Meat and Muscle Biology™

Muscle Protein Oxidation and Functionality: A Global View of a Once-Neglected Phenomenon

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Abstract: Muscle is a highly organized apparatus with a hierarchic microstructure that offers the protection of cellular components against reactive oxygen species (ROS). However, fresh meat immediately postmortem and meat undergoing processing become susceptible to oxidation due to physical disruption and the influx of molecular oxygen. Upon the activation by endogenous prooxidants, oxygen species are rapidly produced, and both myofibrillar and sarcoplasmic proteins become their primary targets. Direct ROS attack of amino acid sidechains and peptide backbone leads to protein conformational changes, conversion to carbonyl and thiol derivatives, and subsequent aggregation and polymerization. Interestingly, mild radical and nonradical oxidation enables orderly protein physicochemical changes, which explains why gels formed by ROS-modified myofibrillar protein have improved rheological properties and binding potential in comminuted meat and meat emulsions. The incorporation of phenolic and other multifunctional compounds promotes gel network formation, fat emulsification, and water immobilization; however, extensive protein modification induced by high levels of ROS impairs protein functionality. Once neglected but now recognized to be a natural occurrence, protein oxidation has drawn much interest and is being intensively studied within the international community of meat science. This review describes the history and evolution of muscle protein oxidation, the mechanism and functionality impact hereof, and innovative oxidant/antioxidant strategies to control and manipulate oxidation in the context of meat processing, storage, and quality. It is hoped that the review will stimulate in-depth discussion of scientific as well as industrial relevance and importance of protein oxidation and inspire robust international collaboration in addressing this underappreciated challenge.

Key words: muscle protein oxidation, free radicals, protein carbonyls, gelation, emulsification, water-holding capacity
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Introduction

According to the International Union of Pure and Applied Chemistry, oxidation is the "loss of electrons, loss of hydrogen, or gain of oxygen." In a biological system, oxidation refers to the loss of electrons by a molecule, atom, or ion during a chemical or enzymatic reaction. The process usually involves free "radicals" i.e., chemical species that have an unpaired electron. Both free radicals and nonradical species can initiate oxidation. Meat is a rather complex postmortem biological system that contains abundant oxidation-labile phospholipids, hemes, transitional metal ions, and oxidative enzymes (Love and Pearson, 1971). In meat processing and storage, disruptions of muscle cells, incorporation of molecular oxygen, comminution and mixing, and cooking all contribute to the oxidation of muscle components (Domínguez et al., 2019).

Lipid oxidation is readily perceived due to off-flavor development when unsaturated fatty acids are broken down to volatile secondary products. Similarly, heme oxidation is easily noticed due to discoloration. In contrast, protein oxidation generally occurs undetected because the products usually do not sensitize olfactory or gustatory cells and are rarely

visible. Yet, pioneering research and recent development in the field of free radical chemistry, pathology, and gerontology have established the ubiquity and health implications of protein oxidation taking place in human bodies (Stadtman, 1990; Kehm et al., 2021). As well, it has become increasingly recognized that food proteins present in raw and processed animal and plant food stuffs are very susceptible to reactive oxygen species (ROS), and this is particularly true in muscle foods (Xiong and Guo, 2021). Studies have demonstrated both desirable and undesirable consequences when food proteins are exposed to an oxidizing environment. For example, functional myosin (or actomyosin) aggregates produced by the reaction with low concentrations of free radicals can promote protein gel formation via cross-linking of cysteine, lysine, and tyrosine residues leading to an improved product texture (Xiong et al., 2010; Xia et al., 2019). Conversely, extensive oxidative modification and carbonylation impair muscle protein functionality (Utrera and Estévez, 2012; Zhou et al., 2014).

Chemically, protein oxidation involves the initial modification of amino acid side chain groups and subsequent conversion to thiol, carbonyl, and other reactive derivatives. The chemistry of protein oxidation occurring *in vivo* has been intensively studied, and the pathological consequences have been defined (Stadtman, 1993; Berlett and Stadtman, 1997; Shacter, 2000; Hawkins and Davies, 2019; Kehm et al., 2021). In comparison, protein oxidation research in food systems has a rather short history. Much of the current understanding of oxidative effects on food quality and nutrition is from meat protein oxidation studies, although similar oxidation studies have been conducted in milk, cereal, legume, and other food commodities (Xiong and Guo, 2021). In meat products, disulfide, dityrosine, and amine-carbonyl cross-links as well as noncovalent forces, especially hydrophobic association, have been identified in the process of protein aggregate formation. Carbonyl groups formed by ROS-modified amino acid side chains are electrophiles; they are highly reactive with nucleophilic amines and thiol moieties to produce covalent linkages between myofibrillar proteins (MP; Lund et al., 2011). These covalent and noncovalent forces play a collective role in affecting the functionality of muscle proteins. Direct cross-linking of protein radicals, which are otherwise long lived, is another polymerization pathway.

Protein oxidation in live muscle, organs, and cells occurs in a virtually constant pH, ionic, and oxygen environment and is regulated by thermal homeostasis. In contrast, protein oxidation in food generally happens under extremely variable conditions, and the corresponding chemical reactions and products therefore can differ profusely. For example, variable alkalinity, salt concentration, and redox potential between bakery, dairy, legume, and meat products due to specific processing procedures and ingredient requirements have a significance influence on the chemical pathway, aggregation pattern, and functionality of oxidatively modified proteins. The present review provides a historical perspective of the evolving research field of muscle protein oxidation and meat quality and discusses its global relevance and engagement. The chemistry of protein oxidation is discussed, the technological functionality (gelation, emulsification, and water-binding) is highlighted, and antioxidant strategies to modulate protein oxidation and functionality are described.

Evolution and Global Participation in Muscle Protein Oxidation Research

Lipid oxidation has been intensively investigated well over a century, and the true origin of empirical observations is lost in antiquity. According to Hammond and White (2011), the phenomenon described by Duclaux (1886), i.e., sun-exposed butter developed rancid off-flavors and the degradation of triacylglycerols, was probably the very first scientific study in the field of lipid oxidation research. Subsequently, especially since the early 1970s, scientific reports on lipid oxidation based on more advanced analytical instrumentation had increased more than 100-fold (Ayala et al., 2014). Today, the mechanism of lipid oxidation in relation to food quality has become well defined, and the seminal book written by Frankel (1980) provides one of the comprehensive descriptions of lipid oxidation. Of muscle food relevance, the occurrences and control of lipid oxidation in meat and meat products, arguably the most oxidation-susceptible group of food, have been extensively investigated since the 1980s. Progresses in this field have been reviewed by a number of researchers, for example, Ladikos and Lougovois (1990) and Domínguez et al. (2019).

In contrast, protein oxidation has had a relative short history of scientific investigation. As aforementioned, protein oxidation research started in health-related sciences. In particular, neurological science researchers, notably Stadtman and colleagues from the National Institute of Health, are credited for pioneering and prolific studies of the biochemical and molecular mechanisms of protein oxidation (Levine

et al., 1981; Taylor and Davies, 1987; Stadtman, 1990; Stadtman, 1993; Stadtman and Berlett, 1997). These early investigations led to the foundational discovery that oxidation of cellular enzymes and structural proteins had a significant role in cell aging and Alzheimer's disease development. Other neurological disorders and cell debilitations, such as Parkinson's disease, cataracts, muscle dystrophy, and atherosclerosis, have also been linked to protein oxidation (Shacter, 2000). In food, the first mentioning of oxidative damage to muscle protein was made by Buttkus (1967), who described the insolubilization and aggregation of fish (trout) myosin when exposed to malonaldehyde, a lipid oxidation marker. Subsequently, Jarenbäck and Liljemark (1975) reported fish protein denaturation in the presence of lipid hydroperoxides. Later, Smith (1987) presented evidence that loss of solubility and functionality of protein in mechanically separated frozen chicken meat was related to lipid oxidation.

Systematic studies of protein oxidation—chemical processes, mechanisms, and potential impact on protein functionality in postmortem fresh meat and processed meats—started around 1990. Inspired by the early scientific findings that protein oxidation occurring in humans and animals is a primary cause for pathological changes and aging (Levine et al., 1981; Taylor and Davies, 1987), meat scientists in the United States began to explore physicochemical changes in oxidatively affected muscle proteins and the possible link to meat quality (Decker et al., 1993; Wan et al., 1993). These early studies, which concentrated on meat protein structure–functionality relationship, led to the recognition that MP, including myosin and actomyosin, are extremely susceptible to naturally occurring ROS and those generated during conventional meat processing. Specifically, free radical-induced amino acid sidechain modifications were confirmed to be the causative factors for the formation of protein carbonyls, disulfide bonds, dityrosine cross-links, hydrophobic association, and the carbonyl-amine complex. A strong linkage of such reactions to functional behavior of muscle proteins was therefore established (Xiong and Decker, 1995; Xiong, 1996).

Over the ensuing decades, worldwide participations in muscle protein oxidation research followed, and meat scientists from France, Demark, Spain, Israel, China, Finland, Australia, Thailand, and numerous other countries besides the United States have individually or collaboratively made tremendous contributions to the advancement of meat protein oxidation research (Renerre et al., 1996; Martinaud et al., 1997; Estévez and Cava, 2006; Bertram et al., 2007; Lund et al., 2007; Maqsood et al., 2012; Xia et al., 2012). Of special note is a series of scholarly works out of Estévez' lab in Spain during the progressive decade of 2000s. In 1998, "Dietary Strategies for Improving Animal-Based Food Products," an international symposium held in Marid, Spain, the topic of meat protein oxidation was first introduced and debated by speakers from more than 10 different countries. And in 2012, a follow-up global discussion, "Protein Oxidation and Quality Implications in Processed Muscle Food," was held at the Institute of Food Technologists (IFT) meeting in Las Vegas, USA. In more recent years, there is a notable rising trend of research output in the field by meat scientists in Asian countries, reflecting a high demand for meat and meat products and vast expansion of research capacity with strong infrastructural support. The international collaborations have enabled a holistic and broad approach to addressing this once-neglected oxidation phenomenon. The ubiquity of protein oxidation across all animal species is now well recognized (Xiong and Guo, 2021).

The global effort had expanded into various aspects of meat quality, including tenderness of beef and pork (Rowe et al., 2004a; Lametsch et al., 2008; Bao and Ertbjerg, 2015), activity of endogenous enzymes and regulators (Lametsch et al., 2008), histidine-related myoglobin color stability (Suman et al., 2007; Yin et al., 2011), fresh meat cooking yield (Traore et al., 2012; Delles and Xiong, 2014), and quality of fresh and fermented sausages (Jongberg et al., 2013; Fuentes et al., 2014). A host of ROS formed *in situ*, i.e., postmortem aging due to the depletion of indigenous antioxidant defense systems, were found to affect beef aging (Renerre et al., 1996; Martinaud et al., 1997). In particular, the oxidation of calpain, a key endogenous peptidase, by the oxidative modification of intrinsic thiol groups has been implicated in decreased tenderness of aged beef when exposed to an oxidative environment, such as high-oxygen (60% to 80%) modified atmosphere packaging (Rowe et al., 2004a; Lund et al., 2007; Lametsch et al., 2008). In early postmortem meat, nitric oxide (NO) has also been found to react with superoxide to form peroxynitrite ($ONOO^-$), which in turn activates μ-calpain through (a) complex pathway(s) (Warner et al., 2005). The S-nitrosylation effect of NO as well as alterations of the redox state of MP were thought to play a role in postmortem tenderization of beef and pork (Liu et al., 2019b; Hou et al., 2020). Furthermore, both oxidized sarcoplasmic and myofibrillar proteins exhibit decreased digestibility and reduced nutritional value

(Kamin-Belsky et al., 1996; Sante-Lhoutellier et al., 2007; Sun et al., 2011).

Meanwhile, studies on the technological functionality of muscle proteins (gelation, emulsification, water-holding capacity [WHC], and meat particle binding) under the influence of ROS, oxidative enzymes, and other oxidative stressors continued. Relevant findings have been reported in numerous publications, for example, by (chronologically) Srinivasan and Hultin (1997), Park et al. (2007), Parkington et al. (2000), Estévez et al. (2008), Xiong et al. (2010), Jongberg et al. (2013), Zhou et al. (2014), Yang and Xiong (2015), Puolanne (2017), Ge et al. (2020), and many other researchers. Since 1990, a series of comprehensive reviews have been published to address the physicochemical and nutritional significance of protein oxidation in meat, providing a collective and "global" view of the importance of protein oxidation in meat and meat product quality. Examples of comprehensive discussions can be found in Xiong (1996, 2000), Lund et al. (2011), Zhang et al. (2013), Soladoye et al. (2015), Estévez and Xiong (2019), Xiong and Guo (2021), and Domínguez et al. (2021). Because of their dominant role in textural quality of meat, MP have been the subject of most of the studies.

Nevertheless, sarcoplasmic protein has also been investigated by several researchers. Studies have shown that sarcoplasmic protein is also susceptible to oxidation. For example, sarcoplasmic and myofibrillar proteins were found equally damaged by free radicals generated in irradiated beef, which was evidenced by the accumulation of protein carbonyls (Rowe et el., 2004b). The oxidative effect was correlated with increased toughness (Warner-Bratzler shear force) of meat. In early postmortem pork, sarcoplasmic proteome was oxidatively modified; the volume intensity of affected protein spots on a 2-D electrophoretic gel was correlated with the concentration of carbonyls formed (Promeyrat et al., 2011). 4-Hydroxynonenal, a secondary carbonyl product from lipid peroxidation, has been shown to bind to a number of histidine residues in myoglobin, thereby changing the fresh meat color stability (Yin et al., 2011; Suman and Joseph, 2013; Viana et al., 2020).

Mechanism of Protein Oxidation and Physicochemical Changes

The chemistry of protein oxidation is rather complex and remains not well defined. The reaction pathways are dependent upon the specific initiators and environmental conditions, promoting many different mechanisms being proposed (Kehm et al., 2021). Oxidation can be initiated by oxygen as well as nitrogen radicals and nonradicals. In biological systems, ROS are of primary importance. Although there have been suggestions to characterize protein oxidation in terms of initiation, propagation, and termination mimicking lipid oxidation, this approach is oversimplified and cannot accurately describe protein molecular changes *in situ*. The diversity of amino acid sidechains, variation of protein structure, and presence of different nonprotein reactants are all contributors to the complexity of oxidation mechanisms. Besides, protein radicals are rather stable and have a long half-life and therefore are not subjecting to spontaneous fragmentation after preoxidation (Østdal et al., 1997). Furthermore, because many nonradical ROS can directly form adducts with proteins instead of generating radicals (Buttkus, 1967; Zhao et al., 2012), oxidation of polypeptides may not propagate as oxidized lipid intermediates. Nevertheless, radical transfer to proteins at the peptide backbone and many of the amino acid sidechain groups is thought to be the first step in protein oxidative reactions in most biological systems (Shacter, 2000; Stadtman and Levine, 2003). When reacting with activated molecular oxygen, unstable carbon-centered free radicals are converted to peroxides that may or may not lead to peptide fragmentation (Stadtman and Berlett, 1997). High yields of protein peroxidation were reported in the initial oxidant flux in ROS-stressed biological materials, and peroxides were formed at cysteine, methionine, and the peptide backbone as well through one-electron reduction (Davies, 2016). Damage of amino acid sidechains by free radicals and binding with oxidizing compounds results in the disruption of intramolecular bonds, leading to conformational transitions and destabilization of protein structure. The resulting exposures of hydrophobic groups can be empirically assessed by measuring fluorescence quenching of tryptophan (Holmgren, 1972), and this spectroscopic method has been applied to the study of muscle protein oxidation (Astruc et al., 2007; Utrera and Estévez, 2012; Cao and Xiong, 2015). Direct oxidant attack of tryptophan residues also contributes to decreased fluorescence emission of tryptophan in affected meat (Figure 1).

The activation of triplet oxygen to an active species can be initiated by photosensitizers, enzymes, ultraviolet light (UVB or UVA), γ-irradiation, or metal-catalyzed one-electron reductions. A wide range of ROS can be produced in biological systems, including both radical and nonradical species, of which hydroxyl

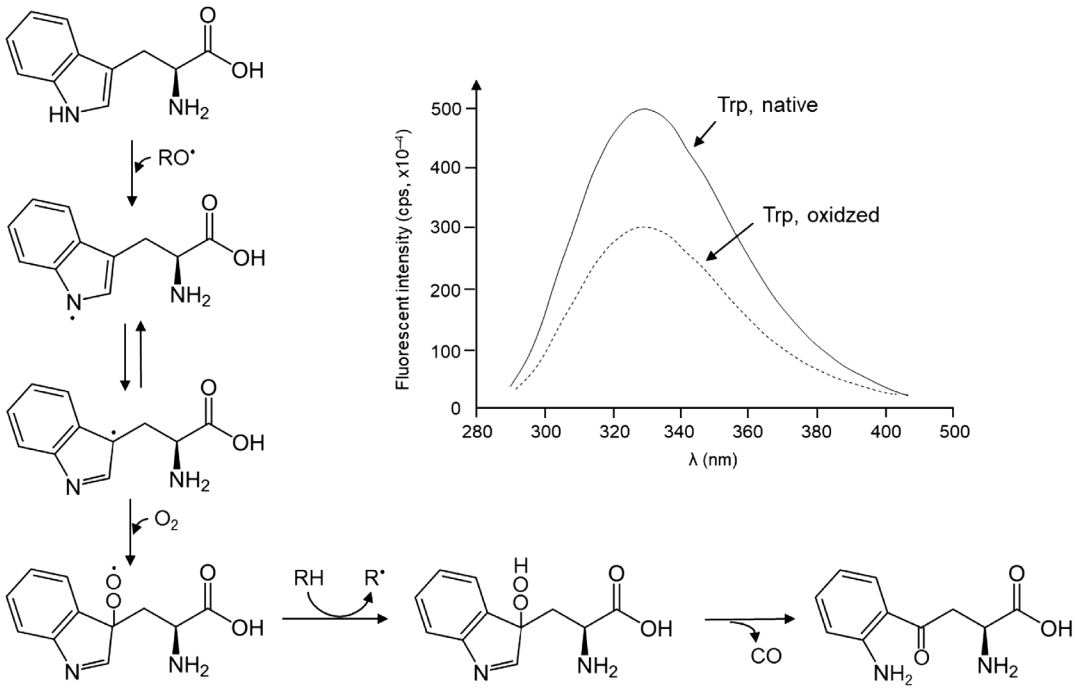

Figure 1. Proposed pathway of tryptophan oxidation and resulting protein conformational changes indicated by fluorescence quenching. Both the oxidation model and the fluorescence plot are drawn based on multiple research studies published in the literature, including results from the author's own lab.

radical ($^{\bullet}OH$), superoxide anion ($O_2^{\bullet-}$), hydroperoxyl radicals ($^{\bullet}OOH$), and hydrogen peroxide (H_2O_2) are commonly reported. In muscle, reactive iron and heme species, such as ferryl radical ($FeOH^{3+}$), perferryl radical (Fe^{2+}-$O_2^{\bullet-}$), and H_2O_2-activated heme are also significant initiators of protein oxidation (Kanner et al., 1988; Park and Xiong, 2007). Metal-catalyzed oxidation, proposed by Stadtman and Levine (2003) to explain protein oxidation in living tissue, is a mechanistic model widely adopted to describe changes in proteins occurring in food, especially meat, due to the abundance of iron (Lund et al., 2007; Xiong and Guo, 2021). When the ferrous ion (Fe^{2+}) is drawn to nucleophiles (e.g., ε-NH_2 in lysine), it catalyzes the formation of $^{\bullet}OH$. The $^{\bullet}OH$ production is through the direct reaction of Fe^{2+} with H_2O_2 (Fenton) or indirect reaction with $O_2^{\bullet-}$ and H_2O_2 (Haber–Weiss). Amino acid residues that can bind with Fe^{2+} to generate $^{\bullet}OH$ and oxidative ferryl ion also include proline, histidine, arginine, and cysteine (Welch et al., 2002). Direct attack by $^{\bullet}OH$ and other ROS has been shown to also occur on other amino acid sidechains, including those of sulfur-containing amino acids (cysteine and methionine) as well as those with bulky side chain groups (tryptophan, leucine, and phenylalanine) to yield sidechain radicals (Stadtman and Berlett, 1997). The one-electron oxidation of methionine by the reaction with $^{\bullet}OH$, which yields a Met-$S^{\bullet+}$ intermediate, is common during iron-catalyzed oxidation of β-amyloid peptide in Alzheimer's disease (Hong and Schöneich, 2001). In addition to modifying sidechains, ROS can attack protein peptide bonds, generating fragments. The cleavage begins with α-hydrogen abstraction to form a carbon-centered radical, which subsequently reacts with O_2 to produce peroxyl radical adducts. The ensuing formation of alkyl peroxide and alkoxy radical sets the stage for peptide bond cleavage (Grimsrud et al., 2008). In muscle foods, oxidation-induced fragmentation is generally not a significant phenomenon unless the oxidation goes beyond the normal meat processing and storage conditions (Bao and Ertbjerg, 2015; Liu and Xiong, 2000).

The formation of carbonyls (aldehydes and ketone) is a hallmark of protein oxidation (Estévez, 2011). A number of chemical pathways can lead to protein carbonyl formation that involves ROS: direct modification of amino acid side chains, peptide backbone cleavages, and adductions of nonprotein carbonyl moieties. γ-Glutamyl semialdehyde and α-aminoadipyl semialdehyde, which are derived from lysine and arginine, respectively, are examples of protein oxidation products that have been detected in meat (Utrera et al., 2011). Amino acid residues possessing nucleophilic elements, such as histidine, cysteine, and lysine, can be indirectly carbonylated through binding with nonprotein reactive carbonyl

species [e.g., malondialdehyde (MDA)] through Michael addition (Zhao et al., 2012; Lv et al., 2021). In addition, Strecker degradation aldehydes can form complexes with proteins to introduce protein-bound carbonyls. Hence, by the adduction to proteins, lipid-derived and carbohydrate-derived carbonyls contribute to the total carbonyl content in oxidized proteins. Although carbon-centered radicals and peroxides at the peptide backbone can cause protein fragmentation and produce carbonylated peptides, this phenomenon is rarely observed in processed meat (Xiong, 1996). Therefore, carbonyl groups formed at amino acid sidechains rather than peptide bond cleavages are responsible for the widely reported protein aggregation in meat and meat products.

Despite the in-depth understanding of the roles that protein oxidation plays in the occurrences and progression of many human chronic diseases, our current knowledge of protein oxidation in muscle foods is extremely limited. Being devoid of oxygen circulation coupled with the addition of various processing ingredients, fresh meat and meat products have a very different redox, pH, ionic, and temperature environment. As a result, the chemistry and theory of protein oxidation in life sciences are not always applicable to food science and should not be simply extrapolated to meat oxidation. Differing from live cells in which the pH, ionic condition, and redox potential are relatively constant for homeostasis, in postmortem meat, the chemistry of muscle protein oxidation is ingredient and processing-dependent (Xiong and Decker, 1995). Oxidative attack on muscle proteins occurs when meat is cut and the surfaces are suddenly exposed to molecular oxygen during chopping and grinding. Due to muscle cell disruption and sudden exposure to molecular oxygen in meat processing, muscle proteins become highly susceptible to oxidative damage, analogous to the hypobaric ischemia-reperfusion injury of live tissue and organs by radicals (Granger and Kvietys, 2015). In meat products, the type and amount of oxidation products are specific to the specific ROS (Park et al., 2006). When exposed to $^{\bullet}$OH, cysteine, methionine, and tyrosine in MP were the most susceptible amino acids; in a linoleic acid/lipoxidase oxidizing system, sulfur-containing amino acids (cysteine and methionine) were the most affected; and in a metmyoglobin-oxidizing system, alanine, glycine, histidine, leucine, lysine, and cysteine were selectively damaged (Park and Xiong, 2007). The sensitivity of methionine, cysteine, tyrosine, and tryptophan residues in MP to peroxyl radical was also reported (Dorta et al., 2019). Due the heat energy input, cooking exacerbates protein oxidation; amino acids residues in myosin were found indiscriminately damaged (Mitra et al., 2018).

Functionality of Oxidatively Modified Muscle Proteins

Protein functionality can be defined as the physicochemical behavior of polypeptides that contributes to the structural and textural characteristics of food. In meat and meat products, the abilities to bind water, form a three-dimensional gel, and emulsify fat are the most relevant functionalities of muscle proteins (Xiong, 2018). In particular, MP as a whole are the principal fraction contributing to gelation, emulsification, and water-binding in cooked meat products, and these properties are often interrelated, providing finished products with an organoleptically acceptable texture, mouthfeel, and juiciness. In comminuted products, gelation of MP is responsible for meat particle binding, hence the sliceability. The gel formed also contributes to emulsion stability and water entrapment. The dynamic changes leading to a stable protein gel are depicted in Figure 2. Myosin is highlighted in the model because it is the most reactive and functional protein in meat. Adsorbed myosin or actomyosin (in the absence of pyrophosphate and tripolyphosphate) at the water-oil interface provides the stabilization of fat particles due to amphiphilic distributions. Although gelation and emulsification are not applicable to fresh meat, water-binding by MP as well as water-holding by myofilaments in the interstitial spaces are the major factors for moisture retention and juiciness of fresh meat.

The most critical step in MP thermal gelation is the interaction between individual protein molecules to form functional particulates (small) or aggregates (large) that would further cross-link into an infinitive network capable of entrapping fat particles and water. Surface-active protein aggregates formed can also bind and stabilize fat particles, which is also an important property of MP in meat emulsions (i.e., batters). In meat processing, protein aggregation can occur through both noncovalent (predominantly hydrophobic association) and covalent forces. The latter are responsible for the widely observed myosin polymerization involving molecular oxygen. Six representative modes that explain MP cross-linking in oxidatively modified meat are proposed (Figure 3). Due to the abundance of cysteine residues (approximately 43 free sulfhydryls) in myosin (Nachmias et al., 1982), disulfide bonds account for the bulk of myosin cross-links found in oxidized meat (Xiong and Guo,

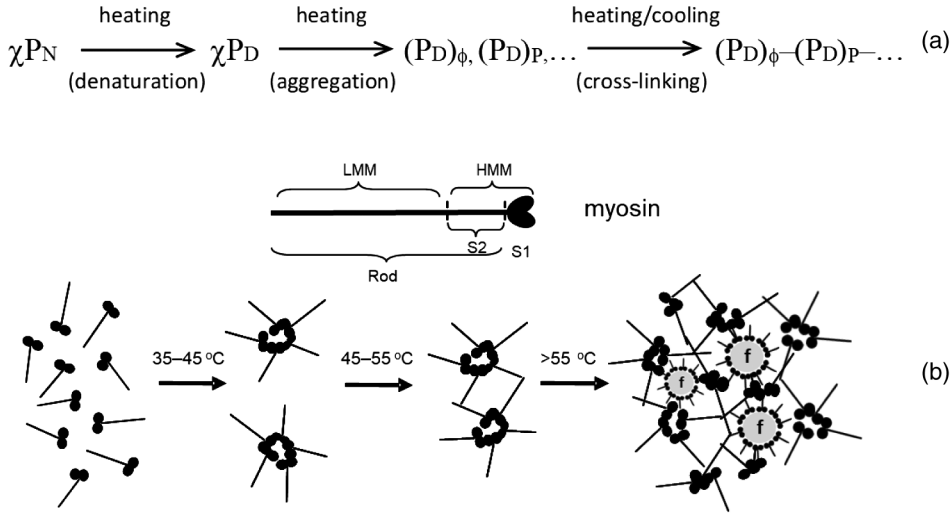

Figure 2. Thermal gelation of myofibrillar protein in comminuted meat products. (a) Sequential changes in proteins from native (P_N) to unfolded (P_D) state that subsequently polymerizes and aggregates; (b) myosin head–head thermal aggregation into a gel matrix that entraps water and immobilizes myosin-coated fat particles (f). Structural segments of myosin are shown, where HMM and LMM denote heavy meromyosin and light meromyosin, respectively, and S1 and S2 represent subfragments 1 and 2, respectively.

2021). Schiff's type complexation between amines or sulfhydryls and carbonyl groups is another important cross-linking mechanism in which donor carbonyls are either modified amino acid sidechains (Estévez, 2011) or oxidized phenols (quinones) from additive plant spices and their phenolic extracts (Tang et al., 2015, 2016).

Oxidation-induced chemical and physical changes have a profound impact on MP functionality, and both detrimental and desirable consequences have been noted. Uncontrolled oxidation can cause excessive structural changes, resulting in reduced gelling, emulsifying, and water-binding properties due to extensive aggregation and insolubility (Bertram et al., 2007; Liu et al., 2010; Bao et al., 2018). This is similar to quality change of whole-muscle meat, such as tissue hardening and decreased tenderness and juiciness due to myofibril shrinkage. However, numerous studies have demonstrated that when limiting the oxidative modification to a relatively low level, oxidation promotes the gelling potential and emulsifying capacity without negatively affecting WHC of muscle proteins (Srinivasan and Hultin, 1997; Xiong et al., 2010; Cheng et al., 2021; Lv et al., 2021). Increased firmness and elastic rigidity of MP gels observed at low concentrations of H_2O_2 (<5 mM), which generates $^{\bullet}OH$ in the presence of Fe^{2+}, was attributed to accentuated hydrophobic association and covalent interactions (disulfide and carbonyl-amine complex) at levels conducive to an isotropic protein gel network rather than random aggregates (Xiong et al., 2009). Disulfide cross-linking of the rod at the hinge zone (part of light meromyosin) instead of the head (heavy meromyosin) was the predominant mode of aggregation for $^{\bullet}OH$ stressed myosin (Ooizumi and Xiong, 2006). This pattern shift favored the development of an ordered scaffold within the MP gel and is conducive to water entrapment (Figure 4). There is not a direct relationship between WHC and the degree of oxidation. Due to the fine capillarity of the MP matrix formed at low concentrations of ROS, the gels tended to have enhanced water-holding potential (Ge et al., 2020; Cheng et al., 2021). The negative effect on WHC, as well as gel strength, by high doses of ROS can be partially offset by the marination of meat with pyrophosphate and polyphosphate, despite the fact that oxidation tends to desensitize the phosphates for dissociating the actomyosin complex, hence myofibril swelling (Liu and Xiong, 2013; Liu et al., 2015).

The generation of MDA and other secondary products from lipid oxidation in meat processing influences the gelling properties of MP and could have a positive effect. As reported by Zhou et al. (2014), treatment of MP with 2.5 mM MDA improved the elasticity, strength, and water-holding capacity of the emulsion composite gel. Similarly, Cheng et al. (2021) showed that MDA in the concentration range of 5 to 20 mM enabled protein cross-linking and a stronger gel with a higher WHC in an MP–emulsion composite system. The inclusion of 2.5% mixed mulberry phenols alleviated the negative effect of high-concentration (40 mM) MDA. Since the mixed phenols under nonoxidizing conditions interfered with MP emulsion but promoted the stability of emulsion-filled gels of MDA-oxidized MP, it can be inferred that covalent protein–quinone

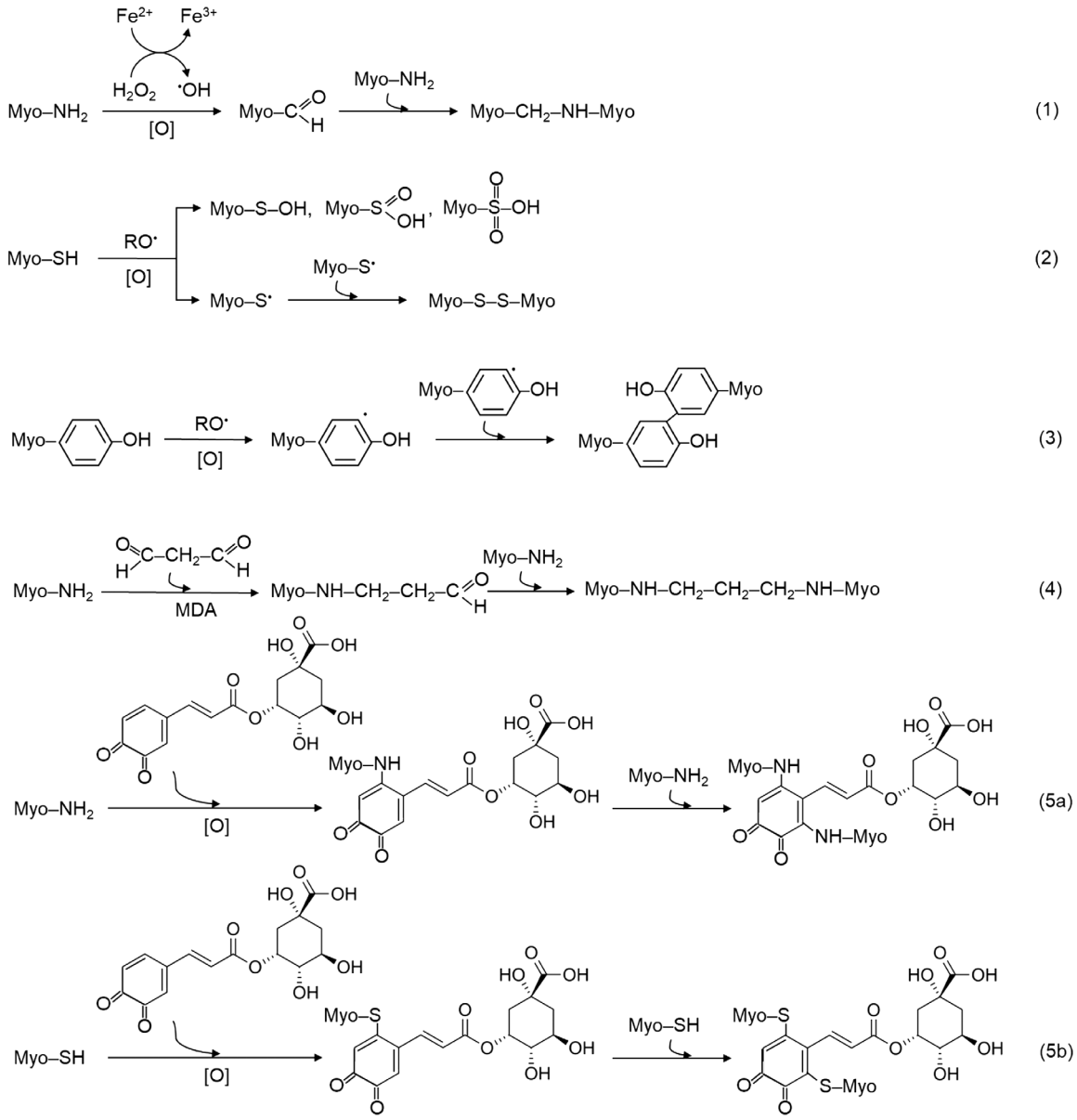

Figure 3. Common myosin cross-linking reactions initiated by reactive oxygen species that involve sidechain groups of lysine (1, 4, 5a), cysteine (2, 5b), and tyrosine (3). Note the cross-linking agents malondialdehyde (MDA) and oxidized phenolic compounds (quinones).

binding altered the mechanism of MDA modification of MP gelation. Gel-enforcement effects were also evidenced in the study by Lv et al. (2021) who claimed that low concentrations of MDA (3 and 6 mM) promoted protein-protein association and improved the emulsifying, gelling, and water-binding properties of MP gels.

In meat processing, muscle tissue disruption, incorporation of molecular oxygen, and the addition of salt and different chemical ingredients will promote chemical production of ROS. Because chemical oxidation tends to be abrupt (a sudden burst of radicals) and difficult to control, enzymatic production of ROS has been proposed to produce possible benefits conferred by limited oxidation. For example, by the application of glucose oxidase, which slowly produces H_2O_2 (precursor of $^{\bullet}OH$) in a glucose concentration-dependent manner, the extent of protein modification may be modulated (Figure 5). MP gels with a range of rheological properties (hardness and elasticity), superior to chemically modified (direct Fenton reaction) MP gels, have been reported (Wang et al., 2016, 2017). The progressive production of ROS in the enzymatic oxidation reaction explains the favorable protein cross-linking pattern, allowing for a more elastic and firmer gel formed. It was subsequently demonstrated

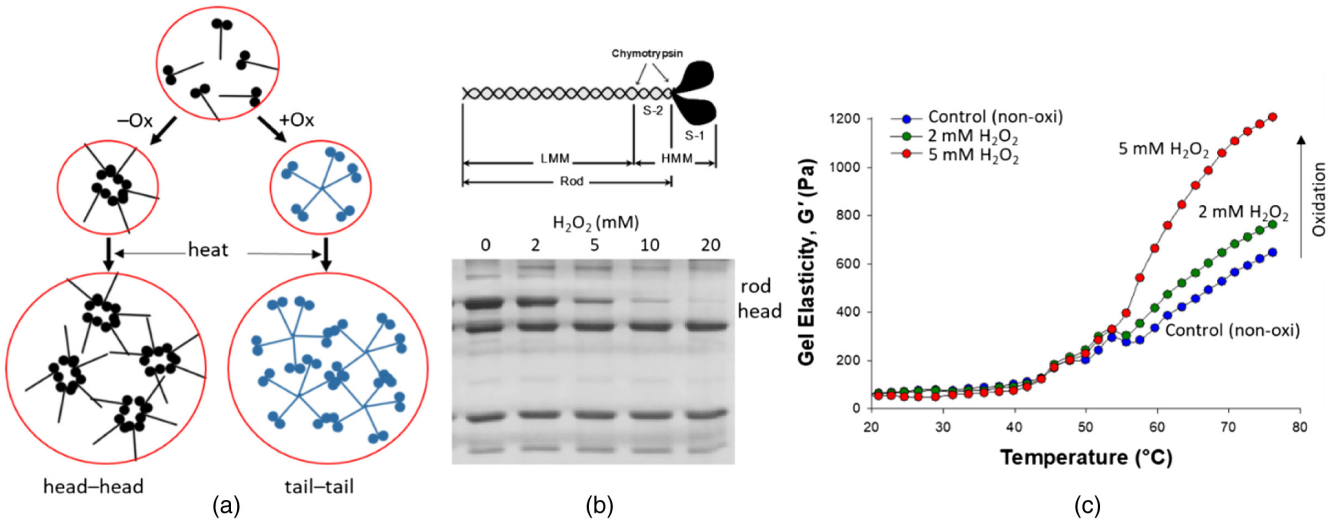

Figure 4. Cross-linking and gel formation of myofibrillar protein under nonoxidizing (−Ox) and oxidizing (+Ox) conditions generated in the presence of mixed 1 mM ferrous ion (Fe^{2+}) and 2–20 mM hydrogen peroxide (H_2O_2) that produces hydroxyl radical ($^{\bullet}OH$). Note the shift from head-head aggregation (−Ox) of heavy meromyosin (HMM) or myosin head (s-1) to tail-tail aggregation (+Ox) of light meromyosin (LMM) or myosin rod. (a) Schematic representation of cross-linking; (b) electrophoretic evidence; and (c) elasticity development during thermal gelation. Data extracted from Ooizumi and Xiong (2006) and Xiong et al. (2010).

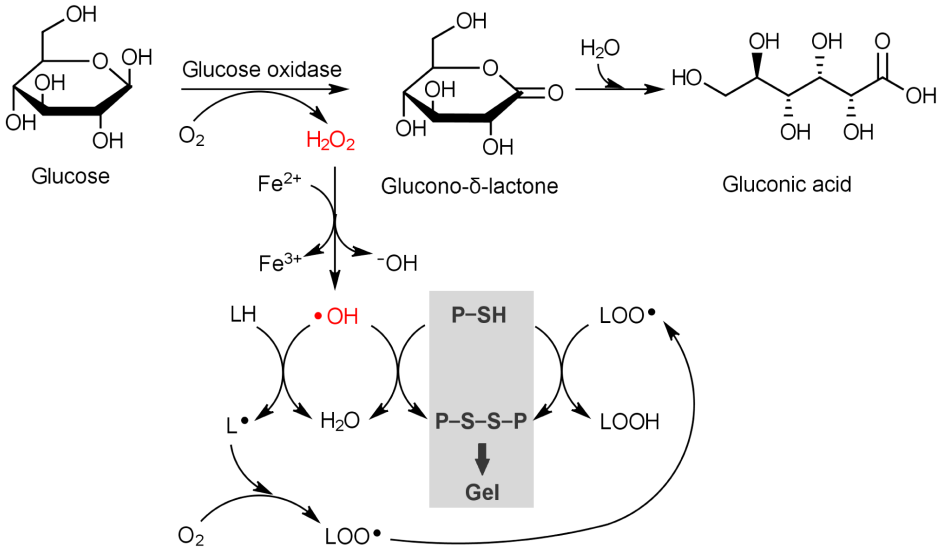

Figure 5. Proposed mechanism of glucose oxidase-induced cross-linking and gelation of myofibrillar protein. LH, lipid molecule; P–SH, protein sulfhydryl; P–S–S–P, disulfide cross-linked protein. Data extracted from Wang et al. (2016).

that structurally modified myosin by glucose oxidase promoted the interaction with plant phenols to further enhance the gelling capacity of MP (Guo and Xiong, 2019). The efficacy of phenol-dependent gelation improvement was related to the specific sidechains of phenolic compounds. For example, esterified with gallic acid, the epigallocatechin group in epigallocatechin-3-gallate appears to be highly reactive with myosin producing a well-structured gel matrix when compared with mono- and di-phenol compounds (Guo et al., 2021).

Antioxidant Strategies to Modulate Protein Functionality

There are 2 principal strategies to modulate protein oxidation: incorporation of antioxidants into muscle through feeding meat animals antioxidant supplements or addition of antioxidants to product formulations through processing (Falowo et al., 2014; Jiang and Xiong, 2016; Jongberg et al., 2017). The 2 very different approaches can both be effective in affecting

muscle protein functionality and meat quality. The feeding strategy has the benefit of improving water-binding and retention in fresh meat. However, there has been minimal research to examine the potential effect on the functional properties of processed meat. Nutritional antioxidants as a part of formulated animal diet can be absorbed and effectively distributed in muscle tissue both inside the cell and at the membrane (Mitsumoto, 2000). Moreover, as feed supplements, antioxidants and synergists, such as α-tocopherol and enzyme cofactors (Se, Cu, and Mn) can activate muscle cellular antioxidative enzymes, thereby enhancing the redox potential of fresh meat (Delles et al., 2014; Liu et al., 2019a). Improved meat WHC and decreased drip loss are common effects of feeding animals phenolic and nonphenolic antioxidants, which have been associated with decreased oxidative damage to MP and the mitigation of protein aggregation (Delles et al., 2014). Meat from broiler chickens fed supplemental microalgae and their extracts had significantly improved WHC and reduced cooking loss (Delles et al., 2015; El-Bahr et al., 2020). Similarly, meat from pigs fed peptide-bound Se and other enzyme cofactors exhibited stronger water-binding and improved cooking yield (Jiang et al., 2017, 2021). It should be noted that dietary antioxidants generally promote lipid oxidative stability but not all are effective as inhibitors of muscle protein oxidation. For example, Delles et al. (2015) observed significant inhibition of protein carbonyl formation in the muscle of broilers fed algae-based polyphenols and Se yeast. Smet et al. (2008), who fed broilers rosemary, green tea, and grape seed phenolic extracts, found no inhibition of protein oxidation in meat during storage. The degree of absorption by animals is known to be likely variable, depending on the chemical structure of the specific phenolic compounds (Surai, 2014).

The use of antioxidant additives in meat product formulation is a more feasible and economical strategy to control and regulate protein oxidation. The focus is on natural compounds, especially plant polyphenols (Guo and Xiong, 2021). The exogenous arginine and lysine were found to mitigate •OH-induced structural changes in myosin, allowing myosin to maintain its emulsifying capacity under oxidative conditions (Zhang et al., 2021). The effect was attributed to the radical scavenging activity of both free amino acids. Yet, because protein carbonyl and dityrosine contents remained high in treated emulsions, it was not clear whether interfacial proteins were affected by either amino acid. On the other hand, the efficacy of plant phenolic acids and flavonoids has been more robustly investigated owing to their abundance, wide availability, and biological activities (Shahidi and Zhong, 2010; Rohn, 2019). Phenolic compounds are capable of scavenging radicals and binding with prooxidant minerals to inhibit lipid oxidation (Shah et al., 2014). Plant seeds, fruits, and dry herbs used as flavor additives are excellent sources of abundant phenolic chemicals (Shan et al., 2005). Polyphenols released from crushed spices and seasonings during meat processing can be oxidized into electrophilic quinone species, which in turn react with MP covalently. By the mechanism of cross-linking with amino and thiol groups in myosin and other muscle proteins via Michael addition, protein dimers, oligomers, and polymers are formed, which serve as the building blocks for protein gel networks. When MP are mixed with gallic acid and exposed to glucose oxidase, the rate of MP cross-linking and gel formation increased remarkably, and myosin tail–tail cross-linking rather than head–head association appears to be favored (Figure 6). Similarly, chemically induced (Fenton type) oxidation of MP formed highly viscoelastic gels when subjected to low concentrations (6 and 30 μM) of gallic acid (Cao et al., 2016) or chlorogenic acid (Cao and Xiong, 2015). Disulfide bridges as well as amine-carbonyl and sulfur-carbonyl complexes formed by the MP reaction with quinone derivatives were implicated in the improved gelling activity. Through the adduction to cysteine, arginine, and histidine, low and moderate concentrations of rosemarinic acid (50 and 250 μM) promoted MP gelation (Tang et al., 2017).

All these studies showed a dose-dependent effect of phenolic compounds. High concentrations were generally detrimental to MP gelation due to blockages of protein thiol and amine groups by carbonyls rather than quinone carbonyls acting as bifunctional cross-linking agents. The efficacy of phenolic compounds is generally observed under oxidative conditions because of the high reactivity of quinone species. In systems that are deficient in ROS, polyphenols interact with MP noncovalently and largely behave as antioxidants (Estévez et al., 2008). For fish protein (surimi), treatment with oxidized caffeic acid significantly increased gel structural properties and WHC, consistent with many of the findings on red meat and poultry, and the addition of arginine further improved the gel properties (Xiong et al., 2021). There is limited information on phenolic compound effects on MP emulsification. Lv et al. (2021) reported that in a moderate-to-strong oxidizing environment, epigallocatechin-3-gallate mainly acted as an antioxidant to inhibit protein polymerization and aggregation caused by oxidation, thereby

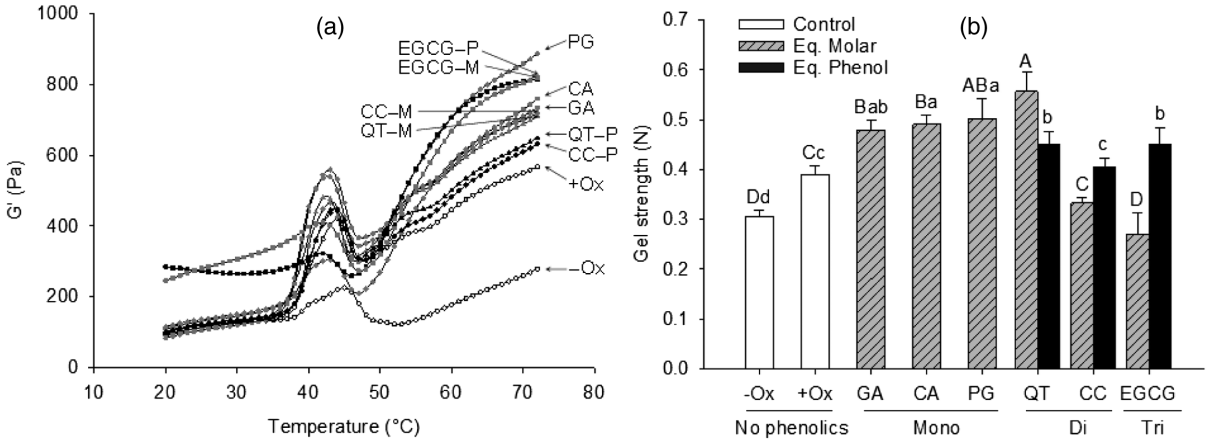

Figure 6. Elastic deformability (storage modulus, G′) of myofibrillar protein (MP) during thermal gelation (a) and the breaking strength of the set gel (b). MP sols were treated with different phenolic compounds under oxidative conditions. Nonoxidized (−Ox) and oxidized (+Ox) controls and MP modified with gallic acid (GA), chlorogenic acid (CA), propyl gallate (PG), quercetin (QT), catechin (CC), and (−)-epigallocatechin-3-gallate (EGCG) were tested. Phenolics were added at an equal molar concentration (−M; 60 μmol/g MP) or an equal phenol concentration (−P; 60, 30, and 20 μmol/g MP). Means with different letters (A–D or a–d) differ significantly ($P < 0.05$). Data extracted from Guo and Xiong (2019).

improving emulsion stability. Li et al. (2019) also claimed that emulsifying activity of MP was improved by the addition of sage extract. Jongberg et al. (2013) found that low dosages of green tea extract protected the texture as well as improved oxidative stability of protein emulsion *in situ*. The interfacial behavior or phenol-modified MP in meat emulsion products remains poorly understood. It may be presumed that protein-polyphenol complexation would promote steric hindrances and the amphiphilicity of interfacial membrane, leading to an improved emulsion stability. However, it is clear that the structure-function relationship of the individual phenolic compounds (polyol, carboxyl, and quinone carbonyl groups of sidechains) is among the determinant factors for their effects, which should be methodically investigated in future studies.

Conclusions

Protein oxidation in fresh meat and, more so, meat products is a significant chemical phenomenon that is increasingly recognized to influence the quality of muscle foods. The mechanism and exact chemistry are still poorly defined. Different from *in vivo* oxidation occurring in humans and animals, which has a rather invariable or closely constant physiological condition (temperature ∼37°C, pH ∼7.0, ionic strength ∼0.15, and homeostatic redox state), postmortem muscle proteins are subjected to extensive variations in environmental factors associated with processing and product formulations. Therefore, research findings in health sciences and from any physiological condition should not be simply extrapolated to muscle food processing or used directly to explain oxidative changes and functionality impact in meat proteins. Nevertheless, basic and applied studies conducted over the past 3 decades in meat systems have led to the indisputable conclusion that both free radicals and nonradical species formed in meat during postmortem storage and processing can cause irreversible damage of proteins leading to intensive aggregation and loss of organoleptic quality. However, controlled and mild oxidative modification of amino acid sidechains could significantly improve the gelation, emulsification, and water-binding properties of MP. The antioxidant ingredient approach, especially the inclusion of plant phenolic extracts, is a promising strategy to modulate protein oxidation and allows for optimum functionality and meat product quality through covalent and noncovalent interactions while inhibiting lipid oxidation and development of off-flavors. Because the efficacy of polyphenols is variable, future research should explore the structure-function relationship of phenolic chemicals in their reaction with proteins under different redox conditions. This is possible by the robust chemical analyses, such as mass spectrometry and isothermal calorimetry coupled with in silico modeling, that can help decipher the reactivity of specific side groups of quinone species and the consideration of different conformational spaces of MP existing in meat under various processing and storage conditions. Finally, the toxicity of oxidized meat proteins and the safety of antioxidant management, which are already being investigated, should be further promoted.

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