MUSCLE STRUCTURE HIERARCHY: A BIOPHYSICAL MECHANISM FOR RADICAL-INDUCED VARIATION OF PROTEIN OXIDATION AND FUNCTIONALITY IN PROCESSED MEAT PRODUCTS

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Abstract – In meat processing, mechanical means are generally employed to extensively modify muscle structure to facilitate protein extraction and induce functionality in the presence of salt and phosphate. However, the process inevitably predisposes myosin and other cellular proteins to oxidant attack, resulting in chemical changes and functionality variations. In this study, we investigated the impact of muscle structure order on the oxidative susceptibility and functionality of myofibrils, the actomyosin complex, and myosin. Experiments probing physicochemical properties (protein carbonyls, tertiary structure, solubility, and aggregation) and functional performances (water-holding, rheology, and gelation) were conducted on oxidatively stressed (10 μM $FeCl_2$, 0.1 mM ascorbate, 0-5 mM H_2O_2) raw muscle and isolated proteins at pH 6.2, 0.6 M NaCl, and 0-15 mM pyrophosphate. The result showed a distinct relationship of the physical state of muscle and muscle fibers with protein susceptibility to oxygen radicals. The sensitivity of myosin to oxidants paralleled a reversed hierarchical order: muscle < fiber < myofibril < actomyosin ≈ myosin. Depending on the extent of oxidation, myofibrillar proteins, including myosin, exhibited either enhanced or depressed functionality. The findings offer an insight into the structural role of muscle in myosin oxidation and may aid in the meat product quality optimization.

Key Words – Myofibrillar protein, Oxidative stress, Water-holding

I. INTRODUCTION

Oxidative reactions are natural consequences in the processing of comminuted meat products that involves extensive grinding, chopping, and blending. The flavor characteristics and textural properties of such meats are often attributed to lipid and protein oxidation, respectively, among other factors. Oxidative modification of amino acid residues, formation of protein polymers, and changes in protein secondary and tertiary structures can lead to both desirable and undesirable protein functionality changes, including water-holding, gelation, meat particle binding, and fat emulsification in processed meats, thereby affecting product quality and storage stability [1, 2]. Although antioxidants have been used to control protein oxidation, much of the effort is limited to empirical trials due to the poor understanding of the chemical pathway and mode of action of oxidants and the protein environmental factors that affect the efficacy of the applied antioxidants. For example, the presence of salt (NaCl) had a minor catalytic effect on lipid oxidation but a major influence on the oxidative stability of myosin in marinated fresh pork or salted myofibrillar protein concentrate [3, 4]. To explain this discrepancy, we propose that the environment to which myosin is exposed is critical for its vulnerability to oxygen radical attack. To test the hypothesis as the objective, we subjected intact muscle tissue, myofibrils, actomyosin, and myosin (which represent the structure hierarchy) to hydroxyl radical-generating oxidants and analyzed resulting structural and functionality changes of whole, mixed, and individual myofibrillar proteins.

II. MATERIALS AND METHODS

Fresh longissimus muscle from 24-h postmortem pork carcasses was utilized except for the myosin experiment that entailed prerigor muscle. Myofibrils, actomyosin, and myosin were prepared as described by Liu *et al*. [4] and Margossian and Lowey [5]. Muscle tissue and isolated protein samples were incubated in a pH

6.2 buffer containing 0.6 M NaCl, 0-5 mM H_2O_2, 100 μM ascorbic acid, and 10 μM $FeCl_3$ either in the absence or the presence of 15 mM pyrophosphate (PP) and 2 mM $MgCl_2$ at 4°C for 12 h. This oxidizing system is known to produce hydroxyl radicals (•OH) expected to encounter in processed meats [3]. Protein carbonyls, aggregation (SDS-PAGE), meat hydration, water-holding, rheology, and gelation of the samples were tested using the appropriate procedures [2-4]. The key steps are indicated in the specific figure captions. Data were subjected the analysis of variance, and significant differences ($P < 0.05$) between means were identified using the LSD test.

III. RESULTS AND DISCUSSION

Muscle and myofibrils. One of the major consequences of protein oxidation is the accumulation of carbonyl derivatives formed from damaged amino acid side chain groups and cleavages of peptide bonds [1,2]. As indicated in Table 1, when subjected to the same •OH attack, only a small amount of protein carbonyls was generated in muscle tissue; in contrast, the amount was abundant in oxidized myofibrils and even in the control (non-oxidized). The result can be explained because the extracellular matrix and cell membrane serve as a primary defense, but their removal exposed cellular proteins to the oxidants. The oxidation apparently commenced during the myofibril preparation when muscle tissue was homogenized and mixed with molecular oxygen.

Table 1 Protein carbonyl formation in oxidatively stressed muscle vs. in isolated myofibrils. *Difference between muscle and myofibril samples ($P < 0.05$).

Sample	Treat	Control (nmol/kg)	Oxidized (nmol/kg)	Δ (nmol/kg)
Muscle	0.6 M NaCl	0.62	0.70	0.08*
	+15 mM PP	0.65	0.80	0.15*
Myofibrils	0.6 M NaCl	1.48	3.10	1.62*

Oxidation slightly promoted meat hydration in salt solution ($P < 0.05$), which was attributed to the •OH-induced canals between muscle cells [4], but caused no measurable change in cooking loss (Table 2). The enlarged extracellular spaces (canals) in oxidatively stressed muscle can also be seen in Figure 1. The presence of PP did not reduce the cooking loss although it slightly improved hydration ($P < 0.05$). In contrast, when myofibrils were extracted during meat comminution that ruptures the cells, the oxidative effect on hydration became prominent. This was evidenced in Figure 2 where oxidized myofibrils were shown much less capable of absorbing the NaCl brine (150%) when compared with non-oxidized myofibrils (200%), substantiating that processing accentuates the adverse effect of •OH on water-holding by the myofibril matrix in meat.

Table 2 Hydration, cooking loss, and product yield of non-oxidized (control) and oxidized pork muscle samples and the influence of pyrophosphate (PP). a-c Means within the same column that lack a common letter differ significantly ($P < 0.05$).

Marination	Treat	Hydration (%)	Cooking loss (%)	Yield (%)
0.6 M NaCl,	control	19.7c	34.3a	78.7c
	oxidized	25.9b	36.2a	80.2bc
0.6 M NaCl, +15 mM PP	control	22.5b	28.4b	86.8a
	oxidized	34.3a	37.4a	84.1ab

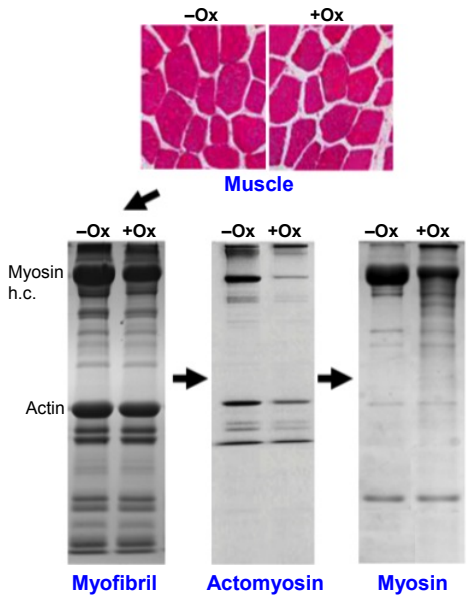

Figure 1. Structure of control (-Ox) vs. oxidized (+Ox) muscle and oxidation-induced disappearance

(aggregation) of myosin in myofibrils, actomyosin, and free myosin solutions.

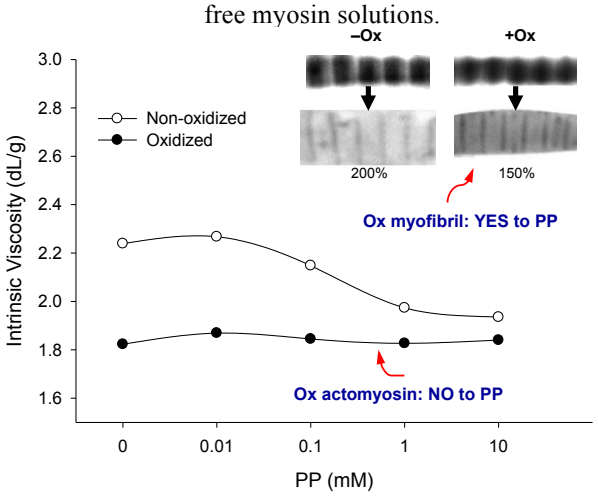

Figure 2. Dissociation (intrinsic viscosity) of actomyosin by PP in control and oxidized samples. Inset: degree (%) of swelling of myofibrils irrigated with 0.6 M NaCl and 15 mM PP, pH 6.2.

Actomyosin and myosin. Although processes that dismantle muscle fibers render myofibrillar proteins vulnerable to radical attack, the association of myofilaments in the integral myofibril bundles still serves to protect the constituents inside myofibrils (myosin, actin, actomyosin, titin, etc.). Therefore, proteins that comprise the surface areas of myofibrils would be more readily oxidized than proteins existing in the interior until the entire myofibril architecture is disintegrated. In support of this hypothesis, the exposure to •OH resulted in a total desensitization of isolated actomyosin to PP, suggesting that PPase, located in the globular head of the myosin moiety, did not bind to PP to initiate actomyosin dissociation (i.e., decline in intrinsic viscosity due to the dissociation) (Fig. 2). The complete loss of actomyosin's response to a range of PP concentrations was indicative of extensive oxidative damage to myosin within the actomyosin complex. Indeed, SDS-PSGE clearly depicted substantial oxidative losses of myosin due to disulfide-linked polymerization and aggregation, similar to that in myosin. However, such covalent aggregation and loss of myosin were much less obvious for myofibrils (Fig. 1).

The evaluation of myosin susceptibility to •OH and consequent functionality variation was carried out through rheological testing of the samples during thermal gelation (Fig. 3). In the absence of PP, oxidation lowered the gelling capacity (storage modulus, G′) by almost 20%, comparing to less than 10% if PP was present in the myosin solution during the oxidation process. The result indicated that binding of PP to myosin protected it from extensive structural damage that usually leads to a reduced gelling potential, as shown in Figure 3. The overall gelation-suppression effect of PP, irrespective of oxidation, has been well documented in the literature; it is attributed to enhanced charge repulsions between myosin that interferes with the protein network formation [6].

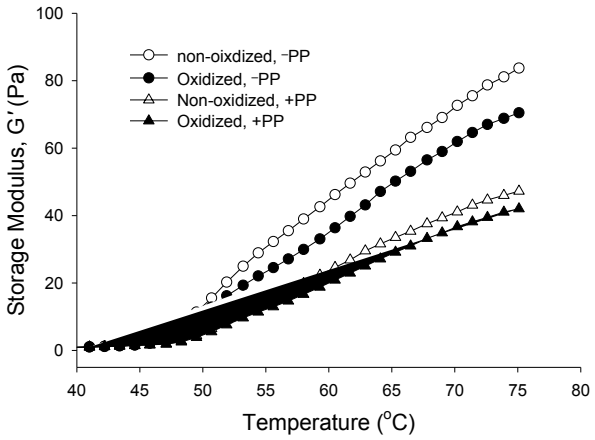

Figure 3. Rheological (shear storage modulus, G′) changes during heat-induced gel formation of control and oxidized myosin (10 mg/mL in 0.6 M NaCl, pH 6.2). For +PP (pyrophosphate), PP (15 mM) was present in the myosin samples during oxidation.

Mixed myofibrillar proteins. Interestingly, upon the oxidative stress by •OH produced with a series of H_2O_2 concentrations, mixed myofibrillar proteins exhibited much more complex rheological changes. In general, mild oxidative attack (with H_2O_2 up to 5 mM) promoted gelation (Figure 4); at higher H_2O_2 concentrations, the G′ began to decline (result not shown). The gel-enhancing effect by low concentrations of H_2O_2 was attributed to the formation of gelation-conducive soluble protein aggregates cross-linked by disulfide and carbonyl-amine bonds. Because similar results were not noted for the gelation of free myosin, which was more oxidizable (Figure 1), the result suggested that the complex myofibril structure may resist the radical penetration, allowing only the surface to be modified. When such changes are limited, which seem to be common in processed meat systems, gelation and

possibly other protein aggregation-based functional properties as well would be promoted.

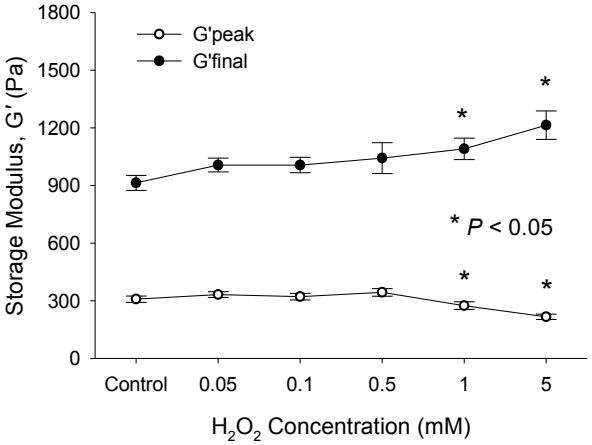

Figure 4. Rheological changes during heat-induced gel formation of control and mildly oxidized myofibrillar proteins (30 mg/mL in 0.6 M NaCl, pH 6.2). The values plotted are peak (~50°C) and final (75°C) shear storage modulus (G′) from the original rheogram.

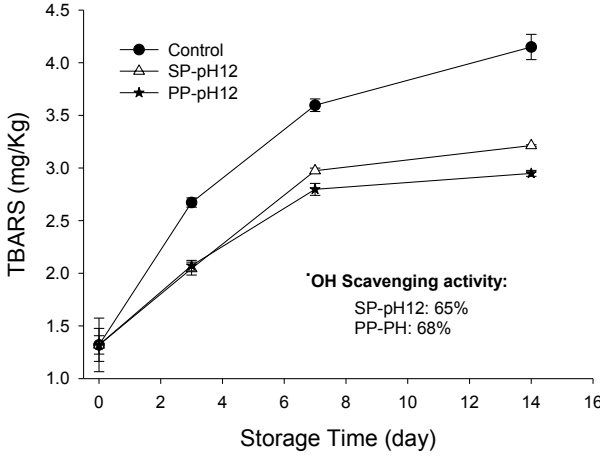

Figure 5. Inhibition of lipid oxidation (TBARS) in cooked pork sausage stored at 4°C by the incorporation of 0.75% radical-scavenging soy (SP) and pea (PP) proteins prepared with an alkaline pH-shifting process.

IV. CONCLUSION

Protein oxidation occurring in muscle food processing is a complex chemical process that remains to be poorly defined. However, results from the present study demonstrate that, with each further step in the processing operation, proteins in meat become increasingly susceptible to oxidants and their functional properties can alter substantially. The whole oxidation process, which is intimately related to the disruption of the muscle structure hierarchy, is intensified by salt and phosphates as processing additives. In order to gain a further insight into the functionality improvement by mild oxidation and avoid unwanted oxidative changes, different antioxidant strategies to control the level of protein oxidation for optimum functionality and product quality are warranted. One of the successes achieved thus far is the application of water-miscible antioxidative peptides and structurally modified plant proteins (e.g., by means of pH-shifting treatments) that can act both as radical scavengers and as physical barriers to limit muscle lipid as well as protein oxidation [7]. This innovative approach, which is most effective for comminuted meats (Figure 5), seems to be promising and may lead to novel ingredient technologies applicable to the food industry.

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