# RELATIONSHIP BETWEEN PROTEIN AND LIPID OXIDATION IN RABBIT HIND LEG MEAT

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## ABSTRACT

The present study aimed at evaluating the effect of the exposure to oxidative conditions as well as of the incubation with different malondialdehyde concentration (MDA) on protein oxidation assessed by measuring carbonyls and free thiol groups on rabbit hind leg meat. For this purpose, five rabbits (10 weeks-old, average live weight of 2.7 kg) were collected from a commercial processing plant and, after deboning the hind legs, the resulting meat was minced and divided into twelve aliquots/each: six exposed to strong oxidant conditions and six considered as fresh. Both fresh and oxidized samples were treated with the addition of different final concentrations of MDA (i.e. 0, 0.25, 0.5, 1.0, 2.5 and 5 mM) and subsequently used to assess carbonyls and free thiol groups content. The same experiment was repeated on turkey (100 days-old, average live weight of 9.7 kg) thigh meat. Data concerning rabbit and turkey meat were separately analyzed according to a 2 × 6 factorial design (ANOVA) to investigate the main effects of the exposure to oxidative conditions and MDA addition. The exposure to oxidative conditions resulted in a 3-fold increase (2.27 *vs.* 6.68 nmol/mg of proteins; $P < 0.001$) in carbonyl content together with a significant reduction (-52%) in free thiol groups (197.5 *vs.* 94.9 nmol/mg of proteins; $P < 0.001$) in rabbit hind leg meat. On the other hand, the incubation with different MDA concentration did not exert any relevant effect on protein oxidation. These results are in overall agreement with those obtained on turkeys' thigh meat subjected to the same experimental design, even if rabbit meat proteins appear to be less prone to a lipid-induced oxidation. Overall, the findings of the present study show that rabbit meat is pretty resistant to main oxidative occurring to proteins. However, the occurrence of oxidative reaction affecting the polypeptide chains might change according cut-up characteristics, thus development of processing strategies aiming at reducing the extent of protein oxidation in rabbit meat and processed products needs to carefully consider its quality traits and attitude for further processing but also the processing steps as well as the storage conditions to which these meats will be subjected.

**Key words**: rabbit meat, malondialdehyde, protein oxidation, carbonyls, free thiols.

## INTRODUCTION

Protein and lipid oxidation in meat and meat products are believed to proceed through similar free radical-mediated chain reactions initiated by Reactive Oxygen Species (ROS) (Guyon *et al*., 2011). In addition, as radicals can be transferred between lipids and proteins, several lipid oxidation products are also ROS for proteins' polypeptide chains and catalyze the oxidative modifications taking place on their amino acids. Lipid oxidation has been extensively investigated in meat as, reacting with proteins, its products can result in the development of off-flavors as well as in a loss of nutritional value (Estévez, 2011). On the other hand, the mechanisms resulting in protein oxidation have raised the attention of the scientific community later on especially when meat is used as raw material for processing. This process, indeed, allows the contact between pro-oxidant agents and their targets thus affecting the quality of the final product (Guyon *et al*., 2016). Singlet oxygen primarily reacts with five amino acids: tryptophan, histidine, tyrosine, methionine, and cysteine to form peroxides. The rate of this reaction strongly depends on the amino acids composition of the proteins as the most reactive

ones are those containing double bonds or an electron-rich sulfur residue (Van Dyck, 2010; Lund *et al*., 2011).

Although the mechanisms resulting in protein and lipid oxidation have been already investigated in different meat types (Lund *et al*., 2011), the knowledge concerning the interaction between these two phenomena in the raw material is limited in rabbits. In detail, the effect of malondialdehyde (MDA) on myofibrillar proteins oxidation was recently investigated only in muscle homogenate (Wang *et al*., 2019). Overall, oxidative muscles are more prone to oxidation if compared to the glycolytic ones (Alasnier *et al*., 1996) and, although rabbit meats are commonly considered as "white" meats, their relatively high PUFA and heme pigment contents make them prone to the development of oxidative reactions (Hernandez and Gondret, 2006; Dalle Zotte and Szendrő, 2011; Petracci and Cavani, 2013). In the past few years, the growing interest of the rabbit industry in developing processed meat products (Cullere and Dalle Zotte, 2018; Petracci *et al*., 2018) highlighted the needs of improving the knowledge concerning the impact of different processing steps (i.e. cooking) on the oxidative modifications to proteins which can affect their functionality (Lund *et al*., 2011).

The present study aimed at evaluating the effect of exposure to oxidative conditions and incubation with different MDA concentration (from 0 to 5 mM) on protein oxidation assessed by measuring carbonyls and free thiol groups on rabbit and turkey minced meat. The same experiment was repeated also thigh meat because of its utmost susceptibility to lipid oxidation within those considered as "white meats" (Mercier *et al*., 1998; Estévez, 2015).

## MATERIALS AND METHODS

### Animals and experimental design

Five rabbit carcasses (10 weeks-old, average live weight of 2.7 kg) were collected after 24 hours *post-mortem* chilling at 4 $^{o}$C from a commercial processing plant. After deboning rabbits' hind legs, the resulting meat was finely minced with a grinder and divided into twelve aliquots/each: six to be added of 100 mM NaClO (that creating strong oxidant conditions would induce oxidative modifications to polypeptide chains), whereas the same volume of distilled $H_2O$ was added to the others (to obtain the same dilution of the protein fraction). Both fresh and oxidized samples were treated with the addition of different final concentrations of MDA (i.e. 0, 0.25, 0.5, 1.0, 2.5 and 5 mM) and incubated in the dark at 37°C for 24 hours. After that, samples were stored at -80°C until analyses carried out to evaluate the oxidation level of the protein fraction through the quantification of carbonyls and free thiol groups. The same experiment was repeated on turkey meat by collecting 5 carcasses (100 days-old, average live weight of 9.7 kg) and dissecting the *Extensor Iliotibialis lateralis* muscles. Samples were prepared and treated as described before.

### Chemical Analyses

Carbonyls were assessed according to the novel DNPH-based method proposed by Soglia *et al*. (2016) in which protein-bound hydrazones are spectrophotometrically determined after derivatization with 2,4-dinitrophenylhydrazine. Free thiol groups were quantified through the reaction of with 5,5'-Dithiobis(2-nitrobenzoic acid) (Ellman, 1959) with the conjugate base of a free sulfhydryl group thus leading to the development of a yellow-colored compound having a maximum absorbance at 412 nm.

### Statistical Analysis

Data concerning rabbit and turkey meat were separately analyzed according to a 2×6 factorial design (ANOVA) to investigate the main effects of the exposure to oxidative conditions and MDA addition on carbonyls and free sulfhydryl groups as well as on the redox state of iron in heme pigments, together with their interaction term. Means were subsequently separated by Tukey-HSD test at $P < 0.05$.

## RESULTS AND DISCUSSION

The results concerning the changes in protein carbonyls and free thiol groups assessed in fresh and oxidized rabbit and turkey meat are summarized in Tables 1 and 2. The exposure to oxidative conditions significantly affected both carbonyls and free thiol groups content in rabbit meat (Table 1). In detail, if compared to fresh meat, a 3-fold increase in carbonyl content was found in oxidized rabbit meat (2.27 *vs.* 6.68 nmol/mg of proteins; $P < 0.001$) together with a significant reduction (-52%) in free thiol groups (197.5 *vs.* 94.9 nmol/mg of proteins; $P < 0.001$) (Table 2). On the other hand, both the main effect related to the addition of different MDA concentrations as well as the interaction term "Ox × MDA" were not significant ($P > 0.05$) (Table 1) in case of rabbit meat.

**Table 1**: Probability values of differences in protein carbonyl content and free thiol groups in rabbit and turkey meat exposed to oxidative conditions (Ox) and different MDA concentrations (MDA) (N = 5 samples/group/type of meat; ** = P < 0.01; *** = P < 0.001; ns = not significant).

| | **Prob.** | | | | | |
|---|---|---|---|---|---|---|
| | **Rabbit meat** | | | **Turkey meat** | | |
| | **Ox** | **MDA** | Ox × MDA | **Ox** | **MDA** | **Ox × MDA** |
| Carbonyls | *** | ns | ns | *** | *** | ** |
| Free thiols | *** | ns | ns | *** | *** | ns |

**Table 2**: Means of protein carbonyl content and free thiol groups in rabbit and turkey meat exposed to oxidative conditions (Ox) and different MDA concentrations (MDA) (N = 5 samples/group/type of meat).

| | **Carbonyls (nmol/mg of protein)** | | **Free thiols (nmol/mg of protein)** | |
|---|---|---|---|---|
| | Rabbit | **Turkey** | **Rabbit** | **Turkey** |
| | Ox | | | |
| Fresh | 2.27b | 3.49b | 197.53a | 109.73b |
| Oxidized | 6.58a | 7.58a | 94.90b | 122.08a |
| | MDA (mM) | | | |
| 0.00 | 1.73 | 2.17 | 225.01 | 123.9 |
| 0.25 | 2.10 | 3.17 | 203.34 | 118.1 |
| 0.50 | 2.61 | 3.20 | 172.49 | 111.8 |
| 1.00 | 2.14 | 3.26 | 213.21 | 114.1 |
| 2.50 | 2.37 | 4.54 | 203.22 | 101.9 |
| 5.00 | 2.68 | 4.61 | 167.89 | 88.6 |

Mean values followed by different letters significantly differ among the groups (Tukey-HSD, $P < 0.05$).

With regard to the effect of MDA concentration, if compared to the control group (0 mM MDA), the attainment of a concentration 5 mM MDA resulted in a 55% increased protein carbonylation. Although not statistically significant, this result corroborates the hypothesis that MDA, as the main product of the secondary oxidation of the lipid fraction, might promote the development of carbonyl compounds, which represents one of the most remarkable changes occurring as a consequence of the oxidative modifications to the polypeptide chains (Estévez, 2011). These results are in overall agreement with those obtained on turkeys' thigh meat subjected to the same experimental design. However, the proteins composing the skeletal muscles of rabbit's hind leg meat appear to be less prone to a lipid-induced oxidation. This phenomenon might be ascribed, aside from the amino acid composition of the polypeptide chains which vary among species, to a lower exposure to pro-oxidant compounds such as hemoproteins (i.e. myoglobin) (Van Dyck, 2010) which are considerably high in turkey meat (Pereira and Vicente, 2013; Estévez, 2015). Indeed, because of its high heme and non-heme iron content (Richardson, 1995) thigh meat of turkey is more vulnerable than breast meat to oxidation and this higher susceptibility further explain the different carbonylation level observed in comparison with rabbit meat. The reduction in free thiol groups found in rabbit's hind leg meat exposed to oxidative conditions is in agreement with the findings of previous studies carried out on oxidized-myosin molecules (Frederiksen *et al*., 2008). Indeed, as cysteine is degraded into cysteine disulfide and sulfenic acid, the loss of free thiol groups in muscle protein can be considered as a reliable marker for oxidation (Winther and Thorpe, 2014). With regard to the effect of MDA

concentration, a 25% reduction in free thiols content was found by comparing the results obtained in the control group (0 mM MDA) with those found with the addition of 5 mM MDA. As previously observed for carbonyls, although not statistically significant ($P > 0.05$), this result supports the hypothesis that the proteins composing rabbit's hind leg meat exhibit an increased ability to withstand to the lipid-induced oxidative modification to the polypeptide chains. In agreement with that, a 2-fold increase in the concentration of free thiol groups was found in fresh rabbit meat (197.5 nmol/mg of protein) in comparison with the turkey one (109.7 nmol/mg of protein) thus suggesting the occurrence of a less enhanced protein oxidation processes.

## CONCLUSION

The findings of the present study suggest that, based on the ability of its constituting proteins to withstand lipid-induced oxidative modifications, rabbit meat has valuable traits that can be effectively exploited for manufacturing processed products. However, the occurrence of oxidative reactions affecting the polypeptide chains strictly depend on the meat-cut taken into account. Thus, the development of processing strategies aiming at reducing the extent of protein oxidation in rabbit meat and rabbit meat products needs to carefully consider not only their quality traits but also the processing steps and storage conditions.

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