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Impacts of Oxidation on Muscle Protein Functionality

YOULING L. XIONG*

Introduction

Oxidation occurs ubiquitously in biological systems, e.g., in both ante- and post-mortem muscle tissues, in pre- and post-harvest plant cells and in food products of animal and plant origins. In muscle tissue of live animals, where cellular substances are highly compartmentalized, oxidation can occur but is relatively minor. In fact, except in pathological conditions, most damages to muscle tissue by radicals are repairable. However, in post-mortem muscle, particularly in meat undergoing processing treatments, i.e., fabrication, comminution and mixing with various ingredients which effect extensive fibril damages and disruption of cell membranes and organelles, oxidation can become a serious problem. Oxidation as a major cause of quality deterioration for a variety of raw and processed foods during storage is now a well-recognized fact. The quality loss results from reactions of active oxygen species or radicals with compounds possessing chemically unstable structures. Thus, in the past few decades, muscle food researchers have extensively investigated chemical changes occurring in meat and meat products, with most efforts being centered on oxidation of lipids, flavor compounds and pigments, all of which contain polyunsaturated bonds susceptible to free radical attack. In contrast, oxidative modification on muscle proteins and its influence on functional properties, particularly those responsible for texture of final meat products, have been dealt with only recently. Therefore, our current understanding of the role of protein oxidation in meat processing and meat quality is still quite limited.

Proteins are made up of amino acids linked together by peptide bonds. The amount of unsaturated bonds in proteins is relatively small and they are only found in the aromatic amino acids Trp, Tyr and Phe and in His, a heterocyclic amino acid. In muscle, the aromatic amino acid residues are mostly occluded in the native protein structure. Hence, muscle proteins would seem to be resistant to oxidative attack. However, recent advancement in nutritional and toxicological research has led to the recognition that many pro-

*Y.L. Xiong, Dept. of Animal Sciences, 206 W.P. Garrigus Bldg., University of Kentucky, Lexington, KY 40546.

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teins located in the muscle cell can be readily modified by active oxygen species and radicals generated via lipid peroxidation, metal- or enzyme-catalyzed oxidative reactions and other chemical and biological processes (Stadtman, 1990, 1995; Signorini et al., 1995). Both aromatic and aliphatic amino acids are susceptible to oxidative substances. Increasingly documented evidence in the biomedical science literature now indicates remarkable relationships between protein oxidation and numerous pathological processes and diseases. These include Alzheimer's and other neurodegenerative diseases, cancer, ischemia-reperfusion injury and lymphocyte proliferations (Carney and Carney, 1994; Tian et al., 1995). In addition, oxidative damages to muscle proteins in living tissue are linked to the acceleration of certain physiological processes, such as aging, protein turnover and loss of immune functions (Stadtman, 1993; Cao and Cutler, 1995). Proteins that are involved in these diseases and decreased body functions are believed to be enzymes and membrane polypeptides responsible for key biochemical processes in the cell.

The observation of protein oxidation in living animal tissue has recently prompted meat scientists to ponder the effects in post-mortem muscle when proteins are exposed to oxidative environments. A particular concern is the impact of protein oxidation on textural quality of processed meats. In meat, where most biological processes have ceased, protein oxidation can still take place. In fact, oxidation of muscle proteins is common in frozen stored meat where lipid peroxidation is extensive and also in those processed meats that are subjected to grinding or comminution during which various endogenous prooxidants and oxidative substances are mixed with molecular oxygen. The major "functional" proteins in meat, e.g., myoglobin which imparts desirable red color and myofibrillar proteins which largely contribute to physical characteristics of meat products by forming gels and emulsions and by holding water, are susceptible to oxidative changes during processing. Once oxidized, these proteins exhibit altered functional behaviors and hence, can influence texture, binding, stability and palatability of final, cooked products. Some studies suggested that the use of proper antioxidants could minimize oxidation-induced protein functionality changes. However, "functional properties" of proteins which have been chemically modified by either prooxidants or antioxidants are extremely sensitive to environmental (processing) conditions. In our early work, we showed a marked improvement in functional properties (e.g., gel-forming ability) of myofibrillar proteins when lipid oxidation was completely inhibited (Wan et al., 1993; Xiong et al., 1993). In several of our more recent studies, however, we noted that mild oxidative modification of myofibrillar proteins could also facilitate protein gelation (Liu and Xiong, 1996a,b; Srinivasan and Xiong, 1996a). The oxidative effect on muscle protein functionality is apparently complex and there seems to exist a relationship between the extent (and type) of protein oxidation and the magnitude of functionality alteration of the affected proteins.

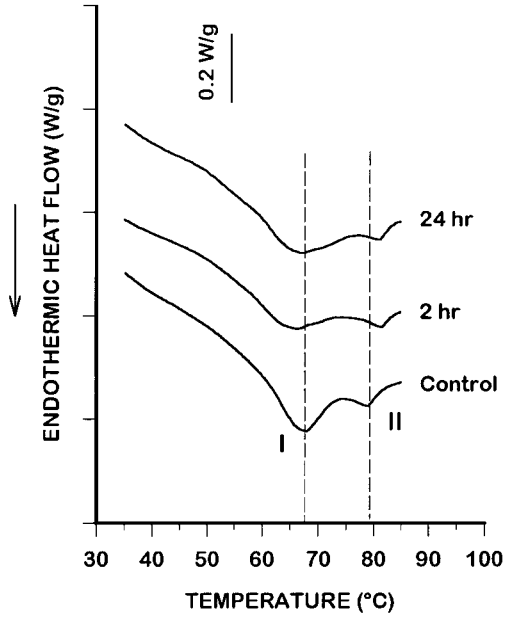
Oxidatively-Induced Changes in Proteins

Functionality of proteins can be loosely defined as the behavior of protein molecules, on a macroscopic scale, before, during and after food processing which determines the quality of final products. Functional properties are regarded as "intermediate" properties in the sense that they are dictated by physicochemical properties of proteins, but they play a critical role in producing desirable product characteristics as shown: physicochemical properties → functionality → product quality. This relationship describes that alterations of protein functionality by oxidative stress result from physicochemical changes of the protein. Hence, to elucidate the impact of oxidation on muscle protein functionality, it is important to define chemical and physical changes involved in the initial stage of oxidative attack. Protein oxidation is accompanied by a variety of physicochemical modifications, including amino acid destruction, unfolding and denaturation of protein molecules, peptide chain scission and insolubilization of protein. When free radicals are involved, molecular polymerization and formation of lipid-protein complexes can occur. A wealth of data on protein oxidation has been published in biochemical and medical science journals.

Muscle proteins are susceptible to free radicals generated during meat processing due to the high concentrations of free radical initiators located in the muscle tissue. In the presence of molecular oxygen, iron (Fe) and copper (Cu), naturally abundant in muscle tissue, are strong catalysts of protein oxidation. Protein oxidation catalyzed by transition metal ions is believed to be site-specific or "caged," i.e., only amino acid side chains at the metal-binding sites are specific targets (Stadtman and Oliver, 1991). Among the most common sites of metal-catalyzed protein oxidation are the alkaline and the sulfur-containing amino acid residues - His, Arg, Lys, Met and Cys, as well as Pro. The aromatic amino acids, Trp, Tyr and Phe, are relatively insensitive to metal ions, presumably because they are not commonly present at the metal-binding sites of proteins. In metal-catalyzed protein oxidation, His residues are converted to Asp or Asn residues; Pro residues to Glu and γ-glutamicsemialdehyde residues; and Lys residues to 2-amino-adipicsemialdehyde residues (Stadtman, 1993). On the other hand, the amino acids most susceptible to lipid oxidation products are those with the following reactive side chains: imidazole ring, indole ring, sulfhydryl, thioether and amino group (Gardner, 1979). Thus, Trp, His, Lys, Cys and Arg residues can be readily oxidized leading to the formation of protein free radicals. Other susceptible amino acids include valine and serine. Since functional properties of proteins are dependent on protein surface properties, i.e., polarity, charge and hydrophobicity, modifications of amino acids by oxidative processes can obviously alter protein functionality.

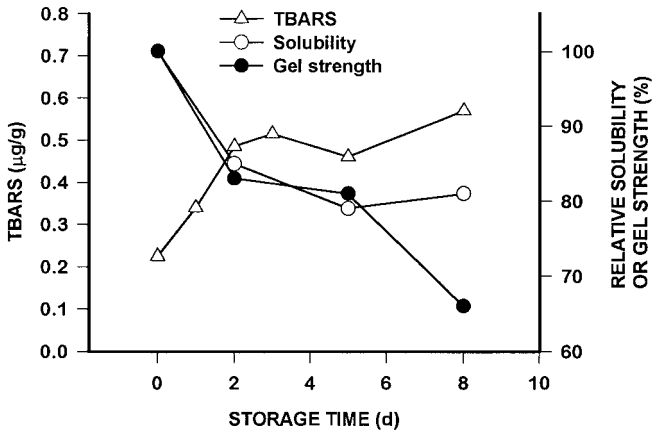
Oxidative modification of surface amino acid residues in muscle proteins can be assessed through analysis of derivatives of oxidized amino acids. For example, oxidation of Lys residues involves deamination and subsequent forma-

FIGURE 1.



Differential scanning calorimetry of beef heart myofibrillar proteins incubated with free radical-generating system (10 mM sodium ascorbate, 100 μM $FeCl_2$, 20 mM H_2O_2) for 2 and 24 h at 2°C. Note the shift in temperatures for the two endothermic transitions marked I and II after oxidation.

FIGURE 2.



Formation of TBA-reactive substances (TBARS) and changes in protein solubility and gel strength for beef heart surimi stored at 0°C for different periods. Surimi was prepared by washing muscle homogenate with 50 mM phosphate buffer at pH 7.0. (Adapted from Wan et al., 1993).

tion of reactive carbonyls. The carbonyls can cross-link with accessible free amino groups producing protein oligomers. Furthermore, intra- and inter-peptide disulfide bonds are typically observed in oxidized meat products. Therefore, the extent of muscle protein oxidation can be estimated by monitoring disappearance of these functional side chain moieties as well as simultaneous formation of their derivatives. Srinivasan et al. (1996) reported a large increase in protein carbonyls and aggregates in beef heart myofibrillar pellet during refrigerated storage and the increase was correlated with lipid peroxidation, i.e., the formation of malonaldehyde. It is likely that oxidized lipids contributed to the initiation of protein oxidation and formation of protein carbonyls. Many proteins exhibit conformational changes after a brief exposure to oxidizing agents. Meucci et al. (1991) reported enhanced absorption of human serum albumin oxidized by 100 mM ascorbate and trace minerals and they postulated that the absorbance increase resulted from the disappearance of ordered protein structure. Similarly, Decker et al. (1993) showed that myofibrillar proteins exposed to oxidizing agents (iron, copper and linoleic acid in the presence of ascorbate) absorbed more within the 250-290 nm range showing an absorption maximum at 265 nm, compared to non-oxidized proteins. The increased absorption can be attributed to increased exposures of the hydrophobic aromatic groups. Although there are some similarities between lipid oxidation and protein oxidation, e.g., both involving hydrogen abstraction, protein oxidation clearly differs from lipid oxidation since the most susceptible sites in proteins are usually not the residues containing double bonds. The mechanisms of protein oxidation in meats have been reviewed by Xiong and Decker (1995).

Myosin, the most abundant myofibrillar component, is very susceptible to oxidizing agents either naturally present in muscle or incorporated during processing. When oxidation occurs, polymerization, rather than molecular scission, of myosin is favored due to the preponderance of moisture or high water activity in the muscle tissue. For example, in the presence of malonaldehyde (a reactive lipid degradation product), fish (trout) myosin molecules polymerize through the amino (myosin) and carbonyl (malonaldehyde) cross-linkages, resulting in an insolubilization and precipitation (Buttkus, 1967). In chicken muscle, myosin is readily oxidized by lipid free radicals and forms large aggregates (Schilder, 1993). Similar observations have been reported by Jarenback and Liljemark (1975) on fish myofibrillar proteins incubated with lipid hydroperoxides. Linoleic acid hydroperoxides were found to be highly destructive to myofibril structure: They caused denaturation and precipitation of the A-band (predominantly myosin) which could not be extracted in salt solution. Within 2.5 h of incubation in 1.5 μmol hydroperoxides/g muscle, protein solubility decreased by 90%. The extent of oxidation is directly related to the number of double bonds in the oxidizing fatty acids (Schilder, 1993). In the simultaneous presence of myoglobin and hydrogen peroxide, myosin forms covalent aggregates undissociable by a reducing agent and myoglobin radicals presumably act as the cross-linkers (Hanan and Shaklai, 1995). Using differential scanning calorimetric (DSC) analysis, we have found that in the presence of iron and hydro-

TABLE 1. Oxidation of Lipids and Proteins and Changes in Gelation Properties of Antioxidant-Washed Chicken Myofibril Pellets During Storage at 0°C.[1]

Parameter	Breast			Leg		
	0	2	6 days	0	2	6 days
TBARS (μg/g pellet)						
Control	0.154	0.182	0.283	0.100	0.164	0.580
PG + AA	0.036	0.084	0.082	0.006	0.045	0.076
PG + TPP	0.058	0.045	0.065	0.042	0.050	0.050
AA + TPP	0.130	0.156	0.438	0.089	0.551	1.291
Mean	0.095	0.117	0.217	0.059	0.203	0.499
Protein carbonyl (nmol/mg protein)						
Control	1.570	1.450	2.053	1.208	0.966	2.536
PG + AA	1.087	2.053	2.416	0.242	1.328	1.690
PG + TPP	1.087	1.328	1.328	1.449	1.570	1.570
AA + TPP	0.362	1.328	1.570	0.483	1.450	2.294
Mean	1.026	1.540	1.840	0.846	1.330	2.023
G′ (Pa)						
Control	472	503	535	224	240	263
PG + AA	367	424	451	193	202	244
PG + TPP	413	419	354	201	209	221
AA + TPP	502	503	533	187	187	204
Mean	438	462	468	201	209	233

[1] PG = propyl gallate; AA = ascorbic acid; TPP = sodium tripolyphosphate; G′ = storage modulus of gels at 70°C. (Data are abstracted from Liu and Xiong, 1996b).

gen peroxide, myosin in washed beef heart muscle was destabilized showing lower transition temperatures and less enthalpy compared to non-oxidized myosin (Fig. 1). The appearance of the first endothermic peak (68°C) is attributed to unfolding of the myosin globular head or heavy meromyosin. The second transition (79°C), tentatively ascribed to actin and light meromyosin, occurred at increased temperatures after oxidation.

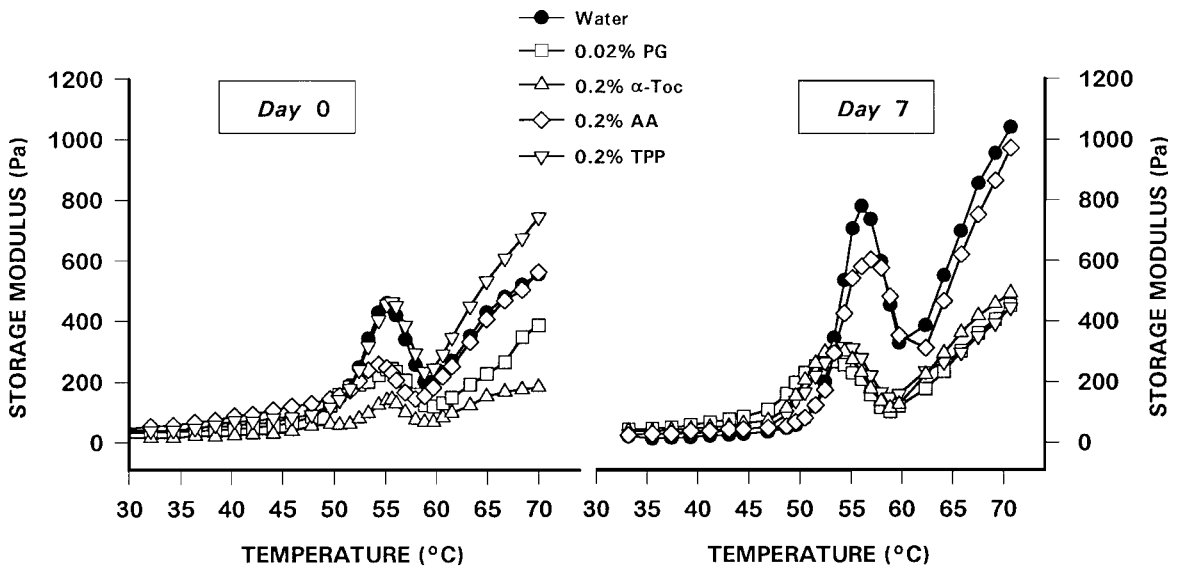
These oxidation-induced physicochemical modifications constitute the principal causal factors for the alterations of protein functionality often observed in oxidized systems. A deviation in functionality of an oxidized protein from its nonoxidized precursor is common because oxidative modifications in the individual protein molecules will lead to major shifts in chemical and physical interactions between protein molecules (as in protein gels and meat bind), as well as between protein and lipid (as in meat emulsions) or water (as in low-fat, water-added luncheon meats). Since physical traits of processed muscle foods (e.g., texture, hardness, cooking yield) are dictated by the functional behavior of proteins, it is suggested that poor quality of processed meats prepared under strong oxidative conditions result, at least in part, from free radical attack on proteins.

Functionality of Oxidized Muscle Proteins

Smith (1987) presented some indirect evidence that myofibrillar proteins under oxidative stress tended to lose their functionality. It was shown that frozen storage of hand and mechanically deboned turkey meat increased lipid oxidation and decreased protein solubility, myosin ATPase activity and myofibril gel strength. Gels prepared from frozen stored proteins consisted of a globular matrix, compared to gels made from fresh muscle proteins which possessed a continuous filamentous microstructure and were capable of entrapping more water. However, since loss in protein functionality could partially result from freeze/thaw stress *per se*, it is unclear exactly what role oxidation had played in the observed deterioration of protein functionality.

Recent studies involved with more purified protein systems have shown complex responses of myofibrillar proteins to oxidizing agents. Depending on the specific experimental conditions, oxidizing agents or stresses can either depress or promote functionality of myofibrillar proteins. Turkey pectoralis myofibrillar proteins mixed with prooxidants exhibited decreased functional properties, which appeared to coincide with changes in protein conformation as well as to the formation of carbonyl compounds (Decker et al., 1993). Proteins oxidized by 25 μM iron or copper in the presence of 10 mM ascorbate decreased solubility by 32% to 36%. When 20 mM oleic, linoleic and linolenic acids were also incorporated into the iron/ascorbate oxidizing system, a further reduction of protein solubility was observed (Schilder 1993). Gel electrophoresis produced strong evidence that both myosin and actin were insolubilized following iron- and copper-catalyzed oxidation, with a concomitant occurrence of many high molecular weight polymers or oligomers. Oxidative reactions catalyzed by both metals severely impaired the gel-forming ability of the myofibrils as seen from weakening of the gel matrix structure in the presence of copper and iron. Because of the high degree of porosity, gels made from oxidized protein were able to hold only 33% to 75% as much water as gels prepared from nonoxidized protein. Overall,

FIGURE 3.



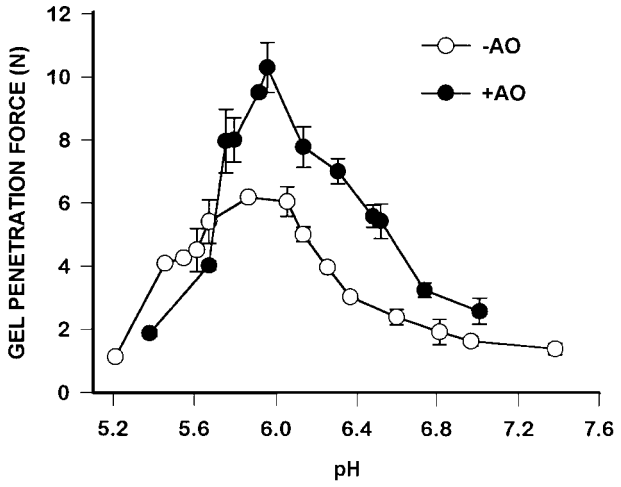
Rheograms of fresh (0 day) and stored (7 days at 0°C) surimi during thermally induced gelation. Surimi was prepared in the presence or absence (water wash) of the following antioxidants: 0.02% propyl gallate (PG), 0.2% -tocopherol (-toc), 0.2% ascorbic acid (AA), or 0.2% sodium tripolyphosphate (TPP) and was suspended in 0.6 M NaCl, pH 6.0 before gelation. (Adapted from Srinivasan and Xiong, 1996a).

iron caused greater changes in protein conformation, carbonyl content, formation of protein polymers and loss of myosin and actin than equal molar concentrations of copper.

Another oxidative model system we have employed in the study of oxidative effects on muscle protein functionality is beef heart surimi (washed muscle mince, i.e., crude myofibril pellet) manufactured under oxidative conditions. Beef heart, which contains a high proportion of polyunsaturated fatty acids such as arachidonic acid and high concentrations of iron, heme and oxidative mitochondrial enzymes, was comminuted, repeatedly blended in buffer (25 mM sodium phosphate, pH 7.0) and centrifuged. Due to vigorous aeration and mixing of molecular oxygen with various endogenous free radical initiators, the final myofibril pellet, which contained a small amount of lipids, was oxidized as evidenced by the production of a large quantity of TBA-reactive substances (TBARS) (Wan et al., 1993). Storage of the myofibril pellet on ice further increased the formation of TBARS (Fig. 2). Increases in TBARS were accompanied by decreases in protein solubility and gel-forming ability (gel strength) of myofibrillar proteins. The decreased functionality most likely resulted from damage of proteins by lipid free radicals and by other radicals produced through metal-, heme-, or enzyme-catalyzed reactions. When the washed myofibril pellet was applied at the junction of meat particles to make structured beef rolls, it produced a bind ten folds weaker than bind produced by the control, i.e., antioxidant-washed myofibril pellet (Xiong et al., 1993). Studies on fish also showed some correlation between lipid oxidation and reduction in gel-forming ability of surimi, more so for whole fish surimi than for surimi made from white muscle only (Kelleher et al., 1994).

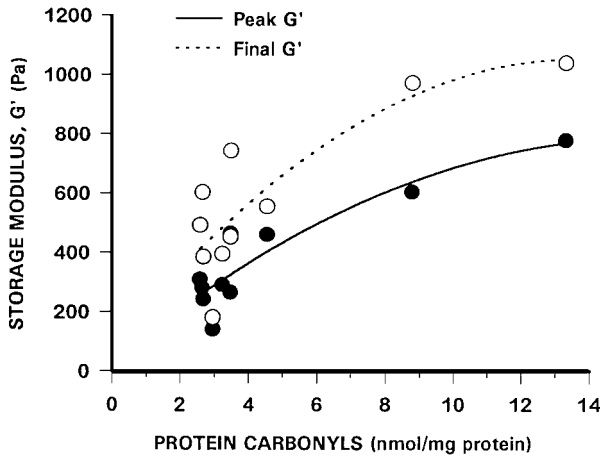
Contrary to the findings described above, however, several more recent investigations have presented evidence of beneficial effects of oxidation on muscle protein functionality. Srinivasan (1995) generated hydroxyl radicals in washed, minced cod muscle by treating the muscle with chelated ferric iron, hydrogen peroxide and ascorbate at 5°C. Protein carbonyl content doubled after a 24-h incubation in the free radical-generating system when compared to the control. The prooxidant treatment decreased protein solubility by 14%, but it improved shear stress and true strain of cooked gels by 70% and 20%, respectively. The emulsification characteristics were also improved as a result of protein oxidation. In our current work with beef heart surimi, we also found that washing muscle mince by water only (without added antioxidants) produced surimi of an improved gel-forming ability when compared to surimi prepared in the presence of added antioxidants such as propyl gallate and -tocopherol (Srinivasan et al., 1996; Srinivasan and Xiong, 1996a). Storage of water- and ascorbate-washed surimi on ice for one week enhanced its gel-forming ability and gel elasticity (G′) remarkably (Fig. 3), with a simultaneous increase in protein carbonyls from 4.5 to 14 nmol/mg protein and in TBARS from 0.02 to 1.3 μg/g pellet. Similar to the findings in beef heart surimi, protein carbonyls were rapidly produced during storage of water-washed chicken muscle mince when compared to samples washed with propyl gallate and tripolyphosphate (Liu and Xiong, 1996b) (Table 1). The final (70°C) G′ values of chicken protein gels increased during storage of the protein pellet, corresponding to increases in protein carbonyls. However, G′ values of chicken protein gels in the intermediate temperature range (45° to 55°C) gen-

FIGURE 4.



Effect of antioxidant (AO) treatments and pH on gelation of beef heart myofibrils. (Adapted from Xiong et al., 1993).

FIGURE 5.



Apparent relationship between protein carbonyl of beef heart surimi and shear storage modulus (G') of its thermally induced gels.

erally decreased during storage, while the protein carbonyl content increased. This seems to indicate that the gelation process of skeletal muscle proteins differs kinetically from that of cardiac muscle proteins under an equal oxidative stress, since for cardiac muscle, oxidation produced a more elastic gel network both in the intermediate temperature range (50° to 60°C) and at the end of cooking (70°C) (Fig. 3).

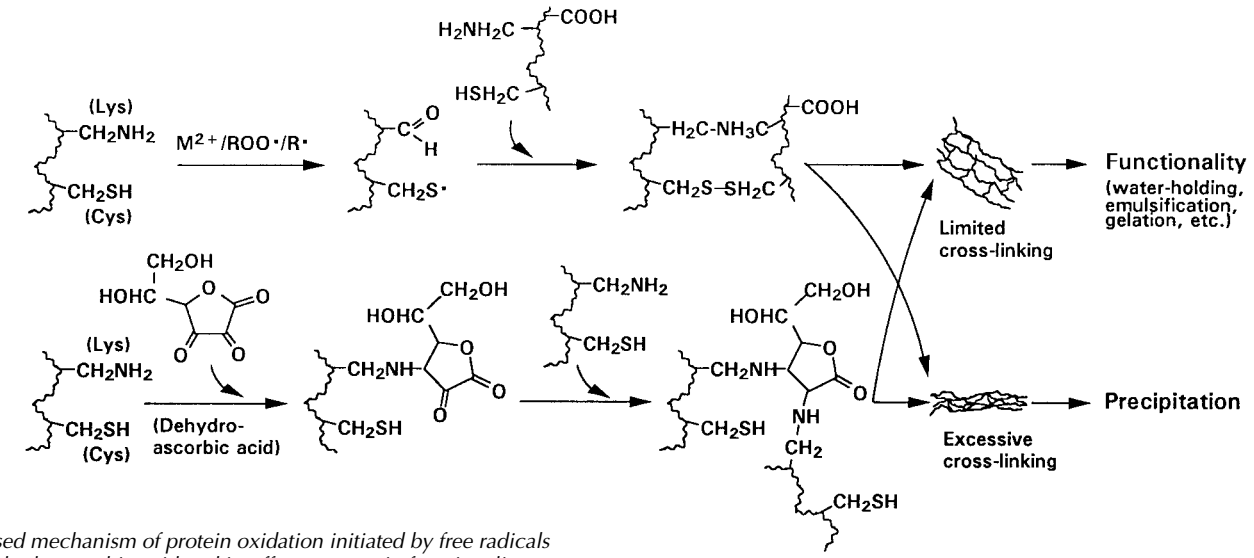
Modification of Muscle Protein Functionality by Antioxidants

One of the major challenges to meat scientists and meat processors is to accurately define roles of different food ingredients or additives intended to produce specific functionalities for a particular meat product. The ability of oxidative compounds to alter muscle protein functionality lends to the speculation that antioxidants, the counterparts, would also be capable of modifying protein functionality in muscle foods. Traditionally, antioxidants are added to fresh and processed foods to prevent oxidative rancidity, retard development of off-flavors and improve color stability thereby extending the product shelf-life. Recent research has proven that antioxidants can indeed alter the functional performance of food proteins. Protection of muscle proteins against functional deterioration by combined antioxidants (0.02% propyl gallate, 0.2% sodium ascorbate, 0.2% sodium tripolyphosphate) was demonstrated in several studies. Unheated suspensions of antioxidant-washed beef heart surimi exhibited greater shear stress values than those of control surimi (Wan et al., 1993). Protection of the protein pellet against oxidation was most evident during storage. Although antioxidants did not completely prevent deleterious changes in protein rheological properties (e.g., shear thinning) during storage, the antioxidant-washed protein nevertheless maintained substantially higher viscosity than waster-washed protein. The reduced loss of viscosity in the antioxidant-treated sample was ostensibly attributed to the inhibition of lipid oxidation which was kept to a minimum throughout storage. TBARS increased from 0.06 to 0.13 μg/g pellet for antioxidant-washed surimi, but they increased from 0.20 to 0.50 μg/g pellet for control surimi. Neither amino acid composition nor the secondary/tertiary structure of antioxidant-washed proteins was analyzed in this study. Hence, it is tempting to surmise that ascorbate, which was incorporated in the washing solution, might have promoted protein oxidation, or partially contributed to the formation of protein carbonyls or protein-bound carbonyls. Overall, the result suggests that antioxidant-washed protein has better hydration and solvation properties, probably due to inhibition of extensive conformational alterations or to less surface charge reduction. This was in agreement with most published findings that strong oxidation causes insolubiliza-tion of proteins due to formation of particulates.

During thermally induced sol → gel transformation, antioxidant-treated myofibrillar protein produced a more viscoelastic gel network than the control counterpart, as manifested by the greater G' values at temperatures less than 60°C (Wan et al., 1993). Loss of protein functionality was minimized by inhibition of oxidation and the individual functional attributes of the protein sample seemed to be equally protected. The effectiveness of antioxidants was dependent on pH. A maximal gel strength was produced at pH 6.0 (Xiong et al., 1993). However, antioxidant-washed myofibrils formed stronger gels than control myofibrils at pH > 5.8 but not at pH < 5.7. This indicates that some charged amino acids were probably modified during oxidation, leading to different electrostatic interactions between peptides (Fig. 4). Due to oxidative reactions, surface charge of proteins can redistribute and this can result in a shift in isoelectric points of proteins.

The majority of muscle proteins, including proteins comprising the myofibrils, are bathed in the aqueous phase. This makes the proteins highly susceptible to surrounding water-

FIGURE 6.



Proposed mechanism of protein oxidation initiated by free radicals and dehydroascorbic acid and its effect on protein functionality.

soluble radicals. To determine the mechanism of oxidative modification of muscle proteins, lipid- and water-soluble antioxidants were added to beef heart mince prior to blending and washing. When propyl gallate (0.02%), α-tocopherol (0.02%), or sodium tripolyphosphate (0.2%) was used alone, lipid oxidation (TBARS; conjugated diene) and protein oxidation (carbonyl; disulfide) in washed mince were substantially curtailed even after 7-day storage (Srinivasan et al., 1996). Thus, oxidation of lipids and proteins occurred most likely at the oil-water interface and the oxidation inhibition was achieved by scavenging radicals and chelating metal ions. Although oxidation was inhibited by propyl gallate and α-tocopherol, neither antioxidant improved the gel-forming ability of the washed myofibrillar proteins. On the other hand, beef heart surimi prepared in the presence of ascorbate had a marked increase in protein carbonyl content, despite the great suppression of lipid oxidation. The carbonyl compounds could be derived from oxidized protein molecules, or from protein-bound dehydroascorbate. There was indirect evidence of protein cross-linking by oxidized ascorbate because some protein polymers formed in the presence of ascorbate could not be dissociated by reducing compounds such as dithiothreitol (Srinivasan and Xiong, 1996b). Gel strength of ascorbate-washed beef heart surimi after 7-day storage was much higher than gel strength of surimi washed with other antioxidants and slightly lower than water-washed surimi (Srinivasan and Xiong, 1996a).

Mechanism of Oxidative Modification

The controversial results obtained, i.e., muscle proteins exposed to oxidizing environments exhibited both improved and reduced functionalities, are a manifestation of complexity of oxidative processes. The effect of oxidation on functionality changes is sensitive to a number of intrinsic and extrinsic factors. Among them are type of prooxidants or antioxidants as well as their combinations, type of muscle or proteins, the specific protein side chains or amino acid residues located on the surface of protein molecules, the extent of oxidative modification and the storage time. In the studies showing functionality improvement by antioxidants (Wan et al., 1993; Xiong et al., 1993; Kelleher et al., 1994), the antioxidative system employed was a combination of propyl gallate (0.02%), tripolyphosphate (0.2%) and ascorbate (0.2%) which effectively inhibited lipid oxidation. However, dehydroascorbate was probably generated from ascorbate during surimi preparation, producing electron-deficient, reactive carbonyls which could readily interact with free amines to form interpeptide cross-linkages (Lee et al., 1992). It is thus possible that this antioxidative system might both prevent oxidative damage to certain protein functional groups and at the same time, facilitate interactions between polypeptides via the Schiff's base linkage. On the other hand, in those studies revealing little beneficial effect by antioxidants (except ascorbate), or functionality improvement in the absence of antioxidants, the extent of protein oxidation was moderate (Liu and Xiong, 1996b; Srinivasan et al., 1996; Srinivasan and Xiong, 1996a).

The most compelling evidence on the involvement and contribution of oxidatively modified protein side chains in the alteration of protein functionality is the notice that when G′ values of beef heart surimi gels are plotted against protein carbonyls, a relationship between them clearly exists (Fig. 5). Regression analysis revealed a poor correlation between gel G′ and protein carbonyl when the extent of oxidation was very low in surimi, as was typical of 0-day samples (r=0.37 for peak G′; r=0.44 for final G′). However, correlation between gel G′ and protein carbonyl was high after the surimi was stored for 7 days (r=0.99 for both peak and final G′), largely owing to the increase in protein and(or) protein-

bound carbonyls in water- and ascorbate-washed surimi pellets. The overall correlation between protein carbonyl and peak G′ (r=0.92) or final G′ (r=0.84) of gels was also strong. The beneficial effect of ascorbate on kamoboko (a fish surimi-based product) has been well-studied and was attributed to polymerization of proteins via dehydroascorbate-catalyzed disulfide bond formation (Lee et al., 1992; Nishimura et al., 1992). We suggest that protein-bound carbonyls can interact with free amines, producing covalent bonds or cross-links between peptides, thereby increasing rigidity of the surimi gel network. This postulation is in line with the recognition that baking quality of wheat flour dough improves as a result of formation of condensed gluten products from dehydroascorbic acid and free amino groups in the protein (Elkassabany and Hoseney, 1980). Furthermore, the formation of intermolecular disulfide linkages is expected to provide additional contributions to strengthening protein networks in a variety of cooked meat products, such as restructured meats and emulsion-based products. It is important to keep in mind that the type of protein side chain groups and the extent of protein modification are important. It is hypothized that a mild-to-moderate modification leads to an improvement in protein functionality, whereas an extensive alteration can result in decreased functionality due to excessive aggregation of protein molecules. Thus, the following model is put forth to elucidate the possible role of oxidation with regard to muscle protein functionality during meat processing (Fig. 6).

Conclusions

Oxidative changes in proteins occurring in fresh and processed muscle foods involve modifications of side chain groups and secondary/tertiary structures of proteins. Oxidatively modified proteins exhibit alterations in functionality as manifested by changes in the gel-forming ability, meat-binding ability, emulsification capacity, solubility, viscosity, and water-holding capacity. Albeit difficult, it is possible to control the type and extent of protein modification to achieve specific product functionality. This can be done by selecting specific prooxidants or antioxidants (e.g., ascorbate) which can inhibit lipid oxidation (thus, preventing off-flavors) and simultaneously, modify functional properties of proteins. Muscle protein functionality can also be improved by the use of antioxidant combinations, varying the incubation time for raw products mixed with pro- or antioxidants, and controlling the atmosphere surrounding the meat product during processing and handling. Future research should concentrate on the identification of a variety of other natural and synthetic antioxidants or prooxidants, as well as processing conditions which allow optimal modification of protein functionality without compromising overall quality of the final meat product. Applicability of the results obtained from model systems to actual meat products needs to be further tested. Research is also needed to examine the potential toxicological effect of oxidized proteins to ensure safety of prooxidant- or antioxidant-treated meat products.

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