation radical, can be generated by metal- or enzyme-catalyzed oxidative reactions, and by lipid oxidation, photosensitizers, ionizing-radiation, singlet oxygen, $H_2O_2$-activated metmyoglobin, and other chemical and biological processes (Asghar, Gary, Buckley, Pearson, & Booren, 1988; Butterfield & Stadtman, 1997; Monahan, 2000; Wolf, Garner, & Dean, 1986).

Modification of amino acid side chain groups is one of the main consequences of protein oxidation. Virtually all the amino acyl side chains in proteins could be modified by ROS (Shacter, 2000; Stadtman & Levine, 2003). For example, oxidation can lead to hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic amino acid residues, nitrosylation of sulphhydryl groups, sulphoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and conversion of some amino acid residue to carbonyl derivatives (Amici, Levine, Tsai, & Stadtman, 1989; Stadtman & Levine, 2003). In muscle foods, such modifications are responsible for peptide scission, loss in myosin ATPase activity, and formation of protein aggregates, which have an implication in meat product quality (Ooizumi & Xiong, 2004; Park, Xiong, & Alderton, 2006a; Park, Xiong, Alderton, & Ooizumi, 2006b; Shacter, 2000;

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Srinivasan & Hultin, 1997; Xiong, Srinivasan, & Liu, 1997).

A number of studies have shown that cysteine (Cys) and methionine (Met) are often the most susceptible amino acid residues to a wide array of oxidative attacks (Lii, Chai, Zhao, Thomas, & Hendrich, 1994; Radi, Bush, Cosgrove, & Freeman, 1991; Vogt, 1995). However, amino acids involved in oxidative modifications were reported to be system specific (Elias, McClements, & Decker, 2005; Hawkins & Davies, 2005; Shacter, 2000; Sharp, Becker, & Hettich, 2004; Suto, Ikeda, Fujii, & Ohba, 2006). Metal-catalyzed oxidizing (MCO) systems are among the most stringent oxidizing conditions that generate many forms of ROS (e.g., hydroxyl radical and ferryl radical). Amino acid residues that have been shown to be readily oxidized by MCO include histidine (His), proline (Pro), arginine (Arg), lysine (Lys), threonine (Thr), tyrosine (Tyr), tryptophan (Trp), and Cys (Amici et al., 1989; Shacter, 2000; Stadtman & Levine, 2003).

The hydroxyl radical is known to rapidly oxidize amino acid side chains. Most notably, it targets sulphur-containing residues and aromatic side chain groups, with the following relative reactivity being observed for amino acid side chain oxidation with hydroxyl radical: Cyc > Trp, Tyr > Met > Phe > His > Ile > Leu > Pro (Sharp et al., 2004). Another oxidizing agent, peroxynitrite, can modify Tyr, Cys, Trp, Met and Phe (Ischiropoulos & Al-Mehdi, 1995). Gamma-irradiation, which causes direct formation of hydroxyl radical in the presence of oxygen, oxidizes hydrophobic amino acyl residues such as valine (Val), leucine (Leu), and Tyr (Fu & Dean, 1997; Simpson et al., 1992). Photo oxidation products of hydrogen peroxide are also known to oxidize Met and Trp residues in catalase, and deactivate the enzyme *in vivo* (Wood & Schallreuter, 2006). In addition, other reactive radicals, such as thiyl radicals and protein radicals, were reported to attack protein molecules, causing irreversible damage to protein structures (Gebicki, 1997; Gebicki & Gebicki, 1993; Nauser, Koppenol, & Gebicki, 2005; Nauser, Pelling, & Schoneich, 2004).

The preference or selectivity of amino acids in the oxidative attack not only depends on the oxidizing systems or matrices, but usually is also specific to protein types. Elias et al. (2005) reported that Cys in β-lactogloblin was oxidized before Trp, and Met was resistant to oxidation. The exposure of soybean trypsin inhibitors and lysozyme to hypobromous acid resulted in their activity loss, and this was associated with the modifications of His, Trp and Tyr residues and not with the change in other amino acids including Met (Hawkins & Davies, 2005). Levine, Mosoni, Berlett, and Stadtman (1996) noted that exposed Met in bacterial glutamine synthetase was readily oxidized to Met sulphoxide in the presence of hydrogen peroxide, resulting in loss of the enzyme activity.

In the present study, the oxidative changes in the amino acid composition of muscle myofibrillar proteins after exposures to three oxidizing systems commonly involved in meat processing (pH ~ 6.0, NaCl ~ 0.6 M, temperature ~ 4 °C) was quantitatively analyzed. The objective was to compare the oxidative susceptibility of different amino acid residues under normal meat processing conditions.

## 2. Materials and methods

### 2.1. Materials

Ten Boston shoulders (4 days postmortem) in five separate vacuum packages were obtained from a commercial packing plant. Serratus ventralis muscle was dissected from each shoulder and diced into approximately 15-g pieces. The dices were mixed well, and subsequently divided into 20 equal portions by hand. Each portion, weighing approximately 200 g, was placed in a Cryovac vacuum bag, vacuum sealed, and stored in a −30 °C walk-in freezer until myofibrillar protein extraction. For each experimental replication, one frozen bag of sample was removed from the freezer and thawed in a 4 °C refrigerator for 16 h before use for myofibrillar protein isolation.

### 2.2. Preparation of myofibrillar protein

Five batches of myofibrillar protein isolate (MPI) were prepared from different bags of muscle samples in a 2 °C walk-in cooler as described elsewhere (Park et al., 2006a). Briefly, minced muscle was homogenized 30 s with a Waring blendor at high speed in 4 vol of isolation buffer (0.1 M NaCl, 10 mM sodium phosphate, 2 mM $MgCl_2$, and 1 mM EGTA, pH 7.0). The slurry was centrifuged at 2000*g* for 15 min and the supernatant was discarded. The crude myofibril pellet was washed two more times with the same isolation buffer, followed by additional two-time washings with 0.1 M NaCl, all with the same homogenization and centrifugation conditions as above. The MPI was stored on ice and utilized within 24 h. The protein concentration in MPI was determined by the Biuret method (Gornall, Bardawill, & David, 1949) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard.

### 2.3. Oxidation

MPI was suspended (26 mg protein/mL) in a 15 mM piperazine- *N*,*N* bis(2-ethane sulphonic acid) (PIPES) buffer (pH 6.0) containing 0.6 M NaCl. Suspended MPI was oxidized 24 h at 4 °C with: (i) an iron-catalyzed oxidizing system (IOS: 10 μM $FeCl_3$, 0.1 mM ascorbic acid, 0.1–10.0 mM $H_2O_2$), (ii) a lipid-oxidizing system (LOS: 0.1–10.0 mM linoleic acid, 3750 units of lipoxidase/mL), or (iii) a metmyoglobin-oxidizing system (MOS: 0.05–0.5 mM metmyoglobin, 0.05–0.5 mM $H_2O_2$). Oxidation was terminated by adding propyl gallate/Trolox C/EDTA (1 mM each). The protein concentration in MPI after completion of oxidation treatment was adjusted to 20 mg/mL with the 15 mM PIPES buffer (pH 6.0) containing 0.6 M NaCl.

### 2.4. Sample hydrolysis

MPI samples were hydrolyzed according to Liu, Chang, Yan, Yu, and Liu (1995) with modifications. For Cys determination, MPI samples were oxidized by performic acid prior to the standard HCl digestion. All glassware were washed with 3 M HCl solution, rinsed with nanopure water, and dried before use.

#### 2.4.1. Acid hydrolysis

Control and oxidized MPI (20 mg protein/g; 1.0 g) and metmyoglobin stock solution (2 mM MetMb, namely, 34 mg MetMb/g stock; 1 mL) were weighed into 15 × 100-mm screw cap test tubes. A 1-mL aliquot of 12 N Optima HCl (Seastar Chemical Inc., Pittsburgh, PA) was slowly added to the sample tube while gentling mixing by vortexing. Head space of the sample tubes was then flushed with $N_2$ gas for 15 s. The sample tubes were tightly capped immediately and placed in a 110 °C oven (Lipshaw Electric Laboratory Incubator-Oven, Lipshaw MFG. Co., Detroit, MI) for 24 h to allow complete hydrolysis. The cooled MPI digests were quantitatively transferred into 100-mL volumetric flasks, and to each, 4.0 mL of an internal standard [2.5 mM DL-α-aminobutyric acid (AABA) in 0.1 M HCl; (Sigma–Aldrich Chemical Co., St. Louis, MO) was added. The solution was then brought to volume with nanopure water. Approximately 4 mL of each of the sample solutions was filtered through a 0.45 μm MCE sterile syringe filter (Fisher Scientific, Hampton, NH). After disposing approximately 1 mL of the initial filtrate, 1 mL of filtered solution was collected into a micro centrifuge tube.

#### 2.4.2. Performic acid oxidation and acid hydrolysis

Performic acid oxidation was done to quantify cysteine. Specifically, 1.0 g of control and oxidized MPI (20 mg protein/g) and metmyoglobin stock solution (2 mM MetMb) was weighed into 30-mL Wheaton serum vials. The vials were placed in an ice bath for 30 min. To each vial, 5 mL of cold performic acid solution (mixture of 1 vol of 30% hydrogen peroxide and 9 vol of 80% formic acid) was added. The vial was capped with 20 mm snap-on butyl stopper, and placed in a −15 °C freezer for 16 h. After taking out of the freezer, 0.75 mL of 48% HBr (Sigma–Aldrich Chemical Co., St. Louis, MO) and five drops of 1-octanol (Sigma–Aldrich Chemical Co., St. Louis, MO) were added. The mixture was placed in an ice bath for 30 min and then dried at 37 °C using a Buchi Rotavapor-R rotary evaporator (Brinkman Instruments, Westbury, NY) under vacuum. Two millilitres of 6.0 M HCl solution was added to the dried samples and the sample hydrolysis was done exactly the same way as described in the acid hydrolysis section.

### 2.5. Derivatization

Prior to amino acid derivatization, a calibration standard and a reconstituted AccQ-fluor reagent (AMQ) were freshly prepared. The calibration standard was a mixture of 100 μM AABA (internal standard; Pierce, Roford, IL) and 50–200 μM of L-amino acids in nanopure water [alanine (Ala), Arg, ammonia, aspartic acid (Asp), Cys, glutamic acid (Glu), glycine (Gly), His, isoleucine (Ile), Leu, Lys, methionine (Met), phenylalanine (Phe), Pro, serine (Ser), (Thr, Tyr, and Val)]. The concentration of each amino acid was 2.5 μmole/mL in 0.1 N HCl solution except Cys whose concentration was 1.25 μmol/mL. A Cys calibration standard for performic acid oxidized samples was prepared by combining 2.5 mM L-cysteic acid stock solution, the AABA stock solution and nanopure water to make the final L-cysteic acid and AABA concentration at 100 μM. The reconstituted AccQ-fluor reagent was prepared by mixing 1.0 mL of AccQ-fluor Reagent Diluents (Waters, Milford, MA) to AccQ-Fluor Reagent Power (Waters, Milford, MA ), and heating at 55 °C in a heating block for 10 min.

A 10 μL aliquot of samples in duplicate was placed into the bottom of a 6 × 50 mm culture tube (Fisher Scientific, Hampton, NH). For standards, the 10 μL calibration standards instead of samples was used. To each culture tube, 70 μL of AccQ-Fluor Borate Buffer (Waters, Milford, MA) was added and vortexed for 5 s; thereafter, 20 μL of the reconstituted AccQ-Fluor reagent was added, mixed with a vortex mixer for 5 s, and heated at 55 °C for 10 min in a heating block.

### 2.6. Chromatography

The LC used to analyze derivatized amino acids was a Waters 2695 Alliance Separations Module equipped with an autosampler and connected to Waters 2996 Photodiode Array Detector and Waters 2475 Multi λ Fluorescence Detector (Waters Corporation, Milford, MA). A Waters Empower Pro (version 5.0) was used to control LC and detectors and collect/analyze data. Eluent A was prepared by diluting AccQ-Tag Eluent A per supplier's instruction and the pH adjusted to 4.95. Eluent B was an HPLC grade acetonitrile (Fisher Scientific, Hampton, NH), and Eluent C was nanopure water containing 5% acetonitrile. All separations were carried out on an AccQ-Tag amino acid analysis column (particle size: 4 μm, dimensions: 150 × 3.9 mm, packing material: Silica base bonded with $C_{18}$) purchased from Waters Corporation, but no guard column was connected.

The column temperature was set at 37 °C for acid hydrolysates, and at 47 °C for performic acid oxidized hydrolysates. With a flow rate set at 1.0 mL/min, 5 μL of standards or hydrolyzed samples was injected and chromatographed for 50 min, and degasser was on throughout the LC operation. There was a 50 min equilibration period prior to the first run, and 10 min equilibration period between sample injections. Gradient conditions for acid hydrolysates and performic acid oxidized hydrolysates were the same as the Waters standard protocol, which are listed in Table 1. The UV detector was operated at 248 nm (for peak identification), and the fluorescence

Table 1
Gradient table for amino acid separation

| Acid hydrolysates | | | | | Performic acid oxidized hydrolysates | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Time (min) | A (%) | B (%) | C (%) | Curve | Time (min) | A (%) | B (%) | C (%) | Curve |
| 0 | 100 | 0 | 0 | – | 0 | 100 | 0 | 0 | – |
| 0.5 | 99 | 1 | 0 | 11 | 17 | 95 | 5 | 0 | 6 |
| 18 | 95 | 5 | 0 | 6 | 23 | 91 | 9 | 0 | 6 |
| 19 | 91 | 9 | 0 | 6 | 37 | 83 | 17 | 0 | 6 |
| 29.5 | 83 | 17 | 0 | 6 | 40 | 0 | 60 | 40 | 11 |
| 33 | 0 | 60 | 40 | 11 | 43 | 100 | 0 | 0 | 11 |
| 36 | 100 | 0 | 0 | 11 | 60 | 100 | 0 | 0 | 6 |
| 50 | 100 | 0 | 0 | 6 | | | | | |

detector was with a 250 nm excitation and a 395 nm emission wavelength (for amino acid quantification).

### 2.7. Peak identification and quantification

Peaks on LC chromatograms of derivatized amino acid standards were identified by comparing the reference chromatographic results provided by Waters (2005a, 2005b) and by Liu et al. (1995). The peaks in derivatized MPI samples were identified by the retention time of individual amino acid and the internal standard on chromatographic results. The quantity of each amino acid in μM was determined from the peak areas of known quantity of amino acid standard mixture and peak areas of individual amino acid in samples that contained 100 μM internal standards. The amount of each amino acid and the total quantity were converted to mg per g protein in MPI. Relative ratios of amino acids were also determined from the total quantity of amino acids.

### 2.8. Statistical analysis

Five batches of MPI were prepared on different days, each used for an independent trial; and all the experiments were repeated with at least three different batches of MPI. The significance of the main effects (concentration of oxidizing reagents) was determined by the analysis of variance (ANOVA) test using SAS/STAT (SAS Institute Inc., Cary, NC). Differences between means were compared by Student–Newman–Kuels (SNK) multiple comparison using SAS at a significance level of 0.05.

## 3. Results

Fig. 1 displays a typical chromatograph of HPLC separation of the MPI digests. A total of 17 amino acids were identified. The Cys peak (Cys*) (an HCl-digested sample) did not accurately reflect its actual content; hence, it was chromatographed and quantified from a separate sample oxidized with performic acid followed by digestion with HCl. On the other hand, tryptophan, which was decomposed during acid digestion of protein samples, was not detectable from the HPLC chromatography. To avoid this problem, protein samples were also digested with 4.2 N NaOH (Allred & MacDonald, 1988) in a separate experiment. However, oxidized protein samples were found to be difficult to be completely dissolved in the alkaline solution, and hence, the experiment was not continued and the

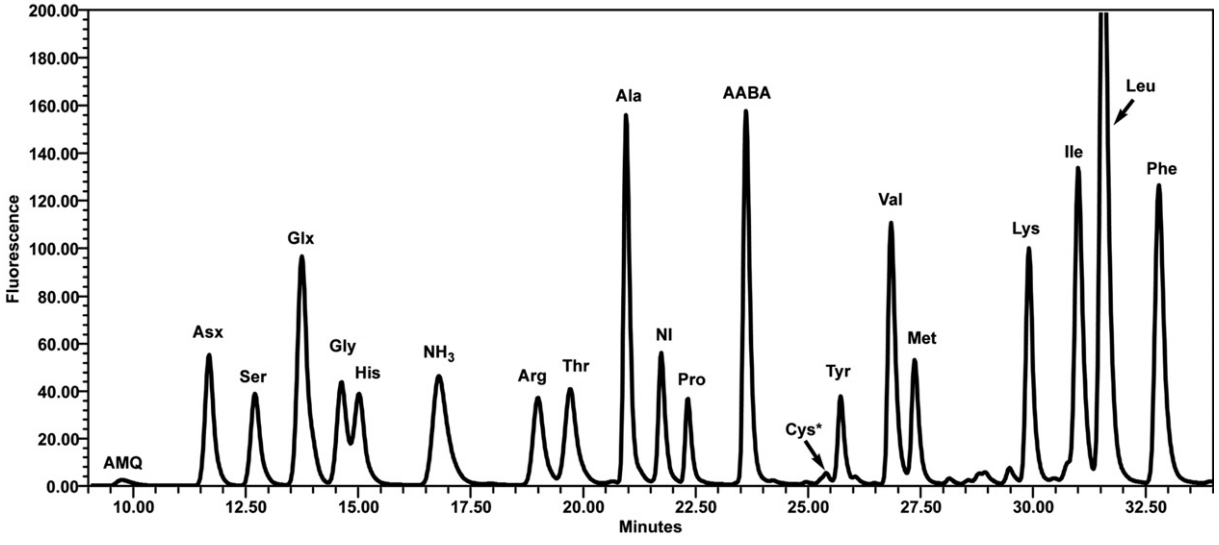


Fig. 1. A typical HPLC chromatograph of oxidized MPI prepared by HCl digestion. On display is the elution profile of the MPI oxidized with 0.5 mM MetMb and digested with 6 M HCl. NI: not identified; AMO: AccQ-fluor reagent; AABA: DL-α-aminobutyric acid. Glx and Asx represent, respectively, combined Glu and Gln, and Asp and Asn.

quantity of tryptophan was not determined. Furthermore, since acid digestion converted glutamine (Gln) to Glu, and asparagine (Asn) to Asp, the Glx and Asx shown in Fig. 1 represent the combined Glu and Gln, and Asp and Asn.

The results of quantitative analysis of control and oxidized MPI with IOS, LOS and MOS are summarized in Tables 2–4, respectively. Both the weight concentration (mg/g protein) and the percentage were used to express the quantitative changes of amino acids resulting from oxidation. In IOS, the quantity of Cys, Met and Tyr decreased steadily upon the addition of $H_2O_2$. The amount reduced in 10 mM IOS was significantly lower ($P < 0.05$) than control and than in 0.1 mM IOS. In terms of relative concentrations, Cys, Met and Tyr also reduced gradually. The other amino acids as well as their total amount in IOS at different $H_2O_2$ levels did not change significantly.

Table 2
Amino acid composition of oxidized MPI in IOS

| Amino acid | Control | | 0.1 mM $H_2O_2$ | | 1.0 mM $H_2O_2$ | | 10.0 mM $H_2O_2$ | |
|---|---|---|---|---|---|---|---|---|
| | mg/g | % | mg/g | % | mg/g | % | mg/g | % |
| Ala | $41.41^{a}$ | $5.69^{z}$ | $39.53^{a}$ | $5.10^{z}$ | $39.95^{a}$ | $5.46^{z}$ | $42.84^{a}$ | $5.76^{z}$ |
| Arg | $57.69^{a}$ | $7.92^{z}$ | $63.83^{a}$ | $8.17^{z}$ | $58.44^{a}$ | $7.98^{z}$ | $58.98^{a}$ | $7.94^{z}$ |
| Asx | $63.84^{a}$ | $8.78^{z}$ | $61.08^{a}$ | $7.90^{z}$ | $63.51^{a}$ | $8.68^{z}$ | $70.78^{a}$ | $9.52^{z}$ |
| Cys | **$11.00^{a}$** | **$1.51^{z}$** | **$10.54^{a}$** | **$1.36^{yz}$** | **$9.36^{b}$** | **$1.28^{yz}$** | **$8.55^{c}$** | **$1.15^{y}$** |
| Glx | $129.99^{a}$ | $17.86^{z}$ | $140.78^{a}$ | $18.08^{z}$ | $134.74^{a}$ | $18.41^{z}$ | $135.21^{a}$ | $18.19^{z}$ |
| Gly | $27.28^{a}$ | $3.74^{z}$ | $27.75^{a}$ | $3.56^{z}$ | $25.92^{a}$ | $3.54^{z}$ | $26.29^{a}$ | $3.54^{z}$ |
| His | $20.11^{a}$ | $2.76^{z}$ | $23.25^{a}$ | $2.97^{z}$ | $21.46^{a}$ | $2.93^{z}$ | $20.94^{a}$ | $2.82^{z}$ |
| Ile | $34.05^{a}$ | $4.64^{z}$ | $38.05^{a}$ | $4.87^{z}$ | $35.40^{a}$ | $4.84^{z}$ | $37.59^{a}$ | $5.06^{z}$ |
| Leu | $63.07^{a}$ | $8.65^{z}$ | $66.14^{a}$ | $8.49^{z}$ | $61.66^{a}$ | $8.43^{z}$ | $64.06^{a}$ | $8.62^{z}$ |
| Lys | $66.16^{a}$ | $9.08^{z}$ | $58.81^{a}$ | $7.61^{z}$ | $62.68^{a}$ | $8.56^{z}$ | $78.35^{a}$ | $10.54^{z}$ |
| Met | **$24.41^{ab}$** | **$3.35^{z}$** | **$28.02^{a}$** | **$3.58^{z}$** | **$24.49^{ab}$** | **$3.35^{z}$** | **$14.42^{b}$** | **$1.94^{y}$** |
| Phe | $31.55^{a}$ | $4.33^{z}$ | $42.27^{a}$ | $5.37^{z}$ | $33.29^{a}$ | $4.55^{z}$ | $33.44^{a}$ | $4.50^{z}$ |
| Pro | $27.83^{a}$ | $3.82^{z}$ | $28.74^{a}$ | $3.69^{z}$ | $27.24^{a}$ | $3.72^{z}$ | $27.75^{a}$ | $3.73^{z}$ |
| Ser | $33.24^{a}$ | $4.57^{z}$ | $34.17^{a}$ | $4.39^{z}$ | $32.89^{a}$ | $4.50^{z}$ | $32.52^{a}$ | $4.38^{z}$ |
| Thr | $36.58^{a}$ | $5.02^{z}$ | $42.73^{a}$ | $5.47^{z}$ | $38.07^{a}$ | $5.20^{z}$ | $37.55^{a}$ | $5.05^{z}$ |
| Tyr | **$27.88^{ab}$** | **$3.84^{yz}$** | **$38.42^{a}$** | **$4.91^{y}$** | **$29.61^{ab}$** | **$4.05^{yz}$** | **$18.93^{b}$** | **$2.55^{z}$** |
| Val | $32.52^{a}$ | $4.44^{z}$ | $35.00^{a}$ | $4.48^{z}$ | $33.09^{a}$ | $4.52^{z}$ | $34.96^{a}$ | $4.71^{z}$ |
| Total | $728.60^{a} \pm 25.80$ | | $779.11^{a} \pm 41.17$ | | $731.79^{a} \pm 10.62$ | | $743.14^{a} \pm 3.55$ | |

[a–c] Means of mg/g protein in the same row without a common superscript letter differ significantly ($P < 0.05$).
[y–z] Means of % in the same row without a common superscript letter differ significantly ($P < 0.05$).
The most significant changes are indicated by bold-faced values.

Table 3
Amino acid composition of oxidized MPI in LOS

| Amino acid | Control | | 0.1 mM Linoleic | | 1.0 mM Linoleic | | 10.0 mM Linoleic | |
|---|---|---|---|---|---|---|---|---|
| | mg/g | % | mg/g | % | mg/g | % | mg/g | % |
| Ala | $41.41^{a}$ | $5.69^{z}$ | $41.16^{a}$ | $5.69^{z}$ | $41.07^{a}$ | $5.69^{z}$ | $34.74^{a}$ | $4.87^{z}$ |
| Arg | $57.69^{a}$ | $7.92^{z}$ | $55.44^{a}$ | $7.87^{z}$ | $56.56^{a}$ | $7.84^{z}$ | $56.67^{a}$ | $7.98^{z}$ |
| Asx | $63.84^{a}$ | $8.78^{z}$ | $66.35^{a}$ | $9.21^{z}$ | $66.61^{a}$ | $9.23^{z}$ | $67.82^{a}$ | $9.56^{z}$ |
| Cys | **$11.00^{a}$** | **$1.51^{z}$** | **$9.12^{b}$** | **$1.26^{y}$** | **$9.19^{b}$** | **$1.27^{y}$** | **$9.12^{b}$** | **$1.28^{y}$** |
| Glx | $129.99^{a}$ | $17.86^{z}$ | $130.38^{a}$ | $18.39^{z}$ | $132.53^{a}$ | $18.36^{z}$ | $134.50^{a}$ | $18.95^{z}$ |
| Gly | $27.28^{a}$ | $3.74^{z}$ | $25.75^{a}$ | $3.56^{z}$ | $25.37^{a}$ | $3.52^{z}$ | $25.89^{a}$ | $3.65^{z}$ |
| His | $20.11^{a}$ | $2.76^{z}$ | $18.98^{a}$ | $2.76^{z}$ | $19.97^{a}$ | $2.77^{z}$ | $19.88^{a}$ | $2.80^{z}$ |
| Ile | $34.05^{a}$ | $4.64^{z}$ | $31.71^{a}$ | $4.95^{z}$ | $36.10^{a}$ | $5.00^{z}$ | $33.58^{a}$ | $4.72^{z}$ |
| Leu | $63.07^{a}$ | $8.65^{z}$ | $60.89^{a}$ | $8.70^{z}$ | $63.05^{a}$ | $8.73^{z}$ | $63.10^{a}$ | $8.89^{z}$ |
| Lys | $66.16^{a}$ | $9.08^{z}$ | $67.07^{a}$ | $9.34^{z}$ | $68.74^{a}$ | $9.52^{z}$ | $57.31^{a}$ | $8.03^{z}$ |
| Met | **$24.41^{a}$** | **$3.35^{y}$** | **$13.51^{b}$** | **$1.53^{z}$** | **$11.15^{b}$** | **$1.54^{z}$** | **$14.70^{b}$** | **$2.08^{z}$** |
| Phe | $31.55^{a}$ | $4.33^{z}$ | $30.24^{a}$ | $4.36^{z}$ | $31.24^{a}$ | $4.33^{z}$ | $31.28^{a}$ | $4.41^{z}$ |
| Pro | $27.83^{a}$ | $3.82^{z}$ | $26.87^{a}$ | $3.81^{z}$ | $27.46^{a}$ | $3.80^{z}$ | $27.46^{a}$ | $3.87^{z}$ |
| Ser | $33.24^{a}$ | $4.57^{z}$ | $32.97^{a}$ | $4.55^{z}$ | $32.77^{a}$ | $4.54^{z}$ | $33.81^{a}$ | $4.76^{z}$ |
| Thr | $36.58^{a}$ | $5.02^{z}$ | $36.06^{a}$ | $5.11^{z}$ | $36.58^{a}$ | $5.07^{z}$ | $37.73^{a}$ | $5.32^{z}$ |
| Tyr | $27.88^{a}$ | $3.84^{z}$ | $30.25^{a}$ | $4.26^{z}$ | $29.77^{a}$ | $4.12^{z}$ | $30.92^{a}$ | $4.35^{z}$ |
| Val | $32.52^{a}$ | $4.44^{z}$ | $31.06^{a}$ | $4.65^{z}$ | $33.71^{a}$ | $4.67^{z}$ | $31.87^{a}$ | $4.48^{z}$ |
| Total | $728.60^{a} \pm 25.8$ | | $725.67^{a} \pm 7.21$ | | $721.84^{a} \pm 0.43$ | | $710.10^{a} \pm 13.64$ | |

[a–b] Means of mg/g protein in the same row without a common superscript letter differ significantly ($P < 0.05$).
[y–z] Means of % in the same row without a common superscript letter differ significantly ($P < 0.05$).
The most significant changes are indicated by bold-faced values.

Table 4
Amino acid composition of oxidized MPI in MOS

| Amino acid | Control | | 0.05 mM MetMb/$H_2O_2$ | | 0.1 mM MetMb/$H_2O_2$ | | 0.5 mM MetMb/$H_2O_2$ | |
|---|---|---|---|---|---|---|---|---|
| | mg/g | % | mg/g | % | mg/g | % | mg/g | % |
| Ala | **41.41**$^{a}$ | 5.69$^{z}$ | **39.85**$^{a}$ | 5.46$^{z}$ | **39.24**$^{a}$ | 5.57$^{z}$ | **31.07**$^{b}$ | 5.66$^{z}$ |
| Arg | 57.69$^{a}$ | 7.92$^{z}$ | 58.86$^{a}$ | 8.05$^{z}$ | 57.95$^{a}$ | 8.22$^{z}$ | 47.62$^{a}$ | 8.63$^{z}$ |
| Asx | 63.84$^{a}$ | 8.78$^{z}$ | 63.82$^{a}$ | 8.75$^{z}$ | 63.72$^{a}$ | 9.05$^{z}$ | 52.42$^{a}$ | 9.54$^{z}$ |
| Cys | **11.00**$^{a}$ | **1.51**$^{z}$ | **9.16**$^{ab}$ | **1.25**$^{yz}$ | **7.78**$^{bc}$ | **1.10**$^{y}$ | **5.91**$^{c}$ | **1.06**$^{y}$ |
| Glx | 129.99$^{a}$ | 17.86$^{z}$ | 134.76$^{a}$ | 18.44$^{z}$ | 130.22$^{a}$ | 18.18$^{z}$ | 106.39$^{a}$ | 19.33$^{z}$ |
| Gly | **27.28**$^{a}$ | 3.74$^{z}$ | **25.05**$^{a}$ | 3.43$^{z}$ | **24.41**$^{a}$ | 3.46$^{z}$ | **18.29**$^{b}$ | 3.33$^{z}$ |
| His | **20.11**$^{a}$ | **2.76**$^{z}$ | **19.62**$^{a}$ | **2.68**$^{z}$ | **17.83**$^{a}$ | **2.53**$^{z}$ | **11.88**$^{b}$ | **2.16**$^{y}$ |
| Ile | 34.05$^{a}$ | 4.64$^{z}$ | 36.41$^{a}$ | 4.99$^{z}$ | 37.09$^{a}$ | 5.27$^{z}$ | 24.00$^{a}$ | 4.34$^{z}$ |
| Leu | **63.07**$^{a}$ | 8.65$^{z}$ | **61.89**$^{a}$ | 8.48$^{z}$ | **60.75**$^{a}$ | 8.62$^{z}$ | **44.91**$^{b}$ | 8.16$^{z}$ |
| Lys | **66.16**$^{a}$ | 9.08$^{z}$ | **63.82**$^{a}$ | 8.75$^{z}$ | **64.63**$^{a}$ | 9.18$^{z}$ | **49.59**$^{b}$ | 9.03$^{z}$ |
| Met | 24.41$^{a}$ | 3.35$^{z}$ | 24.80$^{a}$ | 3.39$^{z}$ | 24.11$^{a}$ | 3.42$^{z}$ | 19.64$^{a}$ | 3.56$^{z}$ |
| Phe | 31.55$^{a}$ | 4.33$^{z}$ | 32.07$^{a}$ | 4.39$^{z}$ | 30.87$^{a}$ | 4.38$^{z}$ | 20.32$^{a}$ | 3.69$^{z}$ |
| Pro | 27.83$^{a}$ | 3.82$^{z}$ | 27.32$^{a}$ | 3.74$^{z}$ | 26.69$^{a}$ | 3.79$^{z}$ | 21.41$^{a}$ | 3.89$^{z}$ |
| Ser | 33.24$^{a}$ | 4.57$^{z}$ | 32.43$^{a}$ | 4.44$^{z}$ | 30.23$^{a}$ | 4.29$^{z}$ | 26.69$^{a}$ | 4.85$^{z}$ |
| Thr | 36.58$^{a}$ | 5.02$^{z}$ | 37.20$^{a}$ | 5.09$^{z}$ | 35.27$^{a}$ | 5.01$^{z}$ | 27.12$^{a}$ | 4.92$^{z}$ |
| Tyr | 27.88$^{a}$ | 3.84$^{z}$ | 29.18$^{a}$ | 3.99$^{z}$ | 24.10$^{a}$ | 3.42$^{z}$ | 20.14$^{a}$ | 3.64$^{z}$ |
| Val | 32.52$^{a}$ | 4.44$^{z}$ | 34.19$^{a}$ | 4.68$^{z}$ | 29.53$^{a}$ | 4.21$^{z}$ | 23.32$^{a}$ | 4.21$^{z}$ |
| Total | **728.60**$^{a}$ ± **25.8** | | 730.45$^{a}$ ± 18.07 | | 704.42$^{a}$ ± 16.60 | | **550.72**$^{b}$ ± **42.94** | |

$^{a–c}$ Means of mg/g protein in the same row without a common superscript letter differ significantly ($P < 0.05$).
$^{y–z}$ Means of % in the same row without a common superscript letter differ significantly ($P < 0.05$).
The most significant changes are indicated by bold-faced values.

In LOS, the quantity and percentage of Cys and Met in oxidation treated samples were significantly less than those in the control sample, while the oxidative effect on the other amino acids was negligible (Table 3). For example, the concentration of Met in MPI was decreased from 24.41 mg/g to 13.51 mg/mL (a 45% reduction) after a 24-h exposure to 0.1 mM oxidizing linoleic acid. There was no further reduction in both Cys and Met with the addition of more linoleic acid, suggesting that oxidation with 0.1 mM of the oxidant reached a saturation level.

For the MetMb oxidation system (MOS), quantitative measurement of the oxidatively induced changes in the specific amino acids was a bit complicated. This was because significant amounts of myoglobin (0.05, 0.1 and 0.5 mM, i.e., 0.85, 1.7 and 8.5 mg/mL) were added as oxidants, and the changes in amino acid concentration of oxidized MPI samples must be attributed to the oxidation of both MPI and myoglobin (to MetMb). Thus, to estimate the amino acid changes due to MPI oxidation, it was necessary to deduct the contribution from MetMb to the resultant amino acid content in the mixed MOS protein system. This was done by calculating the weight ratios of amino acids in nonoxidized MPI (20 mg/mL) to the same amino acids in myoglobin (0.85, 1.7 or 8.5 mg/mL) and then assigning the amino acids (mg) in oxidized samples (the MPI/MetMb mixture in MOS) to MPI and MetMb based on the ratios. For example, the amount of His in control MPI sample (nonoxidized) was determined to be 0.4022 mg His/mL MPI sample (i.e., 20.11 mg/g protein), and the amount of His in the 0.05 mM myoglobin solution was 0.1097 mg/mL (i.e., 129.04 mg/g protein). In the MOS with 0.05 mM MetMb (molecular weight ~ 17,000), 0.85 mg protein due to MetMb was in actuality added to each mL of the oxidizing mixture. Therefore, 0.1097 mg of His was added to each mL of oxidizing mixture. As a result, the weight ratio of His from MPI (0.4022 mg) and His from MetMb (0.1097 mg) in 0.05 mM MOS was 78:22. The total amount of His in MOS containing the 0.05 mM oxidizing MetMb was found to be 25.0 mg/g protein. Using the 78:22 ratio and assuming an equal susceptibility to oxidation of the amino acid in MPI and in MetMb, we ascribed 19.5 mg His/g protein to oxidized MPI and the remainder (5.5 mg His/g protein) to MetMb.

A number of amino acids were affected by the MOS oxidation system. The amount of Ala, Cys, His, Leu and Lys was significantly decreased ($P < 0.05$) as the concentration of oxidants (MetMg/$H_2O_2$) increased. The exposure of MPI to the MOS also resulted in significant reductions in the relative content (%) of Cys and His. Furthermore, unlike the other two oxidizing systems, the total amount of amino acids in MOS was significantly reduced in the 0.5 mM MetMb/$H_2O_2$ oxidizing condition (Table 4).

The oxidative effects of the three oxidizing systems on the relative amounts (%) of amino acids in MPI were compared, and the results are shown in Table 5. Cys appeared to be most susceptible to MOS and Tyr appeared to be most susceptible to high concentrations of $H_2O_2$ in IOS. On the other hand, LOS seemed to be most destructive to Met even at low oxidizing linoleic acid concentrations. Furthermore, while percent Lys was unaffected by LOS and MOS, it increased ($P < 0.05$) with $H_2O_2$ concentration in the IOS, suggesting either a high resistance to hydroxyl radical oxidation or a conversion from other oxidatively modified amino acids.

Table 5
Relative amounts (%) of amino acids in MPI exposed to three oxidizing systems

| Amino acid | Control | IOS, [$H_2O_2$] | | | LOS, [Linoleic] | | | MOS, [MetMb/$H_2O_2$] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.1 mM | 1.0 mM | 10.0 mM | 0.1 mM | 1.0 mM | 10.0 mM | 0.05 mM | 0.1 mM | 0.5 mM |
| Ala | 5.69$^{a}$ | 5.10$^{a}$ | 5.46$^{a}$ | 5.76$^{a}$ | 5.69$^{a}$ | 5.69$^{a}$ | 4.87$^{a}$ | 5.45$^{a}$ | 5.52$^{a}$ | 5.60$^{a}$ |
| Arg | **7.92$^{b}$** | **8.17$^{b}$** | **7.98$^{b}$** | **7.94$^{b}$** | **7.87$^{b}$** | **7.84$^{b}$** | **7.98$^{b}$** | **8.11$^{b}$** | **8.27$^{b}$** | **8.93$^{a}$** |
| Asx | 8.78$^{a}$ | 7.90$^{a}$ | 8.68$^{a}$ | 9.52$^{a}$ | 9.21$^{a}$ | 9.23$^{a}$ | 9.56$^{a}$ | 8.77$^{a}$ | 9.03$^{a}$ | 9.62$^{a}$ |
| Cys | **1.51$^{a}$** | **1.36$^{abc}$** | **1.28$^{abc}$** | **1.15$^{c}$** | **1.26$^{bc}$** | **1.27$^{bc}$** | **1.28$^{bc}$** | **1.26$^{abc}$** | **1.12$^{c}$** | **1.12$^{c}$** |
| Glx | 17.86$^{a}$ | 18.08$^{a}$ | 18.41$^{a}$ | 18.19$^{a}$ | 18.39$^{a}$ | 18.36$^{a}$ | 18.95$^{a}$ | 18.47$^{a}$ | 18.39$^{a}$ | 19.40$^{a}$ |
| Gly | **3.74$^{a}$** | **3.56$^{ab}$** | **3.54$^{ab}$** | **3.54$^{ab}$** | **3.56$^{ab}$** | **3.52$^{ab}$** | **3.65$^{ab}$** | **3.41$^{bc}$** | **3.40$^{bc}$** | **3.25$^{c}$** |
| His | **2.76$^{a}$** | **2.97$^{a}$** | **2.93$^{a}$** | **2.82$^{a}$** | **2.76$^{a}$** | **2.77$^{a}$** | **2.80$^{a}$** | **2.61$^{ab}$** | **2.41$^{b}$** | **2.04$^{c}$** |
| Ile | 4.64$^{a}$ | 4.87$^{a}$ | 4.84$^{a}$ | 5.06$^{a}$ | 4.95$^{a}$ | 5.00$^{a}$ | 4.72$^{a}$ | 4.98$^{a}$ | 5.21$^{a}$ | 4.29$^{a}$ |
| Leu | **8.65$^{a}$** | **8.49$^{ab}$** | **8.43$^{ab}$** | **8.62$^{a}$** | **8.70$^{a}$** | **8.73$^{a}$** | **8.89$^{a}$** | **8.45$^{ab}$** | **8.51$^{ab}$** | **8.03$^{b}$** |
| Lys | **9.08$^{ab}$** | **7.61$^{b}$** | **8.56$^{ab}$** | **10.54$^{a}$** | **9.34$^{ab}$** | **9.52$^{ab}$** | **8.03$^{ab}$** | **8.73$^{ab}$** | **9.06$^{ab}$** | **8.90$^{ab}$** |
| Met | **3.35$^{a}$** | **3.58$^{a}$** | **3.35$^{a}$** | **1.94$^{b}$** | **1.53$^{b}$** | **1.54$^{b}$** | **2.08$^{b}$** | **3.41$^{a}$** | **3.42$^{a}$** | **3.63$^{a}$** |
| Phe | **4.33$^{ab}$** | **5.37$^{a}$** | **4.55$^{ab}$** | **4.50$^{ab}$** | **4.36$^{ab}$** | **4.33$^{ab}$** | **4.41$^{ab}$** | **4.35$^{ab}$** | **4.28$^{ab}$** | **3.58$^{b}$** |
| Pro | **3.82$^{ab}$** | **3.69$^{b}$** | **3.72$^{ab}$** | **3.73$^{ab}$** | **3.81$^{ab}$** | **3.80$^{ab}$** | **3.87$^{ab}$** | **3.75$^{ab}$** | **3.78$^{a}$** | **3.92$^{a}$** |
| Ser | 4.57$^{a}$ | 4.39$^{a}$ | 4.50$^{a}$ | 4.38$^{a}$ | 4.55$^{a}$ | 4.54$^{a}$ | 4.76$^{a}$ | 4.45$^{a}$ | 4.29$^{ab}$ | 4.91$^{a}$ |
| Thr | 5.02$^{a}$ | 5.47$^{a}$ | 5.20$^{a}$ | 5.05$^{a}$ | 5.11$^{a}$ | 5.07$^{a}$ | 5.32$^{a}$ | 5.09$^{a}$ | 4.96$^{a}$ | 4.90$^{a}$ |
| Tyr | **3.84$^{ab}$** | **4.91$^{a}$** | **4.05$^{ab}$** | **2.55$^{c}$** | **4.26$^{ab}$** | **4.12$^{ab}$** | **4.35$^{ab}$** | **4.01$^{ab}$** | **3.41$^{b}$** | **3.68$^{ab}$** |
| Val | 4.44$^{a}$ | 4.48$^{a}$ | 4.52$^{a}$ | 4.71$^{a}$ | 4.65$^{a}$ | 4.67$^{a}$ | 4.48$^{a}$ | 4.70$^{a}$ | 4.94$^{a}$ | 4.20$^{a}$ |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

$^{a–c}$ Means in the same row without a common superscript letter differ significantly ($P < 0.05$).
The most significant changes are indicated by bold-faced values.

## 4. Discussion

Oxidative modification of amino acid side chain groups occurs ubiquitously in living organisms, and the process can lead to a wide array of functional consequences in biological systems, such as inactivation of enzymes, loss of protein solubility, peptide scission, and formation of complexes between protein molecules and other macromolecules in the cell (Shacter, 2000; Stadtman & Levine, 2003). Transition metals, such as $Fe^{3+}$ and $Cu^{2+}$, in the presence of $O_2$ and an electron donor, can readily generate radicals through simple Fenton-type reactions (Stadtman, 1993). In nonenzymatic metal-catalyzed systems, ascorbate or sulphhydryl compounds can serve as electron donors (Kim, Rhee, & Stadtman, 1985; Marx & Chevion, 1986; Oliver, Levine, & Stadtman, 1980; Samuni, Aronovitch, Godinger, Chevion, & Czapski, 1983). In enzymatic systems, reduced forms of NAD(P)H dehydrogenases, xanthine oxidase, and cytochrome $P_{50}$ reductase can generate radicals by forming $H_2O_2$ or transferring electrons to $Fe^{3+}$ (Stadtman & Oliver, 1991). As reviewed by Stadtman (1993), His, Arg, Lys, Pro, Met and Cys are the most common sites of oxidation involving metal ions as the catalysts.

In addition, indirect oxidative modification of amino acid residues in proteins can occur via the formation of adducts with products of oxidized lipids, amino acids, sugars, and glutathione (Shacter, 2000). Lipid peroxidation breakdown products, such as hydroxynonenal (HNE) and malondialdehyde (MDA), bind covalently to Lys, His and Cys residues of proteins (Alderton, Faustman, Liebler, & Hill, 2003; Buttkus, 1967; Haberland, Fong, & Cheng, 1988; Requena et al., 1997; Shimasaki, Ueta, & Privett, 1982; Uchida et al., 1998).

In muscle foods, alterations in protein processing functionality (e.g., hydration, gelation, and emulsification) have also been linked to oxidative attack of amino acid side chains by reactive oxygen species (ROS) (Decker, Xiong, Calvert, Crum, & Blanchard, 1993; Ooizumi & Xiong, 2004; Park et al., 2006a, 2006b; Srinivasan & Hultin, 1997; Xiong et al., 1997). While ROS generated from systems with transition metals, ozone and nitric oxides are more biologically relevant to protein oxidation *in vivo*, ROS produced from metal-catalyzed oxidation systems, oxidizing linoleic acid, and metmyoglobin are likely the primary sources of oxidizing agents in fresh and processed muscle foods.

The present study showed the three reactive oxygen species-generation systems – IOS, LOS, and MOS – had some common target sites on MPI, but they also had specificities for different amino acid side residue side chain groups. On one hand, the sulphur amino acids Cys and Met were the most prone to oxidative modifications in all the three oxidizing systems (in MOS, the quantity of Met was appreciably reduced albeit nonsignificant), which was in agreement with previous findings (Lii et al., 1994; Radi et al., 1991; Vogt, 1995). Oxidation of Cys is known to lead to the formation of disulphide bonds, mixed disulphides (e.g., with glutathione), and thiyl radicals (Hu, 1994; Kalyanaraman, 1995). As shown previously, cross-linking of MPI proteins induced by the same three oxidizing conditions largely originated from disulphide bonds, with other covalent bonds playing only a small role (Park et al., 2006a, 2006b). In the case of Met residues, the major oxidation product under biological conditions is methionine sulphoxide (Vogt, 1995). The current finding clearly indicated that sulphur-bearing amino acids were readily modified by the three oxidizing conditions and

were responsible for changes in the functional properties of MPI.

On the other hand, the content of Tyr was significantly decreased by IOS, but not by LOS or MOS. Radical attack on Tyr residues is known to lead to the formation of intra- and inter-protein Tyr-Tyr cross-links (Fu, Dean, Southan, & Truscott, 1998; Giulivi & Davies, 1994; Heinecke, Li, Francis, & Goldstein, 1993; Vissers & Winterbourne, 1991). In a preliminary experiment, we attempted to isolate dityrosine from IOS-oxidized MPI. Some trials showed signs (HPLC peaks and fluorescence detection) of the formation of Tyr-Tyr, although the data were inconclusive. It is assumed that a small amount of myosin polymers produced in IOS-oxidized MPI, as illustrated in a separate study (Park et al., 2006a), may have resulted from Tyr-Tyr and possibly also the condensation of amino and carbonyl groups (Stadtman & Levine, 2003). Because the Cys content was significantly lowered at 1.0 mM $H_2O_2$ in IOS, but the Tyr loss was not significant until the $H_2O_2$ level was raised to 10 mM, we postulate that under mild $Fe/H_2O_2$/ascobate oxidizing conditions, modifications were initiated on sulphur-bearing amino acids. As IOS became harsher, hydrophobic amino acids such as Tyr were more exposed and vulnerable to radical attack.

The content of Ala, Leu, His, and Lys was reduced significantly in the MOS system but not in the other two oxidizing matrixes. Histidine, a metal-binding amino acid, is very susceptible to oxidative attack due to its proximity to the radical formed (Farber & Levine, 1986; Stadtman & Oliver, 1991). It is surprising that His was susceptible to MOS but not to IOS where hydroxyl radicals ($^{\bullet}OH$) were generated through a metal ($Fe^{2+}$)-catalyzed reaction. It may be suggested that the different oxidizing effects imparted by the three oxidizing conditions resulted from different ROS generated. The IOS mostly produces the highly reactive $^{\bullet}OH$ species (Srinivasan & Hultin, 1997); in LOS, lipid peroxide is generated; and in MOS, metmyoglobin in the presence of $H_2O_2$ generates hydroxyl, ferryl and protein radicals (Davies, 1990; Gatellier, Anton, & Reneree, 1995; Rice-Evans, Okunade, & Kahn, 1989). The different ROS produced presumably could readily modify the exposed, susceptible amino acid residues, notably Cys (myosin contains 41 Cys most of which are readily accessible), which was shown in the present study.

The degree of exposure of an amino acid side chain group was thought to greatly affect its oxidation (Elias et al., 2005; Stadtman & Levine, 2003). However, to react with other susceptible amino acid residues, partial unfolding of MPI proteins appeared to be required. In the present study, after the initial destruction of Cys, the ROS produced from different oxidative systems probably altered the protein structure in different manners. This premise is consistent with our previous observation that MPI samples, when exposed the same three oxidizing systems, underwent structural changes (myosin ATPase; enthalpy of denaturation) that differed slightly between the oxidizing systems (Park et al., 2006b). The structural change would facilitate the penetration of the specific ROS to induce the oxidation of additional, more exposed amino acids (i.e., Met and Tyr in IOS; Met in LOS; and Ala, His, Leu, and Lys in MOS).

It is noteworthy that modification of amino acid residues by these individual oxidizing systems in the present study, when compared with most published literature, was limited to only a few amino acids, probably because of the low temperature (4 °C) and a different protein source (muscle myofibrillar protein) used in the study. As reported by Uchida, Kato, and Kawakishi (1992), metal-catalyzed human collagen oxidation at 37 °C resulted in significant losses in a number of amino acids, particularly Pro, His, Phe, Lys, and Arg. Most published studies on amino acid oxidation have been conducted under ambient or higher temperatures. Because high-temperature conditions are not normally involved in meat processing and preservation (except during cooking), they were not employed in the present investigation.

## 5. Conclusions

The results demonstrated that susceptibility of amino acids in muscle proteins to oxidative modification depended on the source of reactive oxygen species. Sulphur-containing amino acids were the most susceptible even to a mild and indirect oxidizing condition. Additional amino acids were oxidized at high concentrations of prooxidants. This research offered an insight into the relative roles of three common radical-generating systems existing in muscle foods (iron/hydrogen peroxide-, lipoxydase-, and metmyoglobin-catalyzed). Further research is warranted to investigate the collective effect of the three radical-generating systems since they would co-exist in meat and meat products.

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