Inhibition of Oxidation during Washing Improves the Functionality of Bovine Cardiac Myofibrillar Protein†

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The effect of antioxidative treatments during bovine cardiac myofibril isolation on rheological and gelling properties of the myofibrillar protein was examined. Minced beef heart muscle was washed four times in 25 mM phosphate buffer (pH 7.0) without (control wash) or with combined antioxidants (0.02% propyl gallate, 0.2% sodium ascorbate, and 0.2% sodium tripolyphosphate). Washing with antioxidants inhibited oxidation in fresh myofibrillar pellets and in pellets stored for up to 8 days. The antioxidant treatment increased the shear stress and viscosity of protein suspensions by 20–100%. Both control and antioxidant-washed myofibrillar proteins exhibited complex dynamic viscoelastic characteristics during thermal gelation, but the antioxidant-washed protein showed greater storage and loss moduli than the control within 50–60 °C. An extrusion test indicated that the antioxidant-treated, freshly isolated protein formed stronger gels than the control. Thus, the functionality of myofibrillar protein can be protected by the inclusion of antioxidants in the muscle washing process.

INTRODUCTION

Beef heart, a byproduct of the meat-packing industry, is an excellent source of nutrients. However, use of beef heart as a human food is very limited because of its low protein functionality and less desirable flavor, compared with skeletal muscle. Nevertheless, recent studies have suggested that this animal byproduct could be used in meat restructuring to produce value-added muscle foods (Kenney et al., 1992).

Success in utilization of beef heart byproduct largely depends on the muscle protein functionality. Eisele and Brekke (1981) used chemical modification with acid anhydrides to improve the functionality of isolated beef heart myofibrils. Smith and Brekke (1984) found that modification of beef heart with ficin was effective in improving protein solubility and emulsification capacity. More recently, McKeith et al. (1988) and Kenney et al. (1992), using a surimi-like washing process to remove sarcoplasmic constituents and fat, produced myofibrillar protein concentrates from beef cardiac muscle which possessed better functional properties than the raw meat. The improved protein functionality would allow the myofibril-concentrated pellet to be used as a functional ingredient in processed meat products. The surimi processing technique has also been used in mechanically deboned poultry meat to improve its textural properties (Dawson et al., 1989). Yang and Froning (1992) observed a fibrous protein network structure resulting from gelation of washed mechanically deboned chicken meat.

Although addition of beef heart surimi improved the color in precooked restructured beef, its use noticeably increased lipid oxidation and associated off-flavor in cooked products (Kenney et al., 1992). The susceptibility of beef heart surimi to oxidation was probably related to the high proportion of polyunsaturated fatty acids (e.g., arachidonic acid) and the presence of heme protein and iron in the cardiac muscle (USDA, 1990). Conceivably, during surimi manufacturing, in which oxygen is mixed with lipids and different prooxidants, lipid radicals (e.g., the hydroxyl radical) and active oxygen species can be generated, resulting in possible damage to the myofibrillar protein. Smith (1987) reported decreased myofibrillar protein functionality in mechanically deboned chicken caused by radicals produced from oxidized lipids. Decker et al. (1992), using a purified myofibril system, demonstrated that iron was an important catalyst for the oxidative damage to protein structure and the resulting deterioration in physicochemical and gelation properties. Recently, Kelleher et al. (1992) showed that oxidation and associated off-flavor in washed minced Atlantic mackerel could be inhibited by incorporating antioxidants in the washing process. Such an antioxidative treatment appeared to have great potential for producing surimi products with improved sensory characteristics and palatability.

The objective of this study was to determine the effect of combined antioxidants used in muscle washing and myofibril isolation on the solubility, rheological behavior, and gelation properties of the isolated bovine cardiac myofibrillar protein.

MATERIALS AND METHODS

Muscle Source. Fresh beef hearts (24–28 h post-mortem) were obtained from a local packing company. After individual vacuum packaging in polyethylene bags, the hearts were frozen and stored at −29 °C. All heart samples were used within 3 months.

Preparation of Myofibrillar Protein. All experiments were replicated three or four times (unless otherwise specified) using myofibril samples freshly prepared on different days. Mean values from different experiments are reported. Beef hearts were thawed at 2 °C for 18 h. Unless otherwise specified, meat chopping and washing described below were conducted in a 10 °C meat-processing room in the University of Kentucky Meat Laboratory. After removal of caps, vessels, and fat tissue, the lean muscle was diced into approximately 2-cm cubes and subsequently chopped without a vacuum for 60 s in a food processor (CountCraft, Sears & Roebuck Co., Chicago, IL). The minced muscle was washed four times in 4 volumes (v/w) of specific buffers by blending for 30 s (high speed) in a Waring blender and then centrifuged at 2000*g* for 15 min at 2 °C. Control washing was conducted in 25 mM phosphate (Na_2HPO_4/NaH_2PO_4) buffer (pH 7.0) only, and treatment washing was done using

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the same buffer that also contained combined antioxidants (AO): 0.02% propyl gallate, 0.2% ascorbate, and 0.2% sodium tripolyphosphates (STP) (first wash); 0.2% ascorbate and 0.2% STP (second and third washes); and 0.2% ascorbate only (fourth wash). In the fourth wash, the myofibril suspension was adjusted to pH 6.0 using 0.1 N HCl prior to centrifugation. Myofibrillar pellets were stored in ice for specific periods (0, 1, 2, 3, 5, and 8 days) to monitor oxidative reactions and changes in protein functionality. Protein concentration was measured by the biuret method (Torten and Whitaker, 1963) with bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as the standard.

Lipid Oxidation. Thiobarbituric acid-reactive substances (TBARS) produced from lipid oxidation in myofibril isolates were determined using the extraction method described by Witte et al. (1970).

Protein Solubility. Myofibrillar pellets were diluted to 5 mg/mL in 50 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.0), and the protein solubility was determined as previously described (Xiong, 1992).

Viscosity. Samples used for viscometric measurement were diluted to 10 mg/mL with 50 mM phosphate buffer (pH 6.0) containing 0.6 M NaCl. Shear stress and apparent viscosity were determined as described by Robe and Xiong (1993) using a Model VOR Bohlin rheometer (Bohlin Instruments, Inc., Cranbury, NJ).

Dynamic Rheological Measurements. Samples used for gelation studies were prepared by diluting myofibrillar pellets to 20 mg/mL with 50 mM phosphate containing 0.6 M NaCl (pH 6.0). A Model VOR Bohlin rheometer equipped with parallel plates (upper plate diameter 3.0 cm) was used for dynamic rheological measurements during protein gelation as outlined elsewhere (Xiong, 1993). Protein gels were formed by heating the myofibril suspensions from 20 to 65 °C at 1 °C/min, and the gelling samples were sheared at a fixed frequency (0.1 Hz) with a maximum strain of 0.02.

Gel Extrusion Test. Gels for the back extrusion test were prepared by heating 5-g (about 5-mL) myofibril suspensions (20 mg/mL, in 50 mM phosphate, 0.6 M NaCl, pH 6.0) in glass vials (15.5-mm diameter; 8-mm length) from 20 to 65 °C at 0.75 °C/min in a water bath. After setting at 2 °C overnight, gels were equilibrated at 21 °C for 45 min and subsequently extruded using a 12.5-mm probe attached to a Model 1120 Instron (Instron Corp., Canton, MA) (Xiong, 1992). The crosshead speed was set at 20 mm/min. The force required to rupture the gels (first peak) was used to represent the gel strength.

Statistical Analysis. Data were analyzed using the general linear models procedure of the Statistix 3.5 software package (Analytical Software, Inc., St. Paul, MN) for microcomputers. Analysis of variance (AOV) was conducted to determine the significance of the main effects (antioxidant treatment; storage time). Significant ($P < 0.05$) differences between means were identified using the least significant difference procedure (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Oxidation. Isolation of myofibrillar protein in the absence of antioxidants (−AO) resulted in the formation of TBARS during the washing procedure; for each gram of myofibrillar pellet, approximately 0.22 μg of TBARS was generated (Figure 1). However, in the presence of antioxidants (+AO), the TBARS concentration decreased by 74% ($P < 0.05$), indicating that inclusion of the combined antioxidants in the washing solution effectively inhibited lipid oxidation. Kelleher et al. (1992), employing a similar antioxidative system, also observed decreased lipid oxidation and associated off-flavor in surimi prepared from mackerel. Storage in ice slurries for up to 8 days generated very little TBARS in the +AO myofibrillar pellet; however, the TBARS concentration increased by 1.5-fold ($P < 0.05$) in the −AO sample. TBARS were generated most rapidly within the first 2 days and changed very little thereafter. Since bovine cardiac muscle contains a high proportion of polyunsaturated fatty acids (e.g., 10.4% of total lipid is from arachidonic acid) (USDA, 1990)

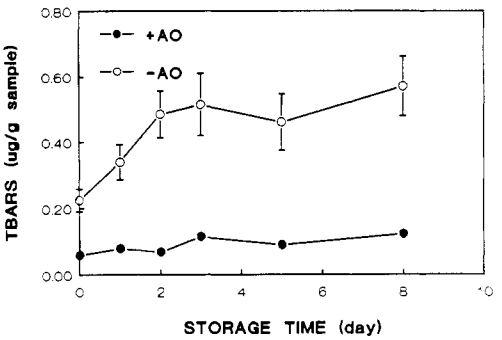

Figure 1. Effect of storage (0 °C) time on TBARS concentration of bovine cardiac myofibrillar protein prepared in the presence (+AO) or absence (−AO) of antioxidants (0.02% propyl gallate, 0.2% ascorbate, 0.2% polyphosphate).

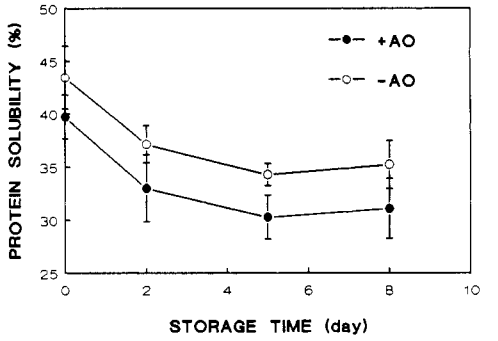

Figure 2. Changes in protein solubility of bovine cardiac myofibrils during storage (0 °C). Myofibrils were prepared in the presence (+AO) or absence (−AO) of antioxidants (0.02% propyl gallate, 0.2% ascorbate, 0.2% polyphosphate).

and high concentrations of heme protein and iron, the inhibited lipid oxidation in the +AO-washed myofibrils probably resulted from a combined effect of free radical scavenging (by PG), metal chelation (polyphosphate), and donation of electrons (ascorbate) (Decker and Hultin, 1992; Shahidi et al., 1992).

Solubility. Several previous studies showed that proteins exposed to low redox potential or an oxidizing environment were susceptible to oxidative damage, resulting in decreases in enzyme activity, protein denaturation, and a loss in functionality in model systems (Meucci et al., 1991; Decker et al., 1993). Smith (1987) reported that myofibrillar protein isolated from oxidized mechanically deboned chicken meat had decreased solubility and gelling ability. Solubility of the −AO myofibrils in the present study decreased ($P < 0.05$) from 43% at day 0 to 37% at day 2 but exhibited no significant change after 2 days (Figure 2). Inclusion of antioxidants in the washing buffer did not improve the protein solubility, which showed values similar to those of the control throughout the storage period. Decker et al. (1993) observed formation of nonsedimentable protein polymers in oxidized turkey breast myofibrillar protein. Thus, it is possible that the centrifugation method used for solubility measurement in the present study was not sensitive enough to detect differences between the −AO and +AO samples.

Viscosity. Dilute suspensions of both oxidized and nonoxidized myofibrillar pellets exhibited rheological characteristics somewhat similar to those of pseudoplastic flow. However, a linearity was attained at >20 s^{-1} shear rates (Figure 3). The oxidized protein notably had smaller shear stress values than the +AO protein at high shear rates, and when viscosity was calculated, differences between the two treatments became evident (Figure 3). It should be pointed out, however, that the viscosity values

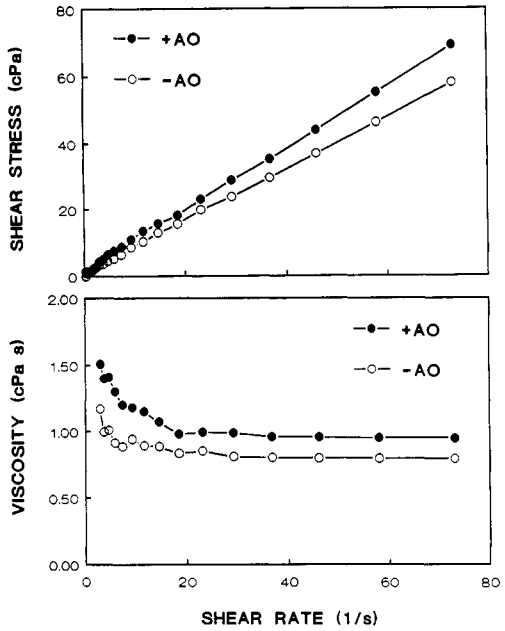

Figure 3. Rheological characteristics of bovine cardiac myofibril suspensions (10 mg/mL protein in 0.6 M NaCl, 50 mM phosphate buffer, pH 6.0). Myofibrils were isolated in the presence (+AO) or absence (-AO) of antioxidants (0.02% propyl gallate, 0.2% ascorbate, 0.2% polyphosphate).

were derived by assuming the viscosity index equal to 1 (Newtonian flow), and hence, some deviations would be expected.

Protein changes due to oxidation and shearing can affect the viscosity in different ways. Decker et al. (1993) showed that iron and copper ions in the presence of low concentrations of ascorbate oxidized myofibrillar protein as indicated by the increased carbonyl content and formation of the disulfide bonds. The oxidation resulted in alterations in protein conformational structure. A partial denaturation of protein would decrease the fluid properties of protein, including interaction with the solvent. However, it can also increase the dynamic volume or bulkiness of the protein, thereby augmenting the viscosity. The overall viscosity increase for the +AO myofibrils suggests that the "thickening" factors prevailed, resulting from antioxidative protection. The "shear-thinning" effect, as manifested by decreases in viscosity with increasing shear rates, can be attributed in part to molecular rearrangement or realignment or dissociation of loose noncovalently bound protein aggregates. Shear stress (at 73 s^{-1}) of the +AO myofibril suspensions did not change during the first 2 days of storage, but it declined steadily afterward; shear stress of the -AO sample generally decreased during storage (Figure 4). Similar shear stress values were shown on day 8 for +AO and -AO proteins, suggesting that differences in hydration properties between the two samples diminished over the extended storage time.

Gelation. Both control and +AO-washed myofibril suspensions formed gels upon heating to above 45 °C, exhibiting a peak during the sol → gel transformation with maximum G' values at 56–57 °C (Figure 5). The sharp decline in G' (an elastic element of the gel or semigel) from 57 to 60 °C was frequently observed on myofibrillar protein of skeletal muscles (Egelandsdal et al., 1986; Xiong, 1993), and this has been suggested to involve rearrangement of cross-linkages between polypeptides (Egelandsdal, 1986) and kinetic constraints for gel network formation within this temperature range (Wu et al., 1991). The G'' peaked at lower temperature (about 55 °C), suggesting

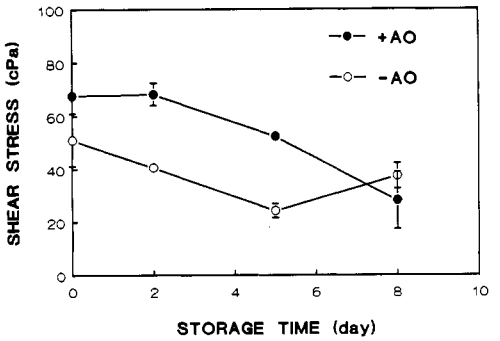

Figure 4. Effect of storage (0 °C) time on shear stress (at 73 s^{-1}) of bovine cardiac myofibril suspensions (10 mg/mL protein in 0.6 M NaCl, 50 mM phosphate, pH 6.0). Myofibrils were isolated in the presence (+AO) or absence (-AO) of antioxidants (0.02% propyl gallate, 0.2% ascorbate, 0.2% polyphosphate).

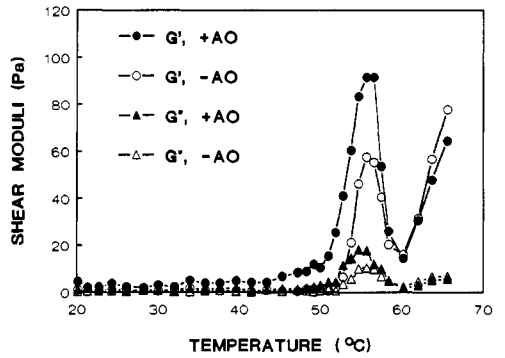

Figure 5. Typical viscoelastic curves showing changes in the shear storage (G') and loss (G'') moduli of myofibril preparations during sol → gel thermal transformation. Myofibrils were isolated in the presence (+AO) or absence (-AO) of antioxidants (0.02% propyl gallate, 0.2% ascorbate, 0.2% polyphosphate) and were suspended (20 mg/mL protein) in a 50 mM phosphate buffer (pH 6.0) containing 0.6 M NaCl for gelation.

Table I. Effect of Storage (2 °C) on Shear Storage Modulus (G') and Loss Modulus (G'') of Myofibrils during Thermal Gelation[a]

viscoelastic parameter[b]	treatment[c]	Pa			
		0 days[d]	2 days	5 days	8 days
peak G'	-AO	**60.2** ± 4.1	**53.4** ± 1.7	**42.9** ± 0	41.1 ± 11.0
	+AO	**91.7** ± 10.0	**98.7** ± 1.7	**54.9** ± 0.7	45.7 ± 7.4
final G'	-AO	**77.8** ± 8.4	78.7 ± 9.4	**76.6** ± 3.8	59.1 ± 1.6
	+AO	**64.5** ± 0.5	75.5 ± 4.5	**57.3** ± 5.8	65.1 ± 11.7
peak G''	-AO	**11.6** ± 0.3	**10.8** ± 1.0	8.7 ± 0.8	9.0 ± 1.4
	+AO	**19.0** ± 1.4	**17.2** ± 0.7	9.8 ± 1.4	9.0 ± 3.0
final G''	-AO	6.9 ± 1.1	7.8 ± 1.3	**8.1** ± 0.2	6.7 ± 0.8
	+AO	5.7 ± 2.2	8.5 ± 1.3	**6.5** ± 1.3	7.4 ± 1.3

[a] Data are means ± standard deviations of two replicated experiments with duplicate measurements. Boldfaced pairs of means (within the same storage time and same parameter group) differ significantly ($P < 0.05$). [b] Peak and final G' refer to those at 55–57 and 65 °C, respectively (see Figure 5). [c] Symbols +AO and -AO denote, respectively, myofibrils prepared in the presence or absence of antioxidants. [d] Days of storage.

that protein unfolding, which resulted in increased viscosity of the sol, preceded the formation of a more elastic semigel network. As summarized in Table I, G' and G'' values at the peak (55–57 °C) were generally greater ($P < 0.05$) for +AO gels than for -AO gels during the first 5 days storage. This corresponded to the flow test that showed a greater shear stress and viscosity of +AO protein than its -AO control (Figure 3). Thus, antioxidant washing appeared to facilitate gel network development during the initial gelling process.

The antioxidant treatment did not affect G' or G'' within the 58–63 °C temperature range. However, it altered the

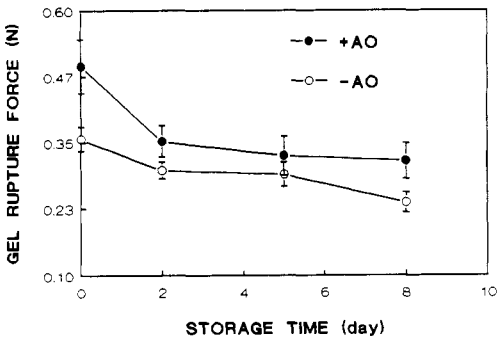

Figure 6. Changes in gel strength (rupture force) of myofibrillar protein during storage (0 °C). Myofibrils were isolated in the presence (+AO) or absence (-AO) of antioxidants (0.02% propyl gallate, 0.2% ascorbate, 0.2% polyphosphate) and were suspended (20 mg/mL protein) in a 50 mM phosphate buffer (pH 6.0) containing 0.6 M NaCl for gelation.

gel viscoelastic properties at higher temperatures. The -AO and +AO myofibrils stored for 2 or 8 days exhibited similar G' and G'' values at 65 °C, but -AO myofibrils stored for 0 and 5 days produced a more elastic gel network (greater G') than +AO myofibrils (Table I). The latter observation contrasts the rheological behavior of the protein gels within 45–57 °C, where the +AO gel displayed a more prominent elastic as well as viscous characteristic than the -AO gel (Figure 5). The difference between -AO and +AO gels at 65 °C might be temporary, and it could reflect kinetic variations in gel matrix formation. Longer heating time, e.g., incubating the gels at the final temperature (65 °C) for 1 h, would seem to be necessary to more precisely differentiate structural differences between the two gels.

Figure 6 illustrates gel rupture strength measured from the extrusion test. Gels that had been set at 2 °C overnight after heating to 65 °C should have reached an equilibrium in network formation and dissociation. The +AO-treated fresh (day 0) myofibrillar protein formed stronger ($P < 0.05$) gels than the control. Storage for up to 2 days decreased ($P < 0.05$) the +AO myofibrillar gel rupture force and diminished ($P < 0.05$) the effect of the antioxidants. Furthermore, there was no significant difference in gel strength between the +AO and -AO myofibrillar proteins stored for 5 and 8 days (Figure 6). It is interesting to note that at 65 °C gels made from 0- or 5-day -AO myofibrils were more rigid (greater G') than gels made from 0- or 5-day +AO myofibrils (Table I), but the opposite was seen for cooled gels (21 °C), as demonstrated by the Instron extrusion test. A number of factors may account for the discrepancy. The different methods—fundamental G' analysis with a nondestructive small strain *vs* empirical extrusion destructive test—employed for gel strength measurement, the different temperatures at which gels were analyzed, and the two equilibrium states could all contribute to the disparity.

Although analysis of TBARS indicated effective inhibition of lipid oxidation in the +AO sample during storage (Figure 1), the antioxidative treatment during washing did not seem to completely eliminate deterioration in protein functionality during storage. The role of the antioxidants was further complicated by the fact that, from day 0 to day 2, the control myofibril pellet (-AO) doubled its TBARS level while the TBARS content in +AO pellet remained unchanged (Figure 1), yet gel rupture force for both myofibrillar samples decreased to a similar value (about 0.32 N) from their original values, 0.49 N (for +AO) and 0.36 N (for -AO) (Figure 6). It is possible that some oxidative damage in protein structure and functional amino acid residues could have occurred at a TBARS level less than that shown in Figure 1 (i.e., 0.064 μg/g for 2-day +AO sample) or were unrelated to the TBARS formation. Decker et al. (1993) have indicated that myofibrillar proteins can be oxidized (forming the disulfide bonds) by trace minerals in the absence of lipids (no TBARS), leading to a decrease in protein functionality. Thus, addition of antioxidants to the myofibrillar isolate (pellet) would seem to be needed to thoroughly curtail the oxidative reactions in protein during storage. Alternatively, a more potent antioxidative system or a protein isolation medium with greater redox potential would be needed to fully protect the functionality of myofibrillar proteins during washing and storage.

Conclusions. The rheological and gelling properties of cardiac myofibrillar protein can be protected by inhibiting oxidative reactions occurring during the muscle washing and myofibril isolation process. Myofibrillar protein prepared under antioxidative conditions also exhibited better gel-forming ability and gel strength during storage, especially within 2 days of protein isolation. Therefore, to maximize the protein functionality during meat processing, or manufacturing of surimi-like products from beef heart and other underutilized animal byproducts, it is important to manipulate the product formulations and regulate the processing procedures to minimize oxidative reactions.

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