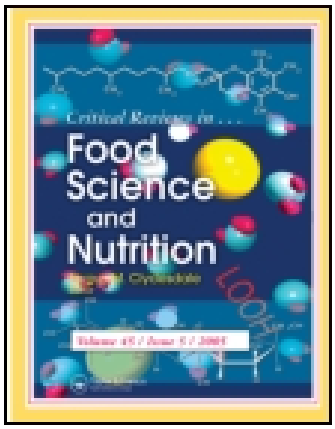

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Protein Oxidation: Basic Principles and Implications for Meat Quality

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Protein Oxidation: Basic Principles and Implications for Meat Quality

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The involvement of oxidized proteins to the development of biological diseases has been studied for a few decades, but the effects and the mechanisms of protein oxidation in food systems are largely unknown. Protein oxidation is defined as the covalent modification of a protein induced either by the direct reactions with reactive oxygen species (ROS) or indirect reactions with secondary by-products of oxidative stress. ROS can cause oxidation in both amino acid side chains and protein backbones, resulting in protein fragmentation or protein–protein cross-linkages. Although all amino acids can be modified by ROS, cysteine, and methionine that are the most susceptible to oxidative changes due to high reaction susceptibility of the sulfur group in those amino acids. Oxidative modifications of proteins can change their physical and chemical properties, including conformation, structure, solubility, susceptibility to proteolysis, and enzyme activities. These modifications can be involved in the regulation of fresh meat quality and influence the processing properties of meat products. Oxidative stress occurs when the formation of oxidants exceeds the ability of antioxidant systems to remove the ROS in organisms. Increased levels of protein oxidation have been associated with various biological consequences, including diseases and aging, in humans and other animal species. The basic principles and products of protein oxidation and the implications of protein oxidation in food systems, especially in meat, are discussed in this review.

Keywords Calpain, carbonyl, meat quality, protein oxidation, reactive oxygen species

INTRODUCTION

Oxidation is one of the major causes for quality deterioration during processing and storage of food products. Lipid oxidation has been extensively studied in food systems for many years, but the influence and the mechanisms of protein oxidation in foods, especially in fresh meat and meat products are largely unknown. Oxidation of proteins results in production of various oxidation derivatives. The main oxidative modifications of protein takes place at the side chains of amino acids, which include thiol oxidation, aromatic hydroxylation, and formation of carbonyl groups (Stadtman, 1990). Among all amino acid side chains, cysteine and methionine are the most susceptible to oxidation because they contain reactive sulfur atoms (Shacter, 2000). Sulfur anion is the most powerful nucleophile and is rich in electrons, which can be easily removed. Reactive oxygen species (ROS) include free radicals ($^{\bullet}OH$, $O_2^{\bullet -}$, $RS^{\bullet}$, and $ROO^{\bullet}$), non-radical species (H_2O_2 and ROOH), and reactive aldehydes and ketones (Butterfield and Stadtman, 1997). Oxidants can directly attack the backbone of a protein to cause fragmentation and conformational changes in the secondary and tertiary structure of the protein. Disulfide, dityrosine, and other intermolecular bridges induced by oxidation can result in protein aggregation and polymerization to change their proteolytic properties (Martinaud et al., 1997; Morzel et al., 2006). This is mainly due to an oxidation-induced unfolding process, which increases the surface hydrophobicity of the oxidized protein during unfolding (Davies and Delsignore, 1987). These alterations can influence physical and chemical properties of proteins including solubility, hydrophobicity, water-holding capacity, meat tenderness, and gelation functions (Srinivasan and Xiong, 1996; Srinivasan and Hultin, 1997; Liu and Xiong, 2000; Rowe et al., 2004a, 2004b). In addition, protein oxidation-induced changes may decrease the bioavailability of amino acid residues and modify the digestibility of proteins, which negatively affects the nutritional values of meat proteins (Lund et al., 2010). Calpain is believed to be the major enzyme for the degradation of myofibrillar proteins and contributes to the development of meat tenderness and water-holding capacity during postmortem aging (Koohmaraie et al., 1986; Taylor et al., 1995; Kristensen and

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Purslow, 2001; Lonergan et al., 2001; Huff-Lonergan and Lonergan, 2005). Calpain oxidation may arrest its proteolytic activity and thus negatively influence fresh meat quality during postmortem refrigerated storage (Rowe et al., 2004a, 2004b). Although great efforts have been exerted in recent years, the occurrence, mechanisms, and effects of protein oxidation on meat and meat products are still largely unknown. This review mainly discusses the basic principles and products of protein oxidation and the implications of protein oxidation in food systems especially in meat.

GENERATION OF REACTIVE OXYGEN SPECIES

ROS can be generated by various processes in biological system. ROS can be produced during normal metabolism including mitochondrial electron transportation reactions, peroxisomal fatty acid metabolism and cytochrome P-450 reactions, and in phagocytic cells especially those involved in terminal respiration (Beckman and Ames, 1998; Shacter, 2000). One- or two-electron reduction from O_2 forms superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), respectively, and eventually generate extremely reactive hydroxyl radical ($^{\bullet}OH$) by reaction with other components, which can initiate the oxidative damage of proteins, lipids, and nucleic acids. The enzymes catalyzing ROS generation include nitric oxide synthases, NADPH oxidase, prostaglandin synthase, xanthine oxidase, lipoxygenases, ribonuclueotide reductase, glucose oxidase, myeloperoxidase, cyclooxygenases, and cytochrome P450 (Shacter, 2000; Davies, 2005). Modification of proteins also can occur through the addition of oxidatively modified products from lipids, amino acids, sugars, and glutathione (Haberland et al., 1988). For example, aldehyde moieties from lipid peroxidation such as malondialdehyde, hydroxynonenal, and acrolein can covalently bind to residues of proteins, resulting in protein oxidation (Uchida and Stadtman, 1994; Requena et al., 1997). In addition, ROS can be generated by exposure of organisms to exogenous agents including ultraviolet (UV) light, irradiation (X-rays, γ-rays, ultraviolet A, and visible light in the presence of a sensitizer), chemical reagents (metal ions such as Fe^{2+} and Cu^+, HONOO, ozone, N_2O_2, deoxyosones, ketoamines, H_2O_2, HOCl, and HOBr), environmental pollutants, drug and their metabolites, xenobiotics, and even cigarette smoke (Shacter, 2000; Davies, 2005; Tetik et al., 2007). In muscle system, pro-oxidative proteins, myoglobin, and hemoglobin can be initiators of protein oxidation as reviewed by Baron and Andersen (2002).

CONSEQUENCES OF PROTEIN OXIDATION

Oxidation of Protein Backbone

Reactions between nonradical oxidants and protein backbone sites are relatively slow. Most of the damages in backbone position are induced by radical oxidants via the $^{\cdot}OH$-dependent hydrogen abstraction (Stadtman, 2001). The reaction occurs at the α-carbon site of amino acid residues and forms stabilized carbon-centered radicals, which react with O_2 to produce alkylperoxyl radicals. These peroxyl radicals either react with HO_2à to form hydroperoxides or are eliminated to generate imines. The hydrolysis of imines can result in backbone fragmentation while the decomposition of hydroperoxides can cleave peptide bonds through alkoxyl-radical-mediated reactions (Davies, 2005). The latter reactions can generate amides at the C-terminal side of the N-terminal part of the protein, and α-keto-acyl residues at the N-terminal side of the C-terminal part of the protein (Stadtman, 1993). In addition, protein fragmentation can be formed via single backbone cleavage by oxidation of glutamyl, aspartyl, and prolyl side chains (Essex et al., 2001). Hydrogen abstraction from γ-carbon atom of a glutamyl residue can lead to the cleavage of peptide bonds and the formation of *N*-pyruvyl derivatives, and oxalic acid intermediates (Garrison, 1987). Oxidation of prolyl residues can produce two protein fragments and an α-amino butyric acid (Uchida et al., 1990). For instance, proline oxidation provides a unique mechanism for peptide bond cleavage via the formation of 2-pyrrolidone, which later is hydrolyzed to 4-aminobutyric acid (Stadtman, 1993; Hawkins and Davies, 2001). Additionally, ROS attack on the β-position via β-scission reaction can give rise to a carbonyl compound and an α-carbon radical susceptible to cleavage via the diamide or α-amidation pathways (Headlam and Davies, 2004).

Oxidation of Amino Acid Residues

Cysteine and methionine are the most susceptible to oxidative modification by all forms of ROS due to their sulfur atoms (Garrison, 1987). One- or two-electron oxidation of cysteine can form similar end products. One-electron oxidation of cysteine with radical oxidant can generate thiyl radicals. These species have two major pathways: reaction with other thiol/thiolate to form disulfide, or reaction with O_2 to generate thiyl peroxyl radicals (RSOO**à**) (Wardman and von Sonntag, 1995; Schoneich, 2008). The two-electron oxidation between cysteine and oxidants can result in the formation of sulfenic acid (CysSOH), sulfinic acid ($CysSO_2H$), and sulfonic acid ($CysSO_3H$) (Claiborne et al., 2003). These species are unstable and can yield oxyacids by hydrolysis reactions or disulfide bonds by reacting with another thiol group (Turell et al., 2008). Similarly, methionine residues can be easily oxidized by various kinds of oxidant species. The major product from methionine oxidation is sulfoxide, which can be further oxidized to sulfone (Vogt, 1995; Barelli et al., 2008). Methionine sulfoxide can be reversed to methionine by enzyme methionine sulfoxide reductase and reducing reagents such as mercaptoethanol and dithiothreitol (Houghten and Li, 1983; Dean et al., 1997). Methionine oxidation can both activate and damage protein functions and regulate homeostasis in biological systems (Vogt, 1995).

Compared to cysteine and methionine, other amino acids need more stringent conditions to be oxidatively modified. For aromatic amino acids, including histidine, phenylalanine, tryptophan, and tyrosine, the major reaction is the addition to

Table 1 Oxidation products of amino acid residue side chains

Amino acid residues	Oxidation products
Arginine	γ-Glutamyl semialdehyde
Cysteine	Disulfides, cysteic acid
Glutamic acid	Oxalic acid, pyruvate adducts
Histidine	Aspartate, asparagines, oxo-histidine
Leucine	3-, 4-, and 5-hydroxyleucine
Lysine	α-Aminoadipic semialdehyde
Methionine	Methionine sulfoxide, methionine sulfone
Phenylalanine	2-,3-,4-Hydroxyphenylalanine, 3,4-dihydroxyphenylalanine
Proline	Glutamic semialdehyde, 4- and 5-hydroxyproline pyroglutamic acid, 2-pyrrolidone
Threonine	2-Amino-3-ketobutyric acid
Tryptophan	2-,4-,5-,6-, and 7-Hydroxytryptophan, formylkynurenine, 3-hydroxykynurenine, kynurenine, and nitrotryptophan
Tyrosine	3,4-Dihydroxyphenylalanine, 3-nitrotyrosine, tyrosine–tyrosine cross-linkages and tyrosine-oxygen-tyrosine

aromatic side chains. The abstraction reactions only occur at heteroatom substitute (Hawkins et al., 2003). The aromatic amino acid residues are not sensitive to metal-catalyzed oxidation systems, but they are the preferred targets of radicals induced by radiolysis (Giulivi et al., 2003). Tyrosine can be converted to dityrosine by UV and γ-irradiation, free radicals, peroxynitrite and lipid hydroperoxides (Giulivi and Davies, 1994). The initial step is the formation of tyrosyl radicals by one-electron oxidation of tyrosine. Two monomeric tyrosyl radicals can combine to form dityrosine, which is relatively stable and resistant to hydrolysis by lytic enzymes (Giulivi and Davies, 1994). This dityrosine can lead to the formation of extensive inter- and intraprotein cross-linkage. Other aromatic amino acids can be oxidatively modified: histidine residues are oxidized to asparagines, aspartic acid residues, and oxo-histidine; phenylalanine can be converted to 2-,3-, and 4-hydroxyphenylalanine and 3,4-dihydroxy-phenylalanine; the oxidation products of tryptophan are 2-,4-,5-,6-, and 7-hydroxy-tryptophan, formylkynurenine, 3-hydroxykynurenine, kynurenine, and nitrotryptophan (Table 1).

Peroxynitrite-Related Protein Oxidation

Nitric oxideis synthesized during the conversion of L-arginine to L-citrulline catalyzed by a family of enzymes called nitric oxide synthase (NOS) in human and other species. Three isoforms of NOS, which include neuronal NOS (NOS1 or nNOS), macrophage (immune)/calcium calmodulin-independent or inducible NOS (NOS2 or iNOS), and endothelial NOS (eNOS or NOS) have been identified (Stamler and Meissner, 2001). In muscle tissues, the nNOS is the major isoform responsible for the production of nitric oxide. Nitric oxide can react with superoxide ($\cdot O_2$) to form peroxynitrite, which can nitrosylate cysteine and nitrate tyrosine, and oxidize methionine, tryptophan, and phenylalanine (Beckman et al., 1994; Ischiropoulos and Ai-Mehdi, 1995; Berlett et al., 1996; Viner et al., 1999). However, oxidation induced by peroxynitrite cannot lead to the formation of carbonyl derivatives (Tien et al., 1999). The function of sulfhydryl-containing proteins can be regulated by nitric oxide (NO)-induced S-nitrosylation. S-nitrosylation is the covalent attachment of a nitrogen monoxide group to the thiol side chain of cysteine (Hess et al., 2005). The formation of S-nitrosothiols and mixed disulfides through S-nitrosylation can influence catalytic activity of cysteine protease in vivo and in vitro (Xian et al., 2000; Ascenzi et al., 2001; Zhang et al., 2008). The proteolytic activity of both m- and μ-calpain can be arrested by NO donors at different pH (Michetti et al., 1995; Koh and Tidball, 2000).

Peroxynitrite can oxidize methionine to both sulfoxide and ethylene by two via the three pathways (Pryor et al., 1994): (i) reactions between methionine and peroxynitrous acid leading to the formation of ethylene by one-electron oxidation pathway and (ii) a direct bimolecular oxidation between peroxynitrite and methionine resulting in methionine sulfoxide by two-electron oxidation pathway. Peroxynitrite can nitrate tyrosine residues to regulate the interconversion of phosphorylation and nucleotidylation, which play critical roles in enzyme activities and signal transduction (Hunter, 1995). However, the nitration of tyrosine and the oxidation of methionine are regulated by the levels of carbon dioxide (CO_2). In the absence of CO_2, peroxynitrite can rapidly oxidize methionine to methionine sulfoxide. In the presence of CO_2, peroxynitrite can react rapidly with CO_2 to form the $ONOOCO_2^-$ or $O_2NOOCO_2^-$ (Lymar and Hurst, 1995; Berlett et al., 1998).

Formation of Protein Carbonyl Derivatives

Generation of carbonyls is the most common damage for oxidized proteins. Four major pathways to generate protein carbonyls include: (i) fragmentation of backbones through the α-amidation pathway and β-scission, (ii) binding of non-protein carbonyl compounds from lipid peroxidation by Michael addition (4-hydroxy-2-nonenal (HNE) and malondialdehyde) to protein amino acid side chains including histidine imidazole, cysteine sulfhydryl, and lysine amino groups (Burcham and Kuhan, 1996; Berlett and Stadtman, 1997; Refsgaard et al., 2000), (iii) direct oxidation of amino acid side chains including arginine, lysine, proline, and threonine (Amici et al., 1989), and (iv) addition of reactive carbonyl derivatives (ketoamines, ketoaldehydes, and deoxyosones) generated by reducing sugars and their oxidation products after reacting with lysine (Decker et al., 2000).

Protein–Protein Cross-linkages

As discussed above, oxidation of protein backbone by hydrogen abstraction can lead to the formation of carbon-centered radicals. In the absence of oxygen, two radicals can combine together to generate cross-linkage within and between proteins

(Stadtman, 2001). Oxidation and nitrosylation of sulfhydryl groups and tyrosine residues can lead to the generation of disulfide and dityrosine bonds, respectively, resulting in cross-linked proteins (Giulivi and Davies, 1993). Carbonyl derivatives arising from direct or indirect oxidation reactions can react with lysine amino groups within a protein or between different proteins leading to the formation of protein cross-linkages (Shen et al., 1996). When myofibrillar proteins of pork were suspended in an iron-catalyzed oxidizing system or an H_2O_2-activated metmyoglobin oxidizing system, the myosin was shifted from head–head associations to tail–tail cross-links due to the formation of disulfide bonds (Xiong et al., 2010). The cross-linking sites of myosin heavy chain was in light meromyosin segment through disulfide bonds, while no cross-linking was found in heavy meromyosin after chicken myofibrils were exposed to hydroxyl radical-generating system (Ooizumi and Xiong, 2006). In three different oxidizing systems (iron-catalyzed oxidizing system, H_2O_2-activated metmyoglobin oxidizing system, and linoleic acid-oxidizing system) for porcine myofibrillar proteins, disulfide bonds were reported to be mainly responsible for protein cross-linkage while malonaldehyde was hardly involved in the formation of cross-linkage (Xiong et al., 2009). These cross-linkages can change the properties of proteins such as structure, function, and susceptibility to degradation as discussed in the following section.

Modification of Conformation, Structure, and Function

Oxidants can attack proteins to disrupt the physical and chemical forces, which contribute to the stability of unmodified proteins. These attacks can change the primary structure of proteins, which can further distort secondary and tertiary structure of proteins. Eventually, changes in primary, secondary, and tertiary structures can induce proteins to unfold to random conformations (Davies and Delsignore, 1987). Amino acid residues exposed on surface are more readily oxidized compared to buried ones, but oxidation of these surface residues seems to have no significant effects on protein conformation (Levine et al., 1999). Oxidation of buried side chains can lead to unfolding, reduced thermal stability, increased or decreased surface hydrophobicity, and conformational changes (Liu and Xiong, 1996; Gao et al., 1998). The formation of disulfide bonds can contribute to the stability of the native conformation of proteins by changing their thermodynamics. Oxidation of aromatic residues can lead to addition of some charged groups, which increase the hydrophilic status for these residues. These changes may force oxidized aromatic residues to be present on protein surface resulting in protein conformation change (Chao et al., 1997). For example, oxidation on catalytic cysteine 252 of tyrosine phosphatase-like phytase could result in conformation changes in other amino acid residues and P-loop of this protein (Gruninger et al., 2008). There are still some arguments about the susceptibility of enzyme-induced degradation of oxidized protein. Generally, heavily oxidized proteins can lower digestibility partly due to protein aggregation, whereas modestly oxidized proteins are easily digested by corresponding proteases (Levine et al., 1981; Wolff and Dean, 1986; Davies et al., 1987; Smuder et al., 2010). It is possible that conformational changes can expose some buried peptide bonds for enzyme hydrolysis and increase degradation susceptibility. Otherwise, strong oxidation environment can result in the formation of protein cross-linkage, which further leads to protein aggregation. These cross-linkages and aggregates are more resistant to enzymatic degradation compared to native protein forms (Davies et al., 1987). Oxidation of methionine especially buried methionine in protein hydrophobic region is highly possible to change protein structures. In actin, chloramine-T-induced methionine oxidation changed its conformation, and increased its surface hydrophobicity. Oxidation of buried methionine can disrupt the non-covalent interactions within actin and decrease the stability of actin filament (Dalle-Donne et al., 2002).

METHODS TO DETERMINE PROTEIN OXIDATION IN FOOD SYSTEMS

2,4-Dinitrophenylhydrazine (DNPH) Derivatization Method

Although carbonyl compounds are not the oxidation products of some amino acid residues such as histidine, phenylalanine, and tryptophan, DNPH derivatization method that detect carbonyl compounds has been developed as a convenient and regular method to quantify the levels of protein oxidation in food system (Oliver et al., 1987; Levine et al., 1994). In this method, DNPH reacts with the carbonyl groups of proteins to generate hydrazones and the absorbance is read at 370 nm (Levine et al., 1990). The carbonyl content was calculated as nmol/mg protein using an absorption coefficient of 22,000 $M^{-1}cm^{-1}$ (Levine et al., 1994). In food systems, carbonyl groups can also be generated by other pathways including metal-catalyzed oxidation of specific amino acid side chains and the addition of sugars or lipid oxidation products such as 4-hydroxynonenal and malondialdehyde (Stadtman and Berlett, 1998), which can lead to overestimation of protein oxidation (Estévez et al., 2008). However, DNPH derivatization method has been utilized as a useful and meaningful marker for the accumulation of oxidized proteins in living tissues of human and other species partly due to the simplicity and convenience of this method. This method has been successfully applied in meat systems including raw fresh meat, meat emulsions, and meat products to evaluate the degree of protein oxidation in the products (Mercier et al., 1998; Haak et al., 2006; Estévez et al., 2007; Lund et al., 2007, 2008; Zhang et al., 2010).

Fluorescence Spectroscopy Method

Fluorescence spectroscopy has been reported to be effective and specific to measure the destruction of tryptophan residues of apolipoprotein B in copper-induced low-density lipoprotein

oxidation (Giessauf et al., 1995). The decrease of tryptophan fluorescence was determined at an emission wavelength of 331 nm and an excitation wavelength of 282 nm. In a lecithin-liposome oxidation system, fluorescence spectroscopy was sensitive to detect the decrease of tryptophan and lysine in bovine serum albumin (BSA) (Heinonen et al., 1998). This fluorescence spectroscopy method could also be used to detect compounds formed by the lipid oxidation products and amino acid groups of proteins with special spectral properties (Viljanen et al., 2004). Recently, fluorescence spectroscopy has been used to study the oxidation of myofibrillar proteins by measuring the loss of tryptophan and the increase of protein carbonyl compounds in an oil-in-water emulsion system (Estévez et al., 2008). Emulsions with rapeseed oil and myofibrillar proteins from porcine *longissimus dorsi* were dissolved in phosphate buffer. The decrease of tryptophan could be determined by the emission spectra recorded from 300 to 400 nm at the excitation wavelength of 283 nm (Estévez et al., 2008). They reported that tryptophan fluorescence was decreased in the early period of 10-day storage while the fluorescence of protein carbonyls was generated in late stage as secondary protein oxidation products (Estévez et al., 2008).

Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS)

Recently, Estévez and his research group have developed a new method to detect special protein oxidation products, α-aminoadipic semialdehydes (AAS) and γ-glutamic semialdehydes (GGS), and applied this method to meat products as well as other food proteins (Armenteros et al., 2009; Estévez et al., 2009). AAS is an oxidative product from the deamination of lysine and GGS is generated from the oxidation of arginine and proline residues through Maillard reaction (Requena et al., 2003). Akagawa et al. (2005) developed a high-performance liquid chromatography (HPLC) method to detect AAS and GGS in BSA in vitro after derivatizing the protein with $NaCNBH_3$ and the *p*-amino-benzoic acid (ABA). For the first time, Armenteros et al. (2009) employed ABA derivatization procedure and a LC-ESI-MS technique to detect the oxidation products of specific amino acids in meat products. AAS and GGS were successfully detected in ground meat, dry-cured ham, dry-cured loin, dry-cured sausage, and cooked sausage. Among the meat products, dry-cured ham and dry-cured sausages showed the highest GGS, while the amount of AAS was higher in ground meat and cooked sausage than other meat products (Armenteros et al., 2009). This indicates that AAS and GGS are the form of oxidation products in lysine, arginine and proline residues of myofibrillar proteins in fresh meat and processed meat products. In other food proteins including α-lactalbumin, soy proteins, and BSA, LC-ESI-MS plus ABA derivatisation procedure was effective in determining the contents of AAS and GGS derived from the oxidation by Fe^{3+} and H_2O_2 (Estévez et al., 2009). The DNPH measurements were significantly correlated with the amount of AAS and GGS determined by LC-ESI-MS. The strong correlation supports that both AAS and GGS can be representative of the total amount of carbonyl contents and thus can be used as the markers for the protein oxidation in food systems (Estévez et al., 2009). Compared to DNPH and fluorescence spectroscopy, the newly developed LC-ESI-MS method can further detect particular compounds that have been proved to be effective and reliable protein oxidation indicators. The detection of these compounds is very significant because it enables us to better comprehend the particular mechanisms involved in the oxidative degradation of myofibrillar proteins and the fate of specific amino acids in food systems.

PROTEIN OXIDATION AND MEAT QUALITY

Protein Oxidation in Fresh Meat During Postmortem Aging

In postmortem muscle, protein oxidation has been gradually recognized as an important factor for meat quality. During postmortem storage, muscle has a decreased ability to maintain its antioxidant defense system, which can lead to the increased accumulation of reactive oxygen and nitrogen species (Renerre et al., 1996). These species can cause increased levels of protein oxidation, which can modify protein structure and function including enzyme activity. These changes are possibly involved in mediating the conversion of muscle to meat, and thus, regulating meat quality.

Protein oxidation can naturally occur during the postmortem aging and refrigerated storage. Using fluorescence microscopy to detect carbonyl content in muscle cells, Astruc et al. (2007) determined the localization of protein oxidation in bovine *rectus abdominis* with three different treatments (chemical oxidation reagents of Fe^{2+}/H_2O_2, refrigerated storage at 4°C, and cooking at 100°C). All of these treatments induced protein oxidation at both the periphery and the inside of muscle cells. Particularly, the amount of protein carbonyl increased fourfold from during 10 days of refrigerated storage. In addition, the levels of protein oxidation at day 10 after refrigerated storage were similar to the levels of oxidation induced by 1 mM of oxidant mixture ($FeSO_4$ and H_2O_2). Protein oxidation was not equally distributed in muscle cells. The peripheral area corresponding to the cell membrane and to a region in close contact with the cell membrane showed higher extent of protein oxidation compared to an inner area containing myofibrils and sarcoplasmic proteins (Astruc et al., 2007). The authors concluded that protein oxidation was initiated from the muscle membrane to the inside of muscle cells during postmortem storage. Lipophilic antioxidants such as vitamin E may be powerful reagents to limit the deterioration by protein oxidation (Rowe et al., 2004a; Astruc et al., 2007). Martinaud et al. (1997) also found that carbonyl content increased in *longissimus lumborum* (70% from 3.1 to 5.1 nmol/mg protein) and *diaphragma pedialis* (44% from 4.8 to 6.9 nmol/mg protein) of beef, respectively, during a 10-day aging period. Lambs raised on concentrated diet produced greater amount of protein carbonyls than those on pasture during

storage (Santé-Lhoutellier et al., 2007). The carbonyl content in turkey *M. sartorius* and *M. pectoralis* increased to 2.53 and 1.83 nmol/mg protein, respectively, after 9 days of postmortem refrigerated storage under oxygen permeable conditions (Mercier et al., 1998).

High-Oxygen Modified Atmosphere Packaging and Protein Oxidation

Modified atmosphere packaging (MAP) with high oxygen content (70–80%) has been widely used in fresh meat retail market due to the bright red color in high-oxygen MAP system (Eilert, 2005; Seyfert et al., 2005). Studies have been focusing on the changes of fresh meat quality and microorganisms induced by MAP, but very limited research on the effects of MAP on protein oxidation. In high-oxygen packaging systems, oxygen could possibly lead to protein oxidation and thus influence fresh meat quality during postmortem chill storage. Kim et al. (2010) reported that beef steaks of *longissimus lumborum* in high-oxygen MAP system showed higher star probe values and lower subjective tenderness scores than samples from vacuum packaging. The decreased tenderness was associated with the formation of cross-linkages between myosin and titin and was independent to the postmortem protein degradation and the activity of μ-calpain (Kim et al., 2010). Similar results were reported in porcine *longissimus dorsi* when muscle samples were packaged in 70% oxygen and 30% CO_2 (Lund et al., 2007). High oxygen in the packaging system resulted in lower tenderness, which could be explained by protein oxidation. Cross-linkage between myosin heavy chains by disulfide bonds and decreased amounts of free thiol groups were shown in high-oxygen package systems compared to skintight packaging (Lund et al., 2007). Clausen et al. (2009) reported that high-oxygen MAP resulted in decreased tenderness, lower values of myofibril fragment index, and higher levels of protein carbonyl compounds in beef *longissimus dorsi*, indicating the possible association between tenderness and protein oxidation and proteolysis. Based on these limited studies, MAP with high oxygen has negative influence on fresh meat quality, especially tenderness and color. However, whether the decreased tenderness is due to the oxidation of proteins or the reduced levels of protein degradation is still inconclusive.

Irradiation-Induced Protein Oxidation and Meat Quality

Irradiation is highly effective in controlling pathogenic microorganism in meat, and thus extending the shelf life of meat. However, irradiation can produce free radicals including $^{\bullet}OH$ and O_2^-, which potentially increase the oxidation of proteins and lipids in meat products (Jo and Ahn, 2000; Nam and Ahn, 2003). Rowe et al. (2004a, 2004b) reported that the oxidation of myoglobin played important roles in the discoloration of irradiated beefsteaks. Carbonyl contents of myofibrillar and sarcoplasmic proteins in irradiated beefsteaks were negatively correlated with meat tenderness at 1, 3, and 7 days of postmortem storage. Increased shear force associated with protein oxidation was suggested to be related to the aggregation of myofibrillar proteins or due to the inhibition of proteolytic enzyme activity (Rowe et al., 2004a). Vitamin E was found to be effective in decreasing the amount and the extent of oxidation of sarcoplasmic proteins. In a parallel study, Rowe et al. (2004b) determined the effects of irradiation-induced oxidation on protein degradation and calpain activity in beef. Compared to nonirradiated beefsteaks, irradiated samples had more intact and less degraded products of desmin after 3, 7, and 14 days of refrigerated storage. The decreased proteolysis was likely due to the inhibition of μ-calpain activity by irradiation (Rowe et al., 2004b). In nonirradiated samples, steaks from vitamin E-supplemented animals showed faster degradation of troponin-T than those from control animals not supplemented with vitamin E. The differences in protein degradations were associated with higher Warner–Bratzler shear force in irradiated steaks than nonirradiated steaks at 1, 3, 7, and 14 days postirradiation. Yoon (2003) reported that low-dose γ-irradiation was associated with higher cook loss and greater shear force in chicken breast meat during 14 days of refrigerated storage.

Protein Oxidation and the Calpain System

The calpain system is generally believed to be responsible for regulating the degradation of proteins in postmortem muscle, and is known to be associated with meat tenderness and water-holding capacity during postmortem storage (Koohmaraie, 1992; Huff-Lonergan et al., 1996; Huff-Lonergan and Lonergan, 2005; Zhang et al., 2006). Three members of the calpain system are the proteases m- and μ-calpain and their inhibitor calpastatin (Goll et al., 2003). Calpains autolyze in the presence of calcium and this autolysis is indicative of their proteolytic activation in postmortem muscles (Geesink and Koohmaraie, 1999). Since both m- and μ-calpain are cysteine protease, oxidation may regulate their proteolytic activities, and thus, influence fresh meat quality.

Guttmann et al. (1997) reported that 100 μM H_2O_2 strongly inhibited μ-calpain-induced proteolysis including fluorescent peptide and the microtubule-associated protein *tau* at all calcium concentrations. The autolysis rate and the extent of either 80 or 30 kDa subunits were not influenced by oxidation conditions regardless of the calcium concentrations. In another study, calpain activity was inhibited in SH-SY5Y human neuroblastoma cells by oxidants doxorubicin. The autolytic rate for large subunit from 80 to 76 kDa also decreased during oxidative stress, but oxidants did not influence the extent of calpain autolysis (Guttmann and Johnson, 1998).

Carlin et al. (2006) further determined the effects of oxidation on the activity of purified m- and μ-calpain and the interaction between μ-calpain and calpastatin. Proteolytic activity of μ-calpain and m-calpain was shown to be inhibited by H_2O_2-induced oxidation at different pH including 6.0, 6.5, and

7.5. Interestingly, H_2O_2 decreased the inhibition of μ-calpain activity by calpastatin at pH 6.5 and 7.5, and allowed μ-calpain to degrade desmin in the presence of calpastatin (Carlin et al., 2006). They reported oxidation decreased the conversion of 80 kDa subunit to 76 kDa without changing the extent of autolysis of large subunit. Both intact 80 kDa and autolyzed 76 kDa products of large subunit of μ-calpain were sensitive to oxidative environment. Inactivation of intact μ-calpain could be reversed by a reducing agent such as dithiothreitol (Guttmann et al., 1997). The inhibition of μ-calpain activity was possibly associated with the formation of a disulfide bond between Cys115 and Cys108 within active site by LC-MS/MS analysis (Lametsch et al., 2008). This disulfide bond was also detected in calpain purified from porcine meat and in calpain incubated with reducing agent β-mercaptoethanol, indicating that the cysteine within the active site is highly susceptible to the formation of intramolecular disulfide bond. All these studies provide strong evidences that protein oxidation is involved in regulating fresh meat quality through mediating the autolysis and proteolytic activity of calpains.

Protein Oxidation and Protein Functionality in Meat and Meat Products

Protein oxidation-induced structural modification can change their functionality and thus influence products quality. During processing of meat products, mechanical strength can destroy the integral structure of cells and break up antioxidant defense systems resulting in high susceptibility to protein oxidation. Afterturkey white muscles wereincubated withoxidants (iron chloride or copper chloride) for 6 hours, the strength of gel formed from myofibrillar proteins decreased from 0.76 to 0.05 and 0.08, respectively. The water-holding capacity of gels oxidized by iron and copper also decreased by 23 and 10%, respectively (Decker et al., 1993). Liu et al., (2010) reported that pork *longissimus* muscle showed lower water-holding capacity after incubation with $FeCl_3$/ascorbate/H_2O_2 at 4°C. Changes in water-holding capacity and product yield were consistent with the increases of protein carbonyl content and cross-linkage among both myofibrillar and sarcoplasmic proteins. The decreased water-holding capacity could be explained by the enlarged extracellular space between adjacent fibers in the oxidized muscle samples (Liu et al., 2010). Effects of protein oxidation on their functional properties were also studied by incubating muscles with antioxidant. In minced beef heart muscle, myofibrillar proteins showed increased shear stress and viscosity after they were washed with combined antioxidants containing 0.02% propyl gallate, 0.2% sodium ascorbate, and 0.2% sodium tripolyphosphate. The antioxidant washing improved other functionality of myofibrillar proteins including greater storage, less moduli within 50–60°C, and stronger gels (Wan et al., 1993). However, protein oxidation is not always associated with deleterious effects on protein functionality. Minced cod muscle showed improved gelation and emulsification characteristics when exposed to three different oxidation systems including chelated ferric iron, H_2O_2, and ascorbate. The shear force and true strength of cooked gels increased by 70 and 20%, respectively (Srinivasan and Hultin, 1997). In beef heart surimi, antioxidant washing (propyl gallate and α-tocopherol) of minced muscle caused a decreased ability of gel formation and increased levels of protein carbonyl compared to water washing treatment (Srinivasan and Xiong, 1996). The increased gel-forming ability due to protein oxidation may be associated with the formation of cross-linkage between polypeptides and between proteins. These cross-linkages can decrease the mobility of gel network and stabilize other non-covalent bonds within the gel matrix (Decker et al., 2000).

Protein oxidation can induce protein polymerization and aggregation, and thus, change their digestibility, which negatively influences the nutritional values of muscle foods. Protein oxidation can change the intermolecular and intramolecular interactions within a protein, and thus, influence their conformation. These changes generally can increase the surface hydrophobicity of a protein due to changes in the tertiary structure. In addition, protein oxidation is associated with the formation of dimmer, trimer, and polymer, and other inter- and intra-cross-linkage, which can further lead to protein aggregation (Hanan and Shaklai, 1995). High levels of oxidation can result in protein denaturation and precipitation, which is associated with decreased protein solubility. Earlier studies showed that linoleic acid and linoleic acid hydroperoxides could destroy A-band structure of thick filament in fish and cause the myosin denaturation. After incubation with hydroperoxides for 2.5 hours, solubility of myofibrillar protein can be decreased by 90% due to the decreased extractability (Jarenback and Liljemark, 1975). The solubility of myofibrillar proteins from turkey white muscle decreased by 32 and 36%, respectively, when the proteins were added in an oxidant system containing iron, copper, and ascorbate (Decker et al., 1993). Sarcoplasmic proteins are also susceptible to oxidative modifications. Rowe et al. (2004a) found that irradiation of beef at 6.4 kGy induced protein oxidation in sarcoplasmic proteins and resulted in decreased protein extractability after 4 and 14 days of refrigerated storage. Lipid free radicals caused the aggregation and denaturation of myoglobin and decreased protein solubility in bovine myosin-methyl linoleate emulsion system (Nakhost and Karel, 1983). Beef *longissimus thoracis* showed high levels of protein oxidation when they were cooked to the surface temperature of 65, 96, and 207°C (Gatellier et al., 2010). High temperature resulted in higher levels of protein carbonyl content and lower contents of tryptophan and tyrosine. The amounts of cysteine, methionine, and tyrosine decreased significantly in ion-catalyzed oxidizing system, while the amount of cysteine, glycine, histidine, alanine, leucine, lysine, and the total amounts of amino acids were significantly lower in metmyoglobin-oxidizing system after the myofibrillar protein isolate was incubated with those oxidants for 24 hours at 4°C (Park and Xiong, 2007). Oxidation of these amino acids along with other essential amino acids including lysine, histidine, arginine, and threonine could decrease the nutritional values of meat and meat products (Liu and Xiong, 2000).

Interactions between Protein and Lipid Oxidation

Oxidation of oxymyoglobin to metmyoglobin results in meat discoloration, while lipid oxidation leads to the production of off-flavor and decrease nutritional values of meat and meat products. The mechanisms behind and the control of interactions between myoglobin and lipid oxidation have been reviewed by Baron and Andersen (2002), and recently by Faustman et al. (2010). Many studies have focused on the interactions between protein and lipid oxidation, and increased evidence supports the idea that oxidation products from protein and lipid can further increase the oxidation in a reciprocal manner (Faustman et al., 2010). Amino acids are more susceptible to damage by secondary products of lipid oxidation such as aldehydes compared to primary products of lipid oxidation such as hydroperoxides. Secondary products derived from lipid oxidation can interact with the amino acid residues of proteins regulating their structure and function. For example, hydroperoxides and aldehydes produced from lipid oxidation can react with lysine residues to lead to the formation of pyrrole derivatives (Hidalgo et al., 1998). The derivatives of interactions between lipid oxidation products and amino acid residues can cause the formation of cross-linkage between proteins. The reaction between 4,5-epoxy-2-alkenals and the amino groups of amino acids results in the formation of N-substituted pyrroles (II), which can polymerize rapidly and lead to the development of brown color (Zamora et al., 2000). Lysine moieties of proteins could react with γ-hydroxy-α,β-unsaturated epoxides generated from lipid oxidation and form some compounds including 4-(propylamino)-*trans*-2-hexene-1,5-diol and N^2-acetyl-N^6-(1,5-dihydroxy-*trans*-2-hexen-4-yl)-l-lysine 4-methylcoumar-7-ylamide in a model system with *trans*-4,5-epoxy-*trans*-2-hexen-1-ol (EH) and propylamine (Lederer, 1996). After beef and porcine oxymyoglobin were incubated with HNE, two histidine residues of porcine myoglobin while four histidine residues of beef myoglobin were attached with HNE via Michael addition (Suman et al., 2006). Adduction of HNE to oxymyoglobin changed the tertiary structure and promoted its susceptibility to oxidation (Maheswarappa et al., 2009). Lipid oxidation as a promoter of protein oxidation can be evidenced by feeding animals with different status of unsaturated fatty acids (Nute et al., 2007). Zhang et al. (2010) reported that consumption of oxidized oil was related to increased levels of protein carbonyl and decreased meat quality including lipid oxidation and drip loss in breast meat of broiler chickens.

Some proteins in muscle foods especially myoglobin can act as pro-oxidants that initiate and accelerate lipid oxidation (Baron and Anderson, 2002). Increased levels of oxygen in packaging systems of beefsteaks were associated with increased lipid oxidation and decreased color stability (Zakrys et al., 2008). Strong negative correlations were found between the content of 2-thiobarbituric acid reactive substances (TBARS) and the concentration of oxymyoglobin especially in high-oxygen packaging groups (Zakrys et al., 2008). In a myoglobin-liposome system, the TBARS values increased by 12.5-, 17.1-, and 19.0-fold, respectively, as the oxymyoglobin concentrations increased from 0.25 to 0.625 and 2.5 mg/ml after 1 hour incubation at 37°C (Chan et al., 1997). Chan et al. (1997) also reported that H_2O_2 instead of O_2^- was directly involved in regulating the interaction of oxidation of oxymyoglobin and lipids. In conclusion, protein oxidation-induced changes can influence fresh meat quality during postmortem aging and subsequent protein functionality of meat products. Addition of antioxidant such as vitamin E in diet and other technological strategies could be effective to limit deteriorative effects of both lipid and protein oxidation in muscle foods as recently reviewed by Lund et al. (2010).

CONCLUSIONS

Protein oxidation induced by ROS can cause the modification of backbones and side chains of proteins. These modifications lead to the structural changes at the levels of primary, secondary, and tertiary structure of proteins. These structural changes can induce conformational and functional modifications of proteins including enzyme activity. Protein oxidation especially μ-calpain oxidation can influence the conversion of muscle to meat and thus contribute to the variation in meat quality including tenderness and water-holding capacity. Oxidative modification of proteins can change their properties including solubility, nutritional values, gel-forming ability, surface hydrophobicity, and water-binding activity. Interactions between protein and lipid oxidation can lead to further oxidation in a concomitant and a reciprocal manner. Although great efforts have been exerted to explore the influence of protein oxidation in muscle foods recently, the occurrence and the mechanisms of protein oxidation in meat systems are still largely unclear. Development of new, accurate methods to detect the modifications of amino acid residues in proteins will help understanding the mechanisms and the products of protein oxidation in meat systems. The roles of protein oxidation on fresh meat quality in early postmortem stage should be further studied especially with the consideration that oxidation of some key enzymes may regulate the rate of glycogen metabolism and the concentration of calcium in cytoplasm of muscle cells in early postmortem stage. In addition, the interaction between protein oxidation and lipid oxidation and how combinations of these modifications modulate muscle food quality should be better explained. Understanding the mechanisms of protein oxidation will enable us to produce muscle foods with better appearance, sensory quality, and nutritional values in future.

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