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CHARACTERIZATION OF THE OXIDATIVE DAMAGE AND ANTIOXIDATIVE PROTECTION OF MYOFIBRILLAR PROTEINS

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INTRODUCTION

Texture and palatability of comminuted and restructured meat products depend on the functionality of protein or physical properties of protein matrix formed during thermal processing. Recent studies have suggested lipid and prooxidants naturally present in meat could critically alter the myofibril gelation process and physical characteristics of resultant gels (Smith, 1987).

It is well documented free radicals generated from oxidized lipids can damage the protein structure and certain amino acids, and change the chemical and biochemical properties of proteins (Levine *et al.*, 1990; Kanner and Karel, 1976). Site-specific protein oxidation can also be caused by metal ions in the absence of lipids (Meucci *et al.*, 1991). During meat processing, where muscle cells are disrupted by comminution, lipid oxidation can readily occur due to the availability of heme protein, iron and active oxygen species. Thus, myofibrillar protein can become highly susceptible to oxidative damage, resulting in changes in physical attributes of processed meat as observed by Smith (1987). Therefore, it is important to elucidate the mechanism of oxidative deterioration in muscle protein. An improved understanding of the oxidative reactions would aid in designing processing procedures for regulating and controlling the functional properties of proteins. Research in this area is also of significance for production of surimi from under-utilized animal by-products, where minced muscle tissue (protein) is fully exposed to various oxidizing agents during the washing and dewatering process.

The objective of this study was to assess changes in conformation and gelling properties of myofibrillar protein exposed to oxidizing agents, and to develop and evaluate certain antioxidative procedures for protecting the protein functionality.

MATERIALS AND METHODS

Myofibrillar protein preparation and oxidation

Protein preparation

Crude myofibrils were prepared from bovine cardiac muscle (28 h postmortem) by washing four times in 4 vol (v/w) of 25mM sodium phosphate buffer (pH7.0) in the absence of antioxidants (-AO or control wash) or in the presence of 0.02% propyl gallate, 0.2% sodium ascorbate and 0.2% sodium tripolyphosphate (+AO wash) as described elsewhere (Xiong *et al.*, 1993). Highly purified myofibrils were prepared from turkey or broiler chicken breast muscle (36 to 48 hours post-mortem) (Decker *et al.*, 1993).

Protein oxidation

Purified skeletal myofibrils (6-20mg/mL) were suspended in 25mM phosphate buffer (pH6.0) containing 0.6M NaCl and incubated six hours in the presence of various oxidizing agents including 0 to 25mM ascorbate, 25M $FeCl_3$, 25M $CuCl_2$ and/or 20mM linoleic acid. Controls contained protein and buffer only. After dialysis in the same buffer to remove antioxidants and prooxidants, the protein samples were immediately used for chemical and physical analyses

and for gel formation.

TBARS analysis

Formation of thiobarbituric acid-reactive substances (TBARS) in fresh myofibril pellets (70mg/mL protein) and pellets during storage (0°C) was monitored using a sensitive colorimetric test developed by Witte *et al.* (1970).

Protein structure and composition

Protein conformational changes were determined by subjecting the protein solution to wavelength scan (250 to 310nm) as described by Decker *et al.* (1993). Carbonyl compounds was determined by incubating protein with dinitrophenylhydrazine (DNPH) reagent for 30 minutes according to the procedure of Levine *et al.* (1990). Changes in the ϵ-amino content resulting from lysyl destruction was followed by the trinitrobenzenesulfonic acid (TNBS) test (Bubnis and Ofner, 1992).

Gelation and evaluation of gel properties

Gelation and gel breaking strength

Myofibril pellets were suspended (20, 50 and 100mg/mL) in 50mM phosphate buffer (pH6.0) containing 0.6M NaCl and heated from 20°C to a final temperature of 70°C at 0.75°C/min as described elsewhere (Xiong *et al.*, 1993). A back-extrusion test (Xiong *et al.*, 1993) in a Model 1122 Instron (Instron Corp., Canton, MA) was used to determine gel breaking strength (penetration strength) which was defined as the force required to disrupt the gels (first peak). Alternatively, gels were compressed between two parallel plates and yield stress was calculated from force at gel structure failure divided by the gel cross-section area. Water holding capacity of gels were determined by the centrifuging method (Decker *et al.*, 1993).

Dynamic gel viscoelasticity

Dynamic viscoelastic measurement was performed by heating myofibril suspensions (20mg/mL in 50mM phosphate, 0.6M NaCl, pH6.0) from 20 to 70°C at 1°C/min between two parallel plates and sheared at a maximal strain of 0.02 with a frequency of 0.1Hz using a Model VOR Bohlin rheometer (Bohlin Instruments, Inc., Cranbury, NJ) (Xiong, 1993). The dynamic storage modulus (G', a measure of elastic attribute) and loss modulus (G", a measure of viscous attribute) were continuously recorded. Non-heated myofibril suspensions (10mg/mL) was also sheared ($73s^{-1}$) in a bob-and-cup device to determine the shear stress of the oxidized and non-oxidized proteins (Wan *et al.*, 1993).

Meat binding test

Myofibrillar protein sols (20, 50mg protein/mL sol) were applied at the junction of meat pieces and heated to form a cohesive bind. Tensile (pull) test was performed to determine the binding strength as outlined elsewhere (Xiong *et al.*, 1993).

RESULTS AND DISCUSSION

Myofibrillar protein exposed to oxidizing agents (iron, copper and linoleic acid, in the presence of ascorbate) exhibited increased absorption within the 250-290nm wavelength range showing an absorption maximum at 265nm. Absorbance of oxidized protein increased with increasing ascorbate concentration up to 25mM (Figure 1). Meucci *et al.* (1991) also reported enhanced absorption of human serum albumin oxidized by 100mM ascorbate and trace minerals, and they postulated the increase resulted from the disappearance of ordered protein structure. Carbonyl content of both iron- and copper-oxidized protein increased substantially when the ascorbate concentration increased from 0 to 5mM, suggesting possible deamination due to oxidative reactions (Fig. 1). To verify this possibility, the ϵ-amine groups of oxidized

protein were analyzed, which showed 16 and 24% reductions in ϵ-amine content for linoleic- and iron-oxidized protein, respectively (data not shown). These results support the observations made Levine *et al.* (1990) who demonstrated that metal-catalyzed formation of active oxygen species (e.g., hydroxyl radicals) led to the conversion of some amino acid residues to carbonyl derivatives. The oxidation in these oxidative systems was probably due to ascorbate-driven redox cycling of the metal ions known to promote lipid oxidation in muscle tissues. It is possible free radicals generated via lipid oxidation were involved in the oxidation of myofibrillar protein since the myofibril samples contained a residual amount of lipid (≈0.5%).

Oxidation-induced changes in protein functionality is illustrated in Figure 2. Oxidized protein (by 25μM iron or copper in the presence of 10mM ascorbate) decreased solubility by 32 to 36%. Oxidation reactions catalyzed by both metals severely impaired the gel-forming ability of the myofibrils as seen from the weakening of gels from 0.76 to 0.08 and 0.05N in the presence of copper and iron respectively. The decreased gelling ability of the oxidized myofibrils was further manifested by 10 and 23% reductions in the water holding capacity of gels oxidized by copper and iron respectively. The decreased protein functionality apparently resulted from oxidative changes in protein structure and composition, which may explain why texture and palatability of many processed meat products often vary from batch to batch.

Much attention has been given in recent years to the utilization of low-value edible meat by-products. For instance, a washing process has been suggested for making surimi-like protein concentrates from beef heart muscle. However, so far, little progress has been made in this area because beef heart surimi readily develops off-flavour and rancidity after the washing process is completed (Kenney *et al.*, 1992). Beef cardiac muscle contains a high proportion of polyunsaturated fat (e.g., arachidonic acid) and high concentration of hemoprotein and iron, compared with skeletal muscle. This oxidizing system is potent for generating free radicals to damage protein structure during the myofibril isolation or washing process. Thus, control of lipid oxidation thereby protecting myofibril functionality during washing of minced muscle seems to be critical for the product success. As shown in Table 1, fresh +AO beef heart myofibril pellet produced less TBARS than -AO myofibril pellet after washing, indicating inclusion of antioxidants in protein isolation buffer inhibited lipid oxidation. The TBARS of -AO sample increased from 0.225 to 0.571μg/g pellet during storage up to eight days, compared to only a 0.063μg increase/g pellet for the +AO sample during the same storage period. The unheated suspension of +AO myofibrils generally exhibited greater shear stress values than that of -AO myofibrils, suggesting a better hydration and solvation property of the +AO protein preparation. During the thermal transition of sol to gel, the +AO myofibrillar protein produced a more viscoelastic gel network than the -AO counterpart, as manifested by the greater G' and G" values. It is possible that +AO washed myofibrillar protein possessed a structure conducive to protein network formation upon heating. The poorer viscoelastic properties of the -AO protein could result from possible conformational changes or destruction of some functional -amine groups as evidenced in Figure 1.

The cardiac myofibrils formed gels over a wide pH range; however, the maximal gel strength was found around pH6.0 (Figure 3). The +AO washed myofibrils formed stronger gels than the control myofibrils (-AO) at pH>5.8 but not at pH<5.7, suggesting possible modification of ionic interactions due to oxidation. One potential use of beef heart surimi-like products is in comminuted and restructured muscle foods in which the surimi serves as a meat and water binding agent and possibly as fat substitute. Tensile stress (at failure) of +AO myofibril gels was greater than that of -AO myofibril gels, suggesting a greater binding ability of the +AO myofibril gels (Table 2). Increasing the protein concentration of gels from 2% to 5% resulted in a ten-fold enhancement in binding strength, which can be due to increased density of the gel networks at higher protein concentration as demonstrated from the gel compression test (Table 2).

CONCLUSIONS

This study clearly shows the reduced protein functionality in processed meats, including the manufacture of surimi-like myofibril concentrate, can be related to changes in the chemical and physical properties of oxidized proteins. However, oxidative changes in protein can be curtailed when proper antioxidative procedures are employed. Therefore, to maximize the functionality of muscle proteins for achieving desirable product texture and consistency and to enhance

the utilization and economic value of animal by-products, it is important to design processing procedures and formulations that would inhibit oxidative reactions.

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Table 1. Changes in thiobarbituric acid-reactive substances (TBARS) and gelation properties of beef cardiac myofibrillar pellets during storage at 2°C. (Adapted from Wan *et al.*, 1993).

Attributes	Treatments	Days of storage 0	2	5	8
TBARS (ug/g)	-AO	0.225	0.485	0.461	0.571
	+AO	0.059	0.069	0.090	0.122
Shear stress (cPa)	-AO	51.1	40.7	24.4	37.6
	+AO	67.7	68.3	52.6	28.3
G' (Pa)	-AO	60.2	53.4	42.9	41.1
	+AO	91.7	98.7	54.9	45.7
G" (Pa)	-AO	11.6	10.8	8.7	9.0
	+AO	19.0	17.2	9.8	9.0
Gel penetration strength (N)	-AO	0.357	0.297	0.289	0.236
	+AO	0.495	0.352	0.324	0.314

Symbols G' and G" are, respectively, shear storage modulus and loss modulus at the peaks (around 56-57°C) during gel network formation.
Symbols +AO and -AO represent, respectively, myofibrils prepared in the presence or absence of antioxidants.

Table 2. Effect of protein concentration on yield stress and meat binding strength (tensile stress) of gels made from beef cardiac myofibrillar protein isolated in the presence (+AO) and absence (-AO) of antioxidants. (Adapted from Xiong *et al.*, 1993).

Gel property	Treatment	Protein concentration (mg/mL) 2	5	10
Yield stress (Pa)	-AO	201	2804	21075
	+AO	1215	4626	39206
Tensile stress (Pa)	-AO	146	1565	--
	+AO	225	2589	--