REVIEW

Protein oxidation in muscle foods: A review

Marianne N. Lund[1], Marina Heinonen[2], Caroline P. Baron[3] and Mario Estévez[4]

[1] Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej, Frederiksberg, Denmark
[2] Department of Food and Environmental Sciences, Food Chemistry, University of Helsinki, Finland
[3] National Food Institute, Technical University of Denmark, Søltofts Plads, Lyngby, Denmark
[4] Department of Animal Production and Food Science, Faculty of Veterinary, University of Extremadura, Cáceres, Spain

Received: September 15, 2010
Revised: November 9, 2010
Accepted: November 9, 2010

Protein oxidation in living tissues is known to play an essential role in the pathogenesis of relevant degenerative diseases, whereas the occurrence and impact of protein oxidation (Pox) in food systems have been ignored for decades. Currently, the increasing interest among food scientists in this topic has led to highlight the influence that Pox may have on meat quality and human nutrition. Recent studies have contributed to solid scientific knowledge regarding basic oxidation mechanisms, and in advanced methodologies to accurately assess Pox in food systems. Some of these studies have provided insight into the reactions involved in the oxidative modifications undergone by muscle proteins. Moreover, a variety of products derived from oxidized muscle proteins, including cross-links and carbonyls, have been identified. The impact of oxidation on protein functionality and on specific meat quality traits has also been addressed. Some other recent studies have shed light on the complex interaction mechanisms between myofibrillar proteins and certain redox-active compounds such as tocopherols and phenolic compounds. This paper is devoted to review the most relevant findings on the occurrence and consequences of Pox in muscle foods. The efficiency of different anti-oxidant strategies against the oxidation of muscle proteins is also reported.

Keywords:
Metal-catalyzed oxidation / Myoglobin-induced oxidation / Protein degradation / Protein–lipid interaction / Protein–phenolics interaction

1 Introduction

Oxidation of food proteins is, currently, one of the most innovative issues of study within the Food Chemistry field. However, the fact that proteins are targets for reactive oxygen species (ROS) was ignored for several decades while the oxidation of other food components, namely lipids, was studied in depth. The lack of suitable and specific methodologies to assess protein oxidation (Pox) and the assumption that lipid oxidation was, together with microbial spoilage, the main cause for food deterioration, slowed down the development of this highly innovative research line. In fact, most of the studies carried out on Pox during the last decades have been conducted to examine the role played by oxidized proteins in a variety of age-related diseases (*i.e.* Alzheimer) [1, 2] whereas the occurrence of Pox in food systems has been largely unexplored. Following the discovery that myofibril proteins are affected by ROS during meat maturation and storage [3], numerous research studies have dealt with the occurrence of Pox in muscle foods and have tried to shed light on the influence of meat origin, composition, and industrial processing on the development and

Correspondence: Dr. Mario Estévez, Animal Production and Food Science, Faculty of Veterinary, University of Extremadura, Avda, Universidad s/n, 10003, Cáceres, Spain
E-mail: mariovet@unex.es
Fax: +34927257110.

Abbreviations: AAS, α-aminoadipic semialdehyde; **GGS,** γ-glutamic acid semialdehyde; **Mb,** myoglobin; **MP,** myofibrillar proteins; **Pox,** protein oxidation; **ROS,** reactive oxygen species; **WHC,** water-holding capacity

intensity of muscle Pox. Most of the initial studies were limited to quantify protein carbonyls in a particular muscle food using a routine and relatively unspecific method (DNPH method) as a complementary analysis within a study mostly devoted to lipid oxidation. Later, the design of experiments on simple model systems and the application of more specific and advanced methodologies (reviewed by Estévez and colleagues, [4]) such as electron spin resonance spectroscopy [5, 6], fluorescence spectroscopy [7] and HPLC coupled to fluorescence detection and MS [8] have contributed toward understanding the role of particular compounds such as iron, myoglobin (Mb), lipids and phenolic compounds in the occurrence of muscle Pox. As a result of some recent advances on this topic, the basic chemical mechanisms involved in the oxidative modification of muscle proteins are being clarified although further studies are still needed. It is known, however, that the reaction of radicals with proteins and peptides in the presence of oxygen gives rise to alterations in both the backbone and of the amino acid side chains. These oxidative changes include cleavage of peptide bonds, modification of amino acid side chains and formation of covalent intermolecular cross-linked protein derivatives [9], as shown in Fig. 1. Some of the most general amino acid modifications are the formation of protein carbonyl groups and protein hydroperoxides, while cross-linking has mostly been described as formation of disulfide and dityrosine through the loss of cysteine and tyrosine residues [6, 8, 10].

The importance of Pox for quality deterioration has only been partially investigated. However, it is known that the modifications caused by ROS in muscle proteins could be implicated in the loss of their functionality and therefore, in the loss of the quality of meat, fish and muscle foods [11]. Oxidation of proteins in processed meat products leads to reduced water-holding capacity (WHC) and texture-forming ability [11]. Several mechanisms have been proposed for the impact of Pox on relevant texture traits in meat such as tenderness and juiciness [12–15]. Oxidation of proteins may cause changes in protein hydrophobicity, conformation, and solubility and altered susceptibility of protein substrates to proteolytic enzymes [16, 17]. This has been regarded as a major cause for the low digestibility and hence, lesser nutritional value of oxidized proteins [18].

The impact of Pox on the quality of muscle foods challenges scientists to develop reliable and effective anti-oxidant strategies to inhibit these reactions and their unpleasant effects on muscle foods. The modification of the composition of the muscle through dietary means in terms of fatty acid composition, carotenoids and tocopherol supplementation has shown to be generally effective to enhance the stability of muscle foods against Pox [12, 19–22]. Processing of muscle foods using herbs, fruits, essential oils and other plant materials usually leads to anti-oxidant and pro-oxidants effects [20–24]. The complex interaction mechanisms between myofibrillar proteins (MP) and phenolic compounds have been recently studied in detail [23, 24]

This review is devoted to examine the most relevant findings regarding Pox in muscle foods and addresses, (i) the mechanisms involved in the oxidation of muscle proteins, (ii) the impact of Pox in muscle foods and (iii) the effectiveness of different anti-oxidant strategies against Pox.

2 Muscle protein oxidation mechanisms

Oxidation of proteins is believed to proceed *via* a free radical chain reaction similar to that of lipid oxidation although, in the former, a higher complexity of the pathways and a larger variety of oxidation products hve been reported [9]. The abstraction of a hydrogen atom by an ROS leads to the generation of a protein carbon-centered radical (P•) which is consecutively converted into a peroxyl radical (POO•) in the presence of oxygen, and an alkyl peroxide (POOH) by abstraction of a hydrogen atom from another molecule. Further reactions with HO_2• lead to the generation of an alcoxyl radical (PO•) and its hydroxyl derivative (POH). As many other macromolecules, MP are susceptible to oxidative

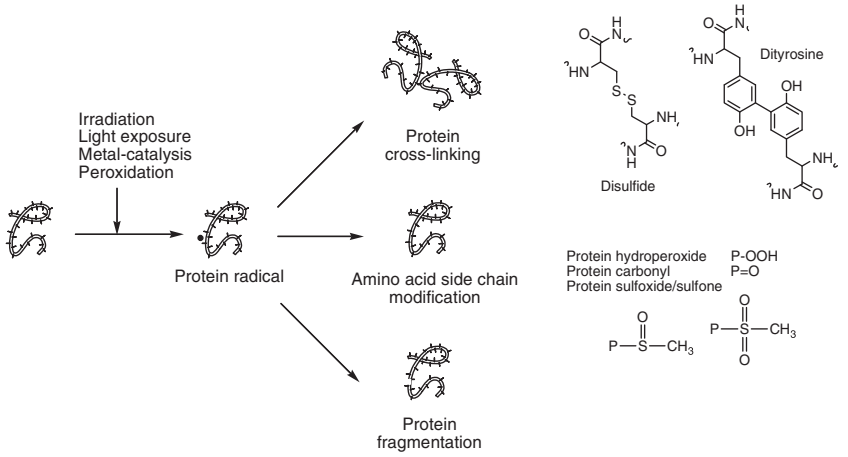


Figure 1. The most common consequences of oxidation of proteins.

reactions with myosin being the most sensitive, followed by troponin T [3]. Among amino acids, cysteine, tyrosine, phenylalanine, tryptophan, histidine, proline, arginine, lysine and methionine have been described as particularly susceptible to ROS [9]. The nature of the Pox products formed is highly dependent on the amino acids involved and how the oxidation process is initiated. The side chains of some particular amino acids such as arginine, lysine and proline are oxidized through metal-catalyzed reactions into carbonyl residues while others such as cysteine or methionine are involved in cross-linking or yield sulfur-containing derivatives. In general, highly reactive radicals are the less selective initiating Pox [9, 25, 26]. However, the level and nature of formed Pox products are also dependent on the naturally occurring prooxidant species present in muscle foods, and this review will focus on initiation of Pox by metals and heme proteins.

Muscle foods naturally contain the pro-oxidative heme proteins Mb or hemoglobin, which have been showed to be able to be good initiators of Pox. The pro-oxidative activity of Mb is directly coupled to the color cycle of meat, and has been reviewed by Baron and Andersen [27], among others. When the reducing enzymes in meat are depleted as a result of slaughter, metmyoglobin (MbFe(III)) accumulates in the meat and by reaction with H_2O_2, hypervalent Mb species perferrylmyoglobin and ferrylmyoglobin ($^{\bullet}$MbFe(IV) = O and MbFe(IV) = O, respectively) are formed (peroxidation cycle). Radical transfer from $^{\bullet}$MbFe(IV) = O to other proteins have been shown to cause the formation of long-lived protein radicals [6, 28]. However, oxidation of proteins is not only initiated by $^{\bullet}$MbFe(IV) = O but in some cases also by MbFe(IV) = O, as demonstrated by the reduction of MbFe(IV) = O by myosin [5]. The presence of hypervalent Mb species in meat has been questioned, but MbFe(IV) = O might be present in meat and also be able to initiate lipid oxidation under the conditions expected to be found in muscles [27], whereas the ability of MbFe(IV) = O to induce Pox in meat yet has to be elucidated. Non-heme iron and other transition metal ions also catalyze both Pox and lipid oxidation in the presence of H_2O_2 in muscle tissue [26, 29, 30]. A finite mechanism of the reaction between iron and H_2O_2 (Fenton reaction) is still not established, but in general, the Fenton reaction is regarded as a common source of the highly reactive hydroxyl radicals ($^{\bullet}$OH). The oxidation of MP in model systems and muscle foods has been linked to the participation of Fe^{3+} as a required initiation factor [8, 31]. The involvement of transition metals generally leads to the formation of carbonyl derivatives from particular amino acids as described below, but formation of cross-links in MP has also been reported [32].

In this section, the most remarkable and measurable changes caused by Pox in muscle foods will be reported and consist of (i) formation of protein carbonyls, (ii) loss of sulfydryl groups and (iii) formation of protein cross-linking. The role played by oxidizing lipids on protein oxidation will also be briefly discussed.

2.1 Formation of carbonyl derivatives

The formation of carbonyl compounds from amino acid side chains is well documented and probably the most outstanding result of metal ion-catalyzed oxidation of MP [29]. The quantification of carbonyl compounds by using the routine DNPH method has been widely employed as a general measure of Pox in multiple muscle foods (reviewed by Estévez and colleagues, [4]) including fish muscle and fish products [33, 34], fresh meat [12, 14, 35] and a variety of meat products [19, 36, 37]. Recently, Estévez and colleagues [8] identified specific carbonyl compounds in oxidized MP namely, α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively) in oxidized MP by using HPLC-MS. According to their proposal, lysine, proline and/or arginine from MP are oxidized in the presence of ferric iron (Fe^{3+}) and H_2O_2 to yield GGS and AAS (Fig. 2). The reaction might be initiated by $OOH^{\bullet}$ radicals derived from the Fenton-like reaction between Fe^{3+} and H_2O_2. The oxidative deamination from the intermediate radical molecule occurs in the presence of Fe^{3+} and yields the semialdehyde. The resulting Fe^{2+} could propagate the oxidative degradation to new amino acid residues by reacting with H_2O_2 to form further hydroxyl radicals. A recent study [24] has confirmed that the H_2O_2-activated Mb and other metals such as Cu^{2+} are also able to promote the formation of AAS and GGS from MP. Both AAS and GGS are thought to account around 70% of the total amount of protein carbonyls formed in oxidized animal proteins [38]. It is worth noticing that the formation of these semialdehydes does not require a previous cleavage of the peptide bond as protein-bound amino acids can be degraded into their corresponding semialdehydes. AAS and GGS have already been detected and employed as indicators of Pox in raw meat and a large variety of processed muscle foods such as cooked patties, frankfurters and dry-cured meats [31, 39, 40].

The amount of non-heme iron in meat has been shown to increase significantly during cooking as iron is released from Mb due to denaturation, increasing, as a result, its pro-oxidative potential [41]. In accordance, Ganhao and colleagues [31] proposed that the intense formation of protein semialdehydes during chill storage of cooked patties was mainly caused by non-heme iron released from the heme molecule during cooking. However, Estévez and Heinonen [24] recently found that H_2O_2-activated Mb promotes the formation of AAS and GGS to a higher extent than non-heme iron. Park and colleagues [42] and Park and Xiong [43] reported that a metmyoglobin-oxidizing system induces a more severe loss of amino acids and a more intense formation of protein carbonyls from MP than Fe^{3+} in the presence of H_2O_2 and ascorbate. To what extent Mb and non-heme iron act individually as pro-oxidants in cooked and uncooked fish and meat remains unclear, but it is generally accepted that both systems induce oxidation in fish and meat [30, 44].

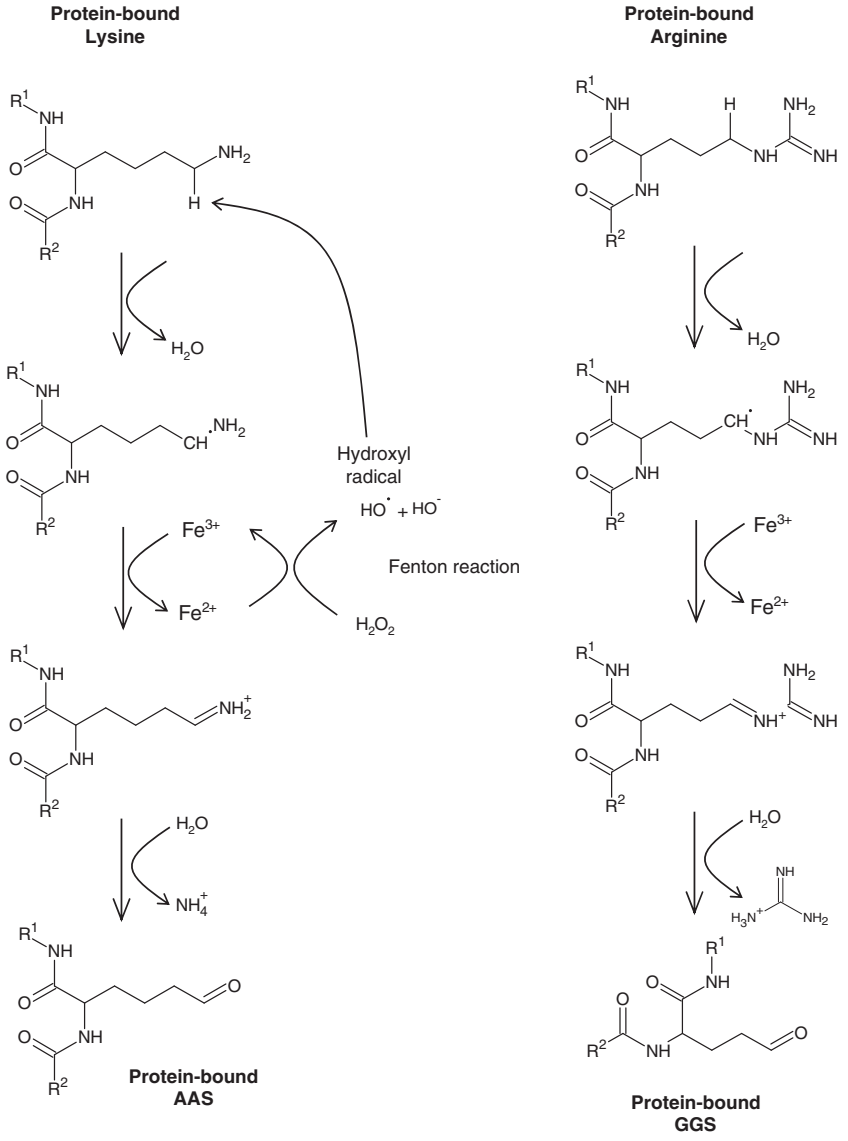


Figure 2. Formation of AAS and GGS in the presence of non-heme iron and hydrogen peroxide. Adapted from Estévez and Heinonen [24].

2.2 Loss of sulfydryl groups

The thiol group of cysteine (RSH) is highly susceptible to oxidation in the presence of hydrogen peroxide [45], which is formed in cells and accumulated in meat *post-mortem* [46]. However, the rate of reaction between H_2O_2 and cysteine-containing peptides or proteins is rather slow, *e.g.* with glutathione the rate constant is $k = 0.87\,M^{-1}s^{-1}$ (pH 7.4, 37°C) [47]. It is also noteworthy that oxidation of myosin (13 μM) with H_2O_2 in the concentration range of 25 μM–10 mM does not cause loss of free thiol groups in myosin in comparison to oxidation with MbFe(III) activated by H_2O_2 [6]. However, oxidation with H_2O_2 of the proteolytic meat enzyme, μ-calpain, containing active sites based on cysteine has been shown to decrease enzymatic activity through formation of a disulfide bond [48, 49]. The oxidation of thiol groups leads to a series of complex reactions resulting in formation of various oxidized products such as sulfenic acid (RSOH), sulfinic acid (RSOOH) and disulfide cross-links (RSSR). Equations (1)–(3) show examples of such reactions [25, 47].

$$RSH + H_2O_2 \rightarrow RSOH + H_2O \quad (1)$$

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (2)$$

$$2RSH + O_2 \rightarrow RSSR + H_2O_2 \quad (3)$$

More complex reactions involving thiyl radicals may also take place, as shown in Eq. (4), but a number of other reactions may also occur during oxidation [9, 25].

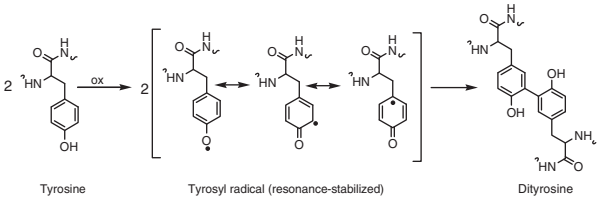
$$RS^{\bullet} + RS^{-} \longrightarrow (RSSR)^{\bullet -} \xrightarrow{O_2 \;\; O_2^{\bullet -}} RSSR \quad (4)$$

The total content of thiol groups in meat does not seem to be affected by *post-mortem* aging [50] suggesting that the content of thiol groups is a good marker of oxidation. Determination of loss of thiol groups in proteins from muscle foods is often based on Ellman's reagent (reviewed by Estévez and colleagues, [4]), and is relatively easy to measure in water soluble and MP fractions. Recently, a protocol has been published for thiol determination based on a fluorescent maleimide-derivative, the ThioGlo-1, which is reported as being more sensitive than Ellman's reagent [51].

The reported losses of thiol groups in fish and meat during storage vary greatly. Only a 6% loss of thiol groups were found in pork slices stored in high-oxygen atmospheres from day 1 to day 14 *post-mortem* [35], while worst-case scenario a 37% loss of thiol groups in minced pork stored in high-oxygen atmospheres for 7 days was observed [52]. In fish it was reported a loss of 50% of the thiol with extensive washing for horse mackerel mince, however, during storage of the different washed fish products the decrease in thiol varied between approx. 0–40% of the initial thiol group content [53]. Other reports showed similar losses of thiol groups dependent on muscle type, experimental conditions and species [3, 53]. A number of studies report larger loss of thiol groups in model systems (up to 92%) with MP or isolated myosin [6, 54, 55] with different oxidants and under different conditions. However, as the model systems are designed to study the effect of oxidation and the concentration of oxidants varied to obtain maximum effect, the extent of oxidation should not be directly compared to real meat products.

2.3 Formation of protein cross-links

The intra- and inter-molecular cross-linking of muscle proteins involves the formation of a variety of cross-linked oxidation products and subsequently polymerization of the proteins. Formation of disulfide cross-links has already been described in Section 2.2 and has been observed in meat model systems, [5, 6, 32] and in fresh meat [14, 15]. Another cross-linked compound, dityrosine, has only been observed in meat model systems [6, 56]. The dityrosine is formed by the oxidation of tyrosine through the formation of a tyrosyl phenoxyl radical [9] as shown below



The tyrosyl radical is resonance stabilized due to its aromatic nature, which may cause the formation of long-lived tyrosyl radicals in some proteins, *e.g.* BSA [28] and myosin [6]. Transfer of oxidative damage from cysteine, tryptophan and methionine to tyrosine is well characterized, and it has therefore been suggested that tyrosine residues act as the ultimate ''sink'' for oxidizing equivalents in proteins [9], but to this date no reports have been published showing the formation of dityrosine in fish and meat.

The hypervalent Mb species have been shown to induce both intra- and inter-molecular protein cross-linking [6, 28, 56, 57]. Park and colleagues [58] have recently reported that the oxidation of MP in a metmyoglobin-oxidizing system also promotes extensive, dose-dependent cross-linking while the effect of other systems (*i.e.* a lipid-oxidizing system) was minimal. •MbFe(IV) = O has been found to react rapidly with myosin to form myosin radicals and cross-linked products in a concentration-dependent manner at physiological pH [6], and the formation of cross-linked myosin induced by H_2O_2-activated Mb does not seem to be greatly affected by lowering pH to values relevant for meat systems [5]. An oxidation mechanism of myosin by H_2O_2-activated heme proteins has been proposed based on detection of radical formation on myosin by electron spin resonance spectroscopy and identification of disulfide and dityrosine cross-links [6] (Fig. 3A). By

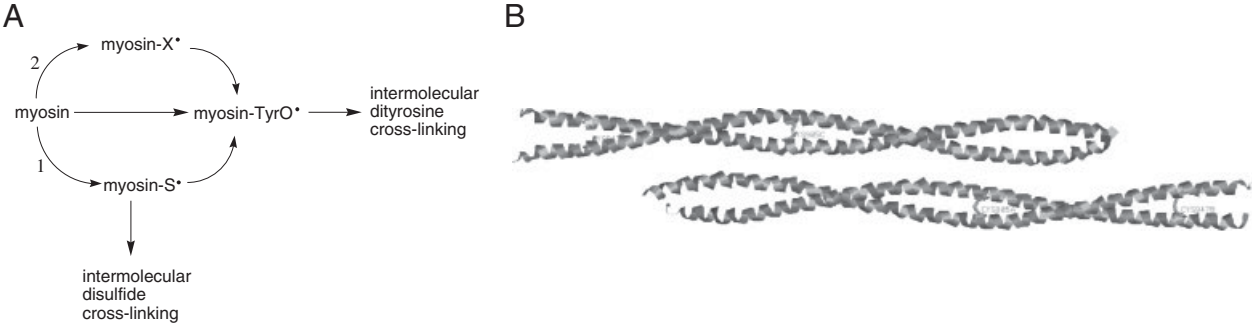


Figure 3. (A) Proposed reaction mechanism for the oxidation of myosin by H_2O_2-activated heme proteins at physiological pH (adapted from Lund and colleagues [6]), (B) Close-up of the myosin heavy chain tail region where the cysteine residues are shown (http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid = 42788, PDB ID: 2FXO).

summarizing all the available reports published on oxidative cross-linking of myosin it seems that oxidation of myosin, irrespective of oxidizing system, results in formation of both disulfide and non-disulfide cross-links in myosin, even though some studies show only formation of disulfide cross-links upon metal-catalyzed oxidation [54, 59], while in other studies the potential formation of both types of cross-links has not been investigated. Even though dityrosine formation has been reported in a number of studies [60], it is suggested that the route to formation of disulfide cross-linking is the most relevant one for meat systems as disulfides are the only cross-linking type identified in fresh meat so far [14, 15].

2.4 Lipid and Pox interactions

The role of oxidizing lipids in the initiation of Pox in muscle foods has been subjected to considerable debate. Some authors have reported the timely interaction of lipid and Pox in several meat systems [23, 61] while others [62] have found that Pox was minimally affected by lipid peroxidation. Park and colleagues [42] further reported that lipid and Pox may be related in certain oxidizing systems (*i.e.* hydroxyl radical-generating system) but not in another (metmyoglobin-oxidizing system). Hence, the environment (muscle types, animal species, type of muscle food, etc.) in which protein and lipid oxidation occur has a major influence on the ''coupling'' of the two oxidative processes. Whereas the oxidative modification of muscle proteins can take place in the absence of lipids (i.e. metal-catalyzed reaction), it is highly unlikely that the oxidation of lipids and proteins takes place independently in meat systems. Numerous papers support the timely coincidence of lipid and Pox in several model systems and muscle foods [19, 20, 23, 61, 63]. Theoretically, the oxidative reactions could be transferred either way between lipids and proteins. However, according to the measurable changes, the onset of lipid oxidation in meat systems would take place faster than the oxidative degradation of MP [7, 23, 37, 63] and hence, it is more likely that lipid-derived radicals and hydroperoxides promote Pox than the other way round. In fact, peroxyl radicals formed during lipid oxidation has been reported to abstract hydrogen atoms from protein molecules leading to a radical-mediated chain reaction similar to that of lipid oxidation [64]. The initiation of lipid oxidation by reaction between hypervalent Mb and lipid (LH) and/or lipid hydroperoxides (LOOH) [30], as well as oxidation of BSA, has been also described and is pH dependent [27, 65]. It is noteworthy that MP could act as redox-active compounds and therefore protects themselves and other food components, such as lipids, from oxidative reactions [7, 66]. The anti-oxidant properties of proteins are attributed to the cooperative effect of a variety of properties including the ability of aromatic and sulfur-containing amino acids to scavenge free radicals, and the capacity to act as metal-ion chelators [66, 67]. According to several studies [7, 23] the loss of tryptophan fluorescence from MP as a result of its oxidative modification is regarded as an early Pox event and coincides with the maximum production of lipid hydroperoxides. In meat model systems, the highest amounts of secondary lipid oxidation products are detected while the formation of protein carbonyls is still growing to reach a maximum several days later [7, 23]. Similar results have been reported for BSA and dairy proteins in liposomes and oil-in-water emulsions [63].

3 Impact of protein oxidation on muscle food quality

The consequences of Pox in muscle food have often been associated with changes in solubility and protein functionality such as gelation and emulsifying properties, or WHC. Most of these studies have mainly been performed in protein model systems and have previously been reviewed by Xiong [11] so this review will mainly cover newly published reports. The impact of processing and storage on Pox in muscle foods has primarily been investigated during the last decade, and the consequences of Pox have so far mainly been identified as deterioration of tenderness and juiciness [12, 14, 15, 20, 36, 40, 53, 60, 68]. Furthermore, oxidative modifications of proteins can lead to loss of essential amino acids and decreased digestibility (18, 21) affecting ultimately the nutritional quality of muscle foods. There are, however, many unexplored areas concerning the consequences of Pox in muscle foods such as the possible impact on flavor due to formation of released carbonyl groups and Schiff bases [7, 8]. Later, Ventanas and colleagues [20] and Fuentes and colleagues [40] have suggested reasonable hypothesis by which protein carbonyls might impact flavor and odor of dry-cured meats although the proposed mechanisms are, currently, subjects of further studies. Other authors [36, 69, 70] have reported likely effects of pigments and myofibrillar Pox on color deterioration of muscle foods but the precise mechanisms have not been clarified. The deterioration of meat color as linked to the oxidation state of heme iron does not affect directly protein structures and will not be covered in this review.

3.1 Water-holding capacity

WHC that is defined as the ability of meat to hold its own or added water during application of any force has an impact on particular quality traits such as juiciness of meat and is implicated in numerous technological processes in which water retention plays a major role. The capability of fresh meat to retain water is affected by various factors but possibly also by *post-mortem* Pox, which has been carefully described by Huff-Lonergan and Lonergan [13]. According

to these authors, reduced proteolytic activity of tenderising enzymes and cross-linking of MP may influence negatively WHC and juiciness of muscle foods. Modified atmosphere packaging with high concentrations of oxygen has been found to decrease sensory-assessed juiciness compared to storage without oxygen, which was suggested to be affected by myosin cross-linking [14, 15]. Reduced WHC of purified myofibrils upon oxidation with Hb and H_2O_2 was found together with an increase in formation of the cross-linked oxidation product, dityrosine, illustrating the effect of cross-linking on water-holding [60]. However, a direct relationship between WHC and degree of oxidation was not found with various pH and ionic strengths indicating a need for further studies. According to recent studies [71, 72] the negative effect of Pox on WHC of meat might affect specific technological processes such as brine irrigation, meat marinating and subsequent cooking. Liu and colleagues [72] reported that disulfide cross-linkages between staggered myosin tails are a major constraint restricting myofibril radial swelling during salt marination. The loss of WHC of strongly oxidized MP is also responsible for high water losses during cooking which may lead to a decreased juiciness [71]. Xia and colleagues [73] highlighted the impact of several freezing–thawing cycles on Pox and that on thawing and cooking losses as a result of altered WHC of pork. Pox may also affect the drying process and characteristics of dry-cured sausages according to a recent study by Sun and colleagues [74]. These authors reported a relationship between Pox, protein aggregation, and the degree of proteolysis.

3.2 Tenderness

Improvement of tenderness in meat occurs mainly during *post-mortem* storage with the rate of tenderization depending on species, gene, age, *etc*. The calpain system is by many considered to be responsible for most of the *post-mortem* tenderization of meat due to its proteolytic activity toward key MP, and details can be found elsewhere [75, 76]. Since it is the only proteolytic enzyme system in meat that has been extensively investigated in relation to oxidation directly in meat it is only the calpain system that will be covered here. The decrease in meat tenderness caused by Pox has mainly been investigated based on two hypotheses, (i) inactivation of μ-calpain [77] and (ii) cross-linking of MP and hereby strengthening of the myofibrillar structure [14, 15, 78].

Increased Pox (measured by carbonyl content) in both sarcoplasmic and myofibrillar protein extracts was found in irradiated steaks compared to the non-irradiated steaks, which was negatively correlated to tenderness [77]. Cross-linking of MP was not investigated in the two studies by Rowe and colleagues [12, 77] and thus it is not possible to determine to what extent μ-calpain inactivation and protein cross-linking individually influence the observed decrease in tenderness caused by irradiation. Whereas the inactivation of calpain as a result of oxidative reactions could have an impact on tenderization of meat, a recent study supports that the oxidative modification of MP enhances their susceptibility to undergo degradation by calpain [79]. It remains unknown whether this mechanism takes place in *post-mortem* muscles that have not been subjected to irradiation.

In pork stored in high-oxygen atmosphere, myosin heavy chain was found to form inter-molecular cross-links while no myosin cross-linking was observed without the presence of oxygen in the packaging [14]. In the same study, storage in high-oxygen atmospheres was also found to cause significantly less tender meat compared to storage without oxygen. Tenderness of pork slices stored in high-oxygen atmosphere decreased significantly further over time, which indicates that the presence of oxygen not only inhibits development of tenderness but also strengthens the myofibrillar structure as well, for example by myosin cross-linking, which was further supported by studies showing an increased strengthening of single muscle fibres stored in high-oxygen atmospheres [78]. When studying the tail region of the myosin molecule (Fig. 3B) it becomes clear that the cysteine residues in one MHC are located very closely to the cysteine residues in the other MHC. This shows that formation of disulfide bonds in the tail region of myosin is highly likely to occur during oxidative conditions, a reaction that could explain the observed reduction in tenderness. The light meromyosin part of myosin has been found to be most susceptible to oxidation and subsequent formation of disulfide cross-links [80]. However, the head region is most likely also susceptible to oxidation, as the ATPase activity of myosin, which is mainly exhibited by the head region, has proven to be strongly affected by oxidation [42]. Myosin cross-linking through disulfide bond formation and reduced tenderness caused by storage in high-oxygen atmospheres was recently confirmed in a study with beef [15]. Interestingly, a possible cross-link between titin and myosin was also observed suggesting that oxidative conditions inducing disulfide cross-links between these two proteins also influence meat tenderness. As opposed to irradiation of meat, no inactivation of μ-calpain has been observed in the studies with high-oxygen atmosphere packaging indicating that inactivation of μ-calpain only occurs when oxidation is induced more dramatically and uniformly as by irradiation and not by exposing meat to elevated concentrations of oxygen, which requires the activity of naturally occurring prooxidants in meat in order to be reactive. In salted herring cross-linking of myosin was found to be responsible for the characteristic texture of herring [81], and further studies have shown that protein carbonyl groups increase with ripening time and despite significant proteolysis, myosin cross-linking correlates with the characteristic texture of salted herring [82]. The increase in hardness during refrigerated storage of processed muscle foods such as cooked sausages and liver pâtés [36, 69] has also been ascribed to onset of Pox and more particularly to

the formation of cross-linking between MP. These authors found significant correlations between instrumental hardness of sausages and their degree of Pox as measured by total protein carbonyls. Similar results have been reported by Fuentes and colleagues [40] while studying the effect of high-hydrostatic pressure on the onset of Pox in dry-cured meats and the impact on particular texture traits such as tenderness and juiciness.

3.3 Nutritional quality

Meat is a major source of protein of high biological significance to human beings. The oxidation of MP may lead to a significant decrease in their nutritional value in terms of availability of essential amino acids and digestibility of oxidized muscle proteins. Oxidation of numerous amino acids leads to the formation of carbonyls groups and other derivatives [1]. Among them, basic amino acids (lysine, histidine and arginine) and threonine are essential amino acids for humans and their oxidation causes a depletion of such amino acids in the dietary muscle food. Phenylalanine and tryptophan are also essential amino acids for humans and their loss in meat under the attack of ROS [31] may lead to a significant depletion of their availability [17], whereas the impact of Pox on protein digestibility has been profusely discussed and opposite arguments have been reported (reviewed by Xiong [11]). The recent studies support that oxidative modification of MP leads to the formation of protein aggregates and a decrease susceptibility to undergo proteolytic degradation, which might have an effect on protein digestibility [18, 21, 22]. Protein carbonylation might not be a reliable indicator of the impact of Pox on protein digestibility according to recent findings by Santé-Lhoutellier and colleagues [21]. It seems that the mechanisms by which oxidation modulates digestibility is complex and may implicate other groups of amino acids, especially aromatic amino acids which are particularly represented in recognition sites of proteases. In further studies, the same authors [83] found direct, significant and quantitative correlations between protein carbonylation and aggregation induced by cooking and proteolytic susceptibility to pepsin. However, no such correlations were observed with other proteolytic enzymes such as trypsin and α-chymotrypsin.

4 Controlling protein oxidation in muscle foods, anti-oxidant strategies

Until recently, most strategies devoted to control Pox in muscle foods were directly adopted from well described and effective procedures against lipid oxidation. These include intervention at the beginning of the food chain by enhancing the oxidative stability of the animal tissues through dietary means or acting at the end of the food chain by adding substances with anti-oxidant activity directly to the food.

4.1 Dietary strategies

It is well known that the feeding regime of the animal and its anti-oxidant status have a great influence on the oxidative stability of the final food product [30, 84]. Muscle tissues of monogastric meat animals and fish reflect, to some extent, the fatty acid composition of the feeds and incorporate in cell membranes certain substances supplemented in the feeds such as the tocopherol. Hence, the dietary strategies against muscle foods oxidation generally involve reducing the ratio PUFA/FA in animal tissues and supplement animal feeds with tocopherol or carotenoids. Feeding animals on pasture and other natural resources such as acorns have also been described as successful strategies owing the high concentration of tocopherols in such feeds [84]. Whereas the supplementation with tocopherol and with carotenoids seems to be effective to inhibit the extent of Pox in muscle foods, the modification of the fatty acid composition has a negligible impact on Pox. For example, Santé-Lhoutellier and colleagues [21] observed a lower level of protein carbonyls in lamb-fed pasture compared to lamb-fed concentrates. The protein carbonyl amount was found to correlate negatively with vitamin E level showing a protective effect of vitamin E on Pox. Lund and colleagues [14] reported that the level of unsaturation of dietary fat in pork was not found to correlate with the development of Pox, and in a study investigating feeding regime and protein and lipid oxidation in rainbow trout it was found that the oil type (fish oil *versus* vegetable oil) did not have a significant impact on the development of Pox [22]. However, the carotenoid canthaxanthin present in the feed was found to prevent Pox in rainbow trout during *post-mortem* storage [22]. Tocopherol supplementation has been shown to decrease the development of protein carbonyls in beef [12] turkey [61] and pork [19] during *post-mortem* storage. Additionally, a study on chicken fed with a high anti-oxidant diet containing apple and broccoli revealed a lower level of protein carbonyl content in the muscle protein soluble fraction compared to chickens fed with a low anti-oxidant diet [85]. According to studies carried out by Ventanas and colleagues [19, 20], the protective role of supplemented tocopherols would remain during long-term processing of meats. The amount of protein carbonyls in dry-cured loins and hams from extensively reared Iberian pigs and from pigs fed on tocopherol-supplemented feeds was significantly lower than that found in products from pigs fed on conventional feeds. In this case, significant positive correlations were found between protein carbonyls and the PUFA/tocopherol ratio. In agreement, meat emulsions (frankfurters and liver pâtés) manufactured with tissues from Iberian pigs fed on pasture and acorns showed a higher stability against the formation of protein carbonyls than products produced from pigs fed on concentrates [69, 86]. Therefore, controlling the extent of Pox during processing and storage of muscle foods by managing animal feeds seems to be an interesting option.

4.2 Technological strategies

The design of new formulations and recipes that generally involves the addition of substances with known anti-oxidant effect has become a popular strategy to inhibit Pox and its unpleasant effects on muscle foods. Some plant phenolics and tocopherols are examples of established anti-oxidants, which have been extensively studied in relation to lipid oxidation in biological and food systems [15, 61, 63, 69]. However, it seems that the conventional lipid anti-oxidant strategies do not necessarily apply to muscle proteins, as compounds that are able to prevent from lipid oxidation are not always able to prevent from Pox. Inhibition of lipid oxidation is expected to prevent Pox to some extent by minimizing the formation of secondary lipid oxidation products and thereby preventing their interactions with proteins. However, in a model system, it has been showed that prevention of Pox using a hydrophilic anti-oxidant also has a protective effect on the lipid fraction [87]. The hydrophilic anti-oxidant Trolox (a vitamin E analogue) was found to prevent oxidation of both protein and lipid fractions but the lipophilic anti-oxidants tested were ineffective at preventing Pox [87]. Plant phenolics may be both hydrophilic and hydrophobic compounds, as anti-oxidants from plant materials can be extracted from both the lipid and water fraction and thus be located in both the aqueous and lipid phase of a food system [88]. The ability of phenolic compounds to act as anti-oxidants depends on intrinsic factors such as its own chemical structure and extrinsic ones such as the composition and characteristics of the substrate, stage and intensity of the oxidative reactions and localization of the phenolic compound [89]. Furthermore, the particular effect of phenolics on food proteins is governed by the result of interactions established through covalent and non-covalent linkages between the phenolic compound and the food protein. These interactions are dependent on the amount and chemical state of the phenolic compound and the size, conformity and overall charge of the protein [89, 90]. The intrinsic mechanisms of the protein oxidation damage, nature of the target, location of the site of attack together with the type of attacking species have an impact on the development of Pox and therefore, on the ability of the compound to prevent the oxidative damage [91]. Therefore, it is difficult to describe the kinetics and mechanisms of the inhibition of Pox by anti-oxidants in detail and the number of studies that can be found in the literature on this matter is rather limited. Most studies that aimed to evaluate the effect of diverse chemical compounds and plant materials against Pox in muscle foods interpreted the overall effect of the tested material without having a complete comprehension of the chemical behind it. Some phenolic-rich plant and fruit extracts have been shown to exert anti-oxidative protection of proteins in cooked pork patties, porcine liver pâté and chicken [36, 37, 70, 92], but the anti-oxidative effect was found to be dependent on the structure and concentration of the phenolic compound. In beef patties, a rosemary extract was found to have no protective effect against Pox and a mixture of ascorbate and citrate promoted Pox, while both anti-oxidant systems protected lipids from oxidation [35]. Furthermore, addition of rosemary oil to frankfurters has been shown to inhibit Pox while addition of higher levels of the rosemary oil resulted in a prooxidative effect when the frankfurters were prepared with meat from white pigs showing that the anti-oxidative effect was dependent on concentration and product characteristics [86]. In model systems, some polyphenols and vitamin E have been reported to be efficient anti-oxidants protecting MP against oxidation, while others promoted Pox [23, 24, 33]. In

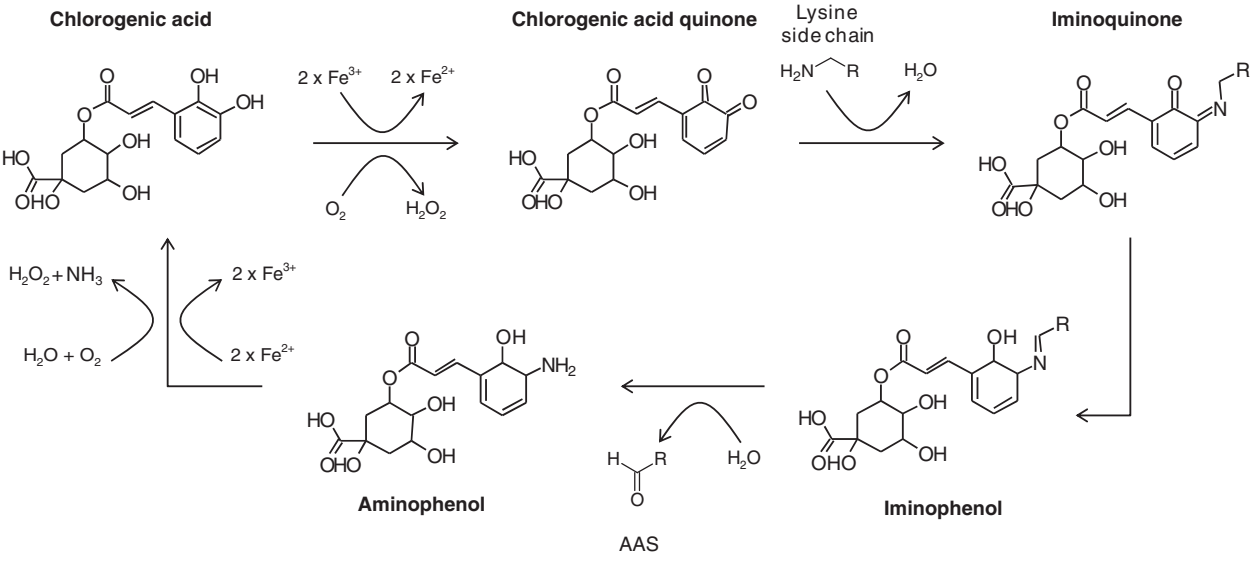


Figure 4. Interaction between a phenolic compound (chlorogenic acid) and a lysine residue in the presence of non-heme iron with the result of AAS formation. Adapted from Estévez and Heinonen [24].

addition, it has been demonstrated that the protein substrate is of importance as the anti-oxidative effect was generally more pronounced on BSA than on MP [23]. A recent study carried out on protein suspensions and using LC-MS to detect the specific protein carbonyls, AAS and GGS, have shed light on particular chemical mechanisms involved in the anti-oxidant and pro-oxidant actions of phenolic compounds on MP [24]. According to this study, phenolic compounds (*i.e* gallic acid, catechin, cyanidin-3-glucoside and rutin) would protect MP from oxidative damage through their ability to act (i) as metal chelators and inactivate the pro-oxidant effect of non-heme iron and (ii) as scavengers of hydroxyl and other radical formed from the iron-mediated Fenton reaction. The protective effect of phenolic-rich fruit extracts against tryptophan depletion and the formation of AAS and GGS in cooked patties has recently been ascribed to these anti-oxidant mechanisms [31]. Based on the original findings by Akagawa and Suyama [93], Estévez and Heinonen [24] also proposed a likely mechanism by which phenolic compounds could promote the formation of AAS and GGS from MP. In the presence of transition metals such as iron or copper, phenolics such as chlorogenic acid would undergo an auto-oxidation process leading to the formation of the corresponding quinones that display amine-oxidase activities. According to this proposal, quinone forms of plant phenolics could catalyze the oxidative deamination of susceptible amino acids to form the corresponding semi-aldehydes (Fig. 4).

The interaction between polyphenols and thiol groups in muscle foods is not completely understood. In washed fish caffeic acid has been shown to protect thiol groups from oxidation, but increased the formation of carbonyl groups [53]. In a storage experiment with beef patties, a decreased formation of carbonyl groups, an increased loss of thiol groups, but decreased amount of disulfide cross-links in myosin were observed when a grape polyphenols extract was added to the patties compared to control patties without addition of polyphenols, suggesting an overall anti-oxidant effect of the polyphenols despite the increased loss of thiol groups [94]. Addition of glutathione reduces the browning of wine caused by oxidation of phenols by forming thiol-quinone adducts [95], and the adduct has been identified for several proteins *e.g.* BSA [96]. A similar reaction could be important for muscle foods with plant phenols added as anti-oxidants, and such reactions are currently being studied.

5 Concluding remarks

Recent studies on Pox have taken a huge step forward to shed light on unknown chemical mechanisms and on the influence of Pox on meat quality. However, a better understanding of the reaction mechanisms leading to Pox in foods is necessary to further progress in this field. In this sense, there is an urge to develop novel selective, reproducible and sensitive methods to characterize the products generated during oxidation of food proteins. More knowledge is needed to reveal the full extent of Pox and its consequence for muscle food quality. In addition, Pox may impact food nutritional value and food digestibility and these aspects deserve attention if a better use of our resources is to be achieved. Nevertheless, oxidation of proteins can be desirable in some food products where it imparts characteristic texture and therefore Pox might be an interesting tool to control texture in food and generate food matrices with new textures and new rheological properties. In addition, the comprehension of the impact of food processing on Pox and the understanding of the chemistry behind it and the interaction of muscle proteins with other food components and other ingredients will provide a solid background to develop muscle foods of higher nutritional and sensory quality.

M. Estévez thanks the Spanish RYC-MICINN program for economical support and the European Community for the Marie Curie Intra-European and Reintegration Fellowships (Pox-MUSCLE and Pox-MEAT). M. N. Lund deeply thanks Prof. Leif H. Skibsted for his continued encouragement and scientific advice to her work on protein oxidation.

The authors have declared no conflict of interest.

6 References

[1] Stadtman, E. R., Berlett, B. S., Reactive oxygen-mediated protein oxidation in aging and disease. *Chem. Res. Toxicol.* 1997, *10*, 485–494.

[2] Davies, M. J., Singlet oxygen-mediated damage to proteins and its consequences. *Biochem. Biophys. Res. Commun.* 2003, *305*, 761–770.

[3] Martinaud, A., Mercier, Y., Marinova, P., Tassy, C. *et al.*, Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *J. Agric. Food Chem.* 1997, *45*, 2481–2487.

[4] Estévez, M., Morcuende, D., Ventanas, S., in: Nollet, L. M. L., Toldrá, F. (Eds.), *Handbook of Processed Meat and Poultry Analysis*, CRC Press, Boca Raton, FL, USA 2008, pp. 141–162.

[5] Frederiksen, A. M., Lund, M. N., Andersen, M. L., Skibsted, L. H., Oxidation of porcine myosin by hypervalent myoglobin: the role of thiol groups. *J. Agric. Food Chem.* 2008, *56*, 3297–3304.

[6] Lund, M. N., Luxford, C., Skibsted, L. H., Davies, M. J., Oxidation of myosin by heme proteins generates myosin radicals and protein cross-links. *Biochem. J.* 2008, *410*, 565–574.

[7] Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., Heinonen, M., Fluorescence spectroscopy as a novel approach for the

assessment of myofibrillar protein oxidation in oil-in-water emulsions. *Meat Sci.* 2008, *80*, 1290–1296.

[8] Estévez, M., Ollilainen, V., Heinonen, M., Analysis of protein oxidation markers – α-aminoadipic and γ-glutamic semialdehydes - in food proteins by using LC-ESI-multi-stage tandem MS. *J. Agric. Food Chem.* 2009, *57*, 3901–3910.

[9] Davies, M. J., Dean, R. T. (Eds.), *Radical-mediated Protein Oxidation*, Oxford Science Publications, Oxford 2003.

[10] Davies, M. J., Fu, S. L., Wang, H. J., Dean, R. T., Stable markers of oxidant damage to proteins and their application in the study of human disease. *Free Radic. Biol. Med.* 1999, *27*, 1151–1163.

[11] Xiong, Y. L., in: Decker, E., Faustman, C. (Eds.), *Antioxidants in Muscle Foods*, Wiley, Chichester 2000, pp. 85–111.

[12] Rowe, L. J., Maddock, K. R., Lonergan, S. M., Huff-Lonergan, E., Influence of early postmortem protein oxidation on beef quality. *J. Anim. Sci.* 2004, *82*, 785–793.

[13] Huff-Lonergan, E., Lonergan, S. M., Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Sci.* 2005, *71*, 194–204.

[14] Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., Skibsted, L. H., High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *Longissimus dorsi* during chill storage. *Meat Sci.* 2007, *77*, 295–303.

[15] Kim, Y. H., Huff-Lonergan, E., Sebranek, J. G., Lonergan, S. M., High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerisation. *Meat Sci.* 2010, *85*, 759–767.

[16] Wolff, S. P., Dean, R. T., Fragmentation of proteins by free radicals and its effect on their susceptibility to enzyme hydrolysis. *Biochem. J.* 1986, *234*, 399–403.

[17] Davies, K. J. A., Lin, S. W., Pacifici, R. E., Protein damage and degradation by oxygen radicals: IV. Degradation of denatured protein. *J. Biol. Chem.* 1987, *262*, 9914–9920.

[18] Morzel, M., Gatellier, Ph., Sayd, T., Renerre, M., Laville, E., Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. *Meat Sci.* 2006, *73*, 536–543.

[19] Ventanas, S., Estévez, M., Tejeda, J. F., Ruiz, J., Protein and lipid oxidation in *Longissimus dorsi* and dry cured loin from Iberian pigs as affected by crossbreeding and diet. *Meat Sci.* 2006, *72*, 647–655.

[20] Ventanas, S., Ventanas, J., Tovar, J., García, C., Estévez, M., Extensive feeding *versus* oleic acid and tocopherol enriched mixed diets for the production of Iberian dry-cured hams: effect on chemical composition, oxidative status and sensory traits. *Meat Sci.* 2007, *77*, 246–256.

[21] Santé-Lhoutellier, V., Engel, E., Aubry, L., Gatellier, P., Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and *in vitro* digestibility. *Meat Sci.* 2008, *79*, 777–783.

[22] Baron, C. P., Hyldig, G., Jacobsen, C., Does feed composition affect oxidation of rainbow trout (*Oncorhynchus mykiss*) during frozen storage? *J. Agric. Food Chem.* 2009, *57*, 4185–4194.

[23] Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., Heinonen, M., Oxidation of skeletal muscle myofibrillar proteins in oil-in-water emulsions: interaction with lipids and effect of selected phenolic compounds. *J. Agric. Food Chem.* 2008, *56*, 10933–10940.

[24] Estévez, M., Heinonen, M., Effect of phenolic compounds on the formation of alpha-aminoadipic and gamma-glutamic semialdehydes from myofibrillar proteins oxidized by copper, iron, and myoglobin. *J. Agric. Food Chem.* 2010, *58*, 4448–4455.

[25] Garrison, W. M., Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chem. Rev.* 1987, *87*, 381–398.

[26] Stadtman, E. R., Berlett, B. S., Fenton chemistry revisited: amino acid oxidation. *Basic Life Sci.* 1988, *49*, 131–136.

[27] Baron, C. P., Andersen, H. J., Myoglobin-induced lipid oxidation. A review. *J. Agric. Food Chem.* 2002, *50*, 3887–3897.

[28] Østdal, H., Skibsted, L. H., Andersen, H. J., Formation of long-lived protein radicals in the reaction between H_2O_2-activated metmyoglobin and other proteins. *Free Radic. Biol. Med.* 1997, *23*, 754–761.

[29] Levine, R. L., Mixed-function oxidation of histidine residues. *Methods Enzymol.* 1984, *107*, 370–376.

[30] Skibsted, L. H., Mikkelsen, A., Bertelsen, G., in: Shahidi, F. (Ed.), *Flavour of Meat, Meat Products and Seafoods* (2nd Ed.), Blackie Academic & Professional, Glasgow 1998, pp. 217–256.

[31] Ganhao, R., Morcuende, D., Estévez, M., Tryptophan depletion and formation of α-aminoadipic and γ-glutamic semialdehydes in porcine burger patties with added phenolic-rich fruit extracts. *J. Agric. Food Chem.* 2010, *58*, 3541–3548.

[32] Decker, E. A., Xiong, Y. L., Calvert, J. T., Crum, A. D., Blanchard, S. P., Chemical, physical, and functional-properties of oxidized turkey white muscle myofibrillar proteins. *J. Agric. Food Chem.* 1993, *41*, 186–189.

[33] Srinivasan, S., Xiong, Y. L., Decker, E. A., Inhibition of protein and lipid oxidation in beef heart surimi-like material by antioxidants and combinations of pH, NaCl, and buffer type in the washing media. *J. Agric. Food Chem.* 1996, *44*, 119–125.

[34] Tokur, B., Polat, A., Myofibrillar and sarcoplasmic protein oxidation and degradation of thin-lipped gray mullet (*Liza ramada*) during refrigerated storage. *J. Muscle Foods* 2010, *21*, 110–118.

[35] Lund, M. N., Hviid, M. S., Skibsted, L. H., The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Sci.* 2007, *76*, 226–233.

[36] Estévez, M., Ventanas, S., Cava, R., Protein oxidation in frankfurters with increasing levels of added rosemary essential oil: effect on color and texture deterioration. *J. Food Sci.* 2005, *70*, C427-C432.

[37] Vuorela, S., Salminen, H., Makela, M., Kivikari, R. *et al.*, Effect of plant phenolics on protein and lipid oxidation in

cooked pork meat patties. *J. Agric. Food Chem.* 2005, *53*, 8492–8497.

[38] Requena, J. R., Levine, R. L., Stadtman, E. R., Recent advances in the analysis of oxidized proteins. *Amino Acids* 2003, *25*, 221–226.

[39] Armenteros, M., Heinonen, M., Ollilainen, V., Toldrá, F., Estévez, M., Analysis of protein carbonyls in meat products by using the DNPH method, fluorescence spectroscopy and liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS). *Meat Sci.* 2009, *83*, 104–112.

[40] Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., Ventanas, S., Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Sci.* 2010, *86*, 510–514.

[41] Schrickler, B. R., Miller, D. D., Effects of cooking and chemical treatment on heme and nonheme iron in meat. *J. Food Sci.* 1983, *48*, 1340–1349.

[42] Park, D., Xiong, Y. L., Alderton, A. L., Ooizumi, T., Biochemical changes in myofibrillar protein isolates exposed to three oxidizing systems. *J. Agric. Food Chem.* 2006, *54*, 4445–4451.

[43] Park, D., Xiong, Y. L., Oxidative modification of amino acids in porcine myofibrillar protein isolates exposed to three oxidizing systems. *Food Chem.* 2007, *103*, 607–616.

[44] Monahan, F. J., Crackel, R. L., Gray, J. I., Buckley, D. J., Morrissey, P. A., Catalysis of lipid oxidation in muscle model systems by haem and inorganic iron. *Meat Sci.* 1993, *34*, 95–106.

[45] Neumann, N. P., Oxidation with hydrogen peroxide. *Methods Enzymol.* 1972, *25*, 393–400.

[46] Harel, S., Kanner, J., Hydrogen peroxide generation in ground muscle tissue. *J. Agric. Food Chem.* 1985, *33*, 1186–1188.

[47] Winterbourn, C. C., Metodiewa, D., Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Radic. Biol. Med.* 1999, *27*, 322–328.

[48] Guttmann, R. P., Elce, J. S., Bell, P. D., Isbell, J. C., Johnson, G. V. W., Oxidation inhibits substrate proteolysis by calpain I but not autolysis. *J. Biol. Chem.* 1997, *272*, 2005–2012.

[49] Lametsch, R., Lonergan, S. M., Huff-Lonergan, E., Disulfide bond within μ-calpain active site inhibits activity and autolysis. *Biochim. Biophys. Acta* 2008, *1784*, 1215–1221.

[50] Hofmann, K., Reiner, H., Sulfhydryl and disulfide groups in meats. *Adv. Food Res.* 1978, *24*, 1–111.

[51] Hawkins, C. L., Morgan, P. E., Davies, M. J., Quantification of protein modification by oxidants. *Free Radic. Biol. Med.* 2009, *46*, 965–988.

[52] Lund, M. N., Hviid, M. S., Claudi-Magnussen, C., Skibsted, L. H., Effects of dietary soybean oil on lipid and protein oxidation in pork patties during chill storage. *Meat Sci.* 2008, *79*, 727–733.

[53] Eymard, S., Baron, C. P., Jacobsen, C., Oxidation of lipid and protein in horse mackerel (*Trachurus trachurus*) mince and washed minces during processing and storage. *Food Chem.* 2009, *114*, 57–65.

[54] Srinivasan, S., Hultin, H. O., Chemical, physical, and functional properties of cod proteins modified by a nonenzymic free-radical-generating system. *J. Agric. Food Chem.* 1997, *45*, 310–320.

[55] Liu, G., Xiong, Y. L., Butterfield, D. A., Chemical, physical, and gel-forming properties of oxidized myofibrils and whey- and soy-protein isolates. *J. Food Sci.* 2000, *65*, 811–818.

[56] Bhoite-Solomon, V., Kessler-Icekson, G., Shaklai, N., Peroxidative crosslinking of myosins. *Biochem. Int.* 1992, *26*, 181–189.

[57] Hanan, T., Shaklai, N., Peroxidative interaction of myoglobin and myosin. *Eur. J. Biochem.* 1995, *233*, 930–936.

[58] Xiong, Y. L., Park, D., Ooizumi, T., Variation in the cross-linking pattern of porcine myofibrillar protein exposed to three oxidative environments. *J. Agric. Food Chem.* 2009, *57*, 153–159.

[59] Liu, G., Xiong, Y. L., Electrophoretic pattern, thermal denaturation, and *in vitro* digestibility of oxidized myosin. *J. Agric. Food Chem.* 2000, *48*, 624–630.

[60] Bertram, H. C., Kristensen, M., Østdal, H., Baron, C. P. *et al.*, Does oxidation affect the water functionality of myofibrillar proteins? *J. Agric. Food Chem.* 2007, *55*, 2342–2348.

[61] Mercier, Y., Gatellier, P., Viau, M., Remignon, H., Renerre, M., Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Sci.* 1998, *48*, 301–318.

[62] Liu, G., Xiong, Y. L., Contribution of lipid and protein oxidation to rheological differences between chicken white and red muscle myofibrillar proteins. *J. Agric. Food Chem.* 1996, *44*, 779–784.

[63] Viljanen, K., Kivikari, R., Heinonen, M., Protein–lipid interactions during liposome oxidation with added anthocyanin and other phenolic compounds. *J. Agric. Food Chem.* 2004, *52*, 1104–1111.

[64] Stadtman, E. R., Levine, R. L., Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* 2003, *25*, 207–218.

[65] Kröger-Ohlsen, M. V., Østdal, H., Andersen, M. L., The effect of pH on the oxidation of bovine serum albumin by hypervalent myoglobin species. *Arch. Biochem. Biophys.* 2003, *416*, 202–208.

[66] Saigas, A., Tanabe, S., Nishimura, T., Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *J. Agric. Food Chem.* 2003, *51*, 3661–3667.

[67] Diaz, M., Decker, E. A., Antioxidant mechanisms of caseinophosphopeptides and casein hydrolysates and their application in ground beef. *J. Agric. Food Chem.* 2004, *52*, 8208–8213.

[68] Baron, C. P., Kjaersgard, I. V. H., Jessen, F., Jacobsen, C., Protein and lipid oxidation during frozen storage of rainbow trout (*Oncorhynchus mykiss*). *J. Agric. Food Chem.* 2007, *55*, 8118–8125.

[69] Estévez, M., Cava, R., Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pâté. *Meat Sci.* 2004, *68*, 551–558.

[70] Ganhao, R., Morcuende, D., Estévez, M., Protein oxidation in emulsified cooked burger patties with added fruit extracts: influence on colour and texture deterioration during chill storage. *Meat Sci.* 2010, *85*, 402–409.

[71] Liu, Z., Xiong, Y. L., Chen, J., Identification of restricting factors that inhibit swelling of oxidized myofibrils during brine irrigation. *J. Agric. Food Chem.* 2009, *57*, 10 009–11 007.

[72] Liu, Z., Xiong, Y. L., Chen, J., Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle. *J. Agric. Food Chem.* 2010, DOI:10.1021/jf102043k.

[73] Xia, X., Kong, B., Liu, Q., Liu, J., Physicochemical change and protein oxidation in porcine *Longissimus dorsi* as influenced by different freeze–thaw cycles. *Meat Sci.* 2009, *83*, 239–245.

[74] Sun, W., Cui, C., Zhao, M., Zhao, Q., Yang, B., Effects of composition and oxidation of proteins on theirs solubility, aggregation and proteolytic susceptibility during processing of Cantonese sausage. *Food Chem.* 2011, *124*, 336–341.

[75] Koohmaraie, M., The role of Ca^{2+}-dependent proteases (calpains) in *post mortem* proteolysis and meat tenderness. *Biochimie* 1992, *74*, 239–245.

[76] Huff-Lonergan, E., Zhang, W., Lonergan, S. M., Biochemistry of postmortem muscle – lessons on mechanisms of meat tenderization. *Meat Sci.* 2010, *86*, 184–195.

[77] Rowe, L. J., Maddock, K. R., Lonergan, S. M., Huff-Lonergan, E., Oxidative environments decrease tenderization of beef steaks through inactivation of milli-calpain. *J. Anim. Sci.* 2004, *82*, 3254–3266.

[78] Lund, M. N., Christensen, M., Fregil, L., Hviid, M. S., Skibsted, L. H., Effect of high-oxygen atmosphere packaging in mechanical properties of single muscle fibres from bovine and porcine *Longissimus dorsi*. *Eur. Food Res. Technol.* 2008, *227*, 1323–1328.

[79] Smudera, A. J., Kavazisa, A. N., Hudsona, M. B., Nelsona, W. B., Powers, S. K., Oxidation enhances myofibrillar protein degradation *via* calpain and caspase-3. *Meat Sci.* 2010, *49*, 1152–1160.

[80] Ooizumi, T., Xiong, Y. L., Identification of cross-linking site(s) of myosin heavy chains in oxidatively stressed chicken myosfibrils. *J. Food Sci.* 2006, *71*, C196–C199.

[81] Andersen, E., Andersen, M. L., Baron, C. P., Characterization of oxidative changes in salted herring (*Clupea harengus*) during ripening. *J. Agric. Food Chem.* 2007, *55*, 9545–9553.

[82] Christensen, M., Andersen, E., Christensen, L., Andersen, M. L., Baron, C. P., Textural and biochemical changes during ripening of salted herrings. *J. Sci. Food and Agric.* 2010, DOI: 10.1002/jsfa.4190.

[83] Santé-Lhoutellier, T., Astruc, V., Marinova, P., Grève, E., Gatellier, P., Effect of meat cooking on physicochemical state and *in vitro* digestibility of myofibrillar proteins. *J. Agric. Food Chem.* 2008, *56*, 1488–1494.

[84] Ruiz, J., López-Bote, C. J., in Toldrá, F. (Ed.), *Research Advances in the Quality of Meat and Meat Products*, Research Singpost, Trivandrum, Kerala, India 2002, pp. 255–271.

[85] Young, J. F., Steffensen, C. L., Nielsen, J. H., Jensen, S. K., Stagsted, J., Chicken model for studying dietary antioxidants reveals that apple (cox's orange)/broccoli (*Brassica oleracea l.* var. *italica*) stabilizes erythrocytes and reduces oxidation of insoluble muscle proteins and lipids in cooked liver. *J. Agric. Food Chem.* 2002, *50*, 5058–5062.

[86] Estévez, M., Cava, R., Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: contradictory effects in different types of frankfurters. *Meat Sci.* 2006, *72*, 348–355.

[87] Baron, C. P., Berner, L., Skibsted, L. H., Refsgaard, H. H. F., Evaluation of activity of selected antioxidants on proteins in solution and in emulsions. *Free Radic. Res.* 2005, *39*, 777–785.

[88] Shahidi, F., Wanasundara, P. K. J. P. D., Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* 1992, *32*, 67–103.

[89] Frankel, E. N., Meyer, A. S., The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants – review. *J. Sci. Food Agric.* 2000, *80*, 1925–1941.

[90] Kroll, J., Rawel, H., Reactions of plant phenols with myoglobin: Influence of chemical structure of the phenolic compounds. *J. Food Sci.* 2001, *66*, 48–58.

[91] Dean, R. T., Hunt, J. V., Grant, A. J., Yamamoto, Y., Niki, E., Free radical damage to proteins: the influence of the relative localization of radical generation, antioxidants, and target proteins. *Free Rad. Biol. Med.* 1991, *11*, 161–168.

[92] Rababah, T., Hettiarachchy, N., Horax, R., Eswaranandam, S. *et al.*, Effect of electron beam irradiation and storage at 5C on thiobarbituric acid reactive substances and carbonyl content in chicken breast meat infused with antioxidants and selected plant extracts. *J. Agric. Food Chem.* 2004, *52*, 8236–8241.

[93] Akagawa, M., Suyama, K., Aminooxidase-like activity of polyphenols. Mechanism and properties. *Eur. J. Biochem.* 2001, *268*, 1953–1963.

[94] Jongberg, S., Tørngren, M. A., Skibsted, L. H., Lund, M. N., Effect of white grape extract and modified atmosphere packaging on protein oxidation in chill stored beef patties. *Food Chem.* 2010, (accepted for publication).

[95] Singleton, V. L., Cilliers, J. J. L. (Eds.), *Phenolic Browning – A Perspective from Grape and Wine Research*, American Chemical Society, Washington 1995.

[96] Alegria, A. E., Sanchez-Cruz, P., Kumar, A., Garcia, C. *et al.*, Thiols oxidation and covalent binding of BSA by cyclolignanic quinones are enhanced by the magnesium cation. *Free Radic. Res.* 2008, *42*, 70–81.

