Contribution of Lipid and Protein Oxidation to Rheological Differences between Chicken White and Red Muscle Myofibrillar Proteins†

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Myofibrils isolated under different antioxidative conditions from three groups of chicken muscle [breast (B_{ctrl}), leg (L), and breast with its iron and fat content adjusted to the level of leg muscle (B_{adj})] exhibited complex viscoelastic characteristics during thermal gelation. Without antioxidants in myofibril isolation buffer, B_{adj} myofibrils had decreased storage moduli (G') and loss moduli (G'') compared with B_{ctrl} myofibrils, but these values were much higher than those of L myofibrils. Lipid oxidation (TBA value) was inhibited in myofibril samples prepared using antioxidant buffers. Antioxidant treatments increased G' and G'' of B_{adj} myofibrils to a level comparable to G' and G'' of the B_{ctrl} counterparts. Different distributions of prooxidants in chicken white and red myofibrillar proteins were not an apparent major factor causing functional discrepancies between the two types of proteins. The discrepancies could be ascribed largely to fiber type-dependent myosin isoforms and polymorphism.

Keywords: *Chicken muscle; fiber type; oxidation; protein gelation; rheology*

INTRODUCTION

Consumption of poultry meat has gradually increased over the past decade, promoted by consumers' health and economic consciousness. The availability of many processed poultry products in the market provides variety to consumers. However, processed poultry meats from different muscle sources often vary in processing quality. Studies have shown that poultry dark (red) and light (white) muscles differ in functional behavior during thermal processing, e.g., the superior texture and binding strength of light meat over dark meat in cooked chicken loaves (Maesso et al., 1970) and the greater swelling ability and protein extractability for turkey light meat than for turkey dark meat (Richardson and Jones, 1987). The disparity in functional properties among different muscle types has been widely attributed to myofibrillar proteins (Asghar et al., 1984; Foegeding, 1987; Morita et al., 1987; Xiong and Blanchard, 1994). Under similar meat-processing conditions, myofibrillar proteins derived from white or fast-twitch glycolytic muscle of both avian and mammalian species form gels that exhibit quite different viscoelastic and rheological characteristics when compared with gels prepared from red or slow-twitch oxidative muscle myofibrillar proteins (Xiong, 1994).

Numerous isomeric forms of myofibrillar proteins have been identified in different muscle types. Asghar et al. (1984) and Morita et al. (1987) attributed the functional discrepancies between red and white muscle to isoforms and polymorphism of myofibrillar proteins, as did Culioli et al. (1993). The magnitude of such isoform-dependent differences is clearly subject to meat-processing conditions, e.g., ionic strength or salt concentration, pH, heating rate, protein concentration, and interactions of myofibrillar proteins with other ingredients in muscle food systems. For instance, Lavelle and Foegeding (1993) found that at a 10% protein concentration, chicken red myofibrillar proteins produced more rigid gels than white myofibrillar proteins, in contrast to the general findings that under similar gelling conditions, but with much lower protein concentrations (<4%), chicken white myofibrils or salt-soluble proteins (SSP) have a greater gelling ability and form stronger gels than red myofibrils or SSP (Xiong, 1994). However, in most of the previous protein functionality studies using myofibrillar protein model systems, the potential role of oxidation, which could occur during protein isolation and purification, was completely ignored. This has left a question to be answered: was myosin isoform the only key factor causing the widely observed functionality differences between white and red myofibrillar proteins?

Compared to white muscle, red muscle contains not only a large amount of lipids but also a high concentration of heme protein and inorganic iron, both of which are catalysts for lipid and protein oxidation (Johns et al., 1989; Levine, 1984; Monahan et al., 1993) and have been shown to decrease the gel-forming ability of myofibrillar proteins (Decker et al., 1993). Isolation of myofibrillar proteins requires extensive homogenization to disrupt cell structures. The homogenization and washing processes could promote oxidative reactions in myofibril pellet by mixing oxidation catalysts with lipids and molecular oxygen, resulting in deteriorations in biochemical and functional properties of myofibrillar proteins such as those in beef heart surimi (Wan et al., 1993; Xiong et al., 1993). Therefore, differences in oxidative changes occurring during the preparation of myofibrillar proteins from different muscle types would be expected, and such changes may contribute to functional differences observed between isolated white and red myofibrillar proteins. Thus, this study was conducted to investigate rheological differences between chicken red and white muscle myofibrillar proteins as influenced by combined antioxidants in washing media,

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so as to improve our understanding of the implication of lipid and protein oxidation in these differences.

MATERIALS AND METHODS

Chemicals. Ferric chloride ($FeCl_3$) and ethylene glycol bis-(β-amino ethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) were purchased from Fisher Scientific (Springdale, NJ). Propyl gallate (PG), sodium ascorbate, sodium tripolyphosphate (TPP), and thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO). Dinitrophenylhydrazine (DNPH) was supplied by Eastman Chemical Co. (Rochester, NY). All other chemicals used in the study were of reagent or high grade.

Muscle Source and Preparation. Six-week-old commercially processed fresh broilers were obtained from a local retailer. Broilers were stored in crushed ice and used within 36−48 h after slaughter. All experiments were replicated twice. For each replicated trial, breast (pectoralis major and minor combined) and leg muscle (thigh and drumstick combined) were excised from two birds and pooled to obtain breast (B_{ctrl}) and leg (L) muscle samples, respectively. After removal of connective tissue and external fat, muscle samples were ground through a 4.5-mm orifice plate in a food grinder. The total iron contents of chicken breast and leg muscle, determined by atomic absorption spectrophotometry using the wet ashing procedure described by Schricker et al. (1982), varied slightly among birds, but averaged around 0.7 and 1.0 mg/100 g of lean tissue, respectively. These data were in agreement with the USDA (1978) values. The total fat contents of chicken breast and leg muscle, determined by fat extraction method using petroleum ether (AOAC, 1990), were 0.59 and 2.3 g/100 g of well-trimmed muscle, respectively. On the basis of these data, precalculated amounts of $FeCl_3$ and separable chicken fat obtained from the backs of the same birds were added to a portion of breast muscle sample. Thus, the iron and fat contents of the sample were adjusted to the level of leg muscle before myofibril isolation, and the adjusted sample was designated B_{adj}. The added ferric ion (Fe^{3+}) can be reduced to ferrous ion (Fe^{2+}) by reducing compounds such as ascorbic acid, NADH, and NADPH, which are commonly found in muscle tissues, or by ascorbate in antioxidant buffers used in the preparation of myofibrils. Ferrous ion is the active form of iron capable of catalyzing lipid oxidation (Kanner et al., 1986).

Preparation of Myofibrils. Myofibril isolation was performed at 2 °C essentially as described by Xiong (1993). Ground muscle was washed four times using 4 volumes (v/w) of isolation buffer (pH 7.0) which contained 0.1 M KCl, 10 mM phosphate (K_2HPO_4/KH_2PO_4), 2 mM $MgCl_2$, and 1 mM EGTA, either with or without combined antioxidants (5 mM PG, 0.2% sodium ascorbate, and 0.2% TPP). The myofibril pellet was then washed with 4 volumes of 0.1 M NaCl solution containing 1 mM NaN_3 as antimicrobial agent. The resulting pellet was suspended in 8 volumes of 0.1 M NaCl solution and was adjusted to pH 6.0 using 0.1 N HCl prior to the final centrifugation. Purified myofibrils were stored on ice and used within 24 h. Protein concentration of the myofibril pellet was measured by the biuret method (Gornall et al., 1949) using bovine serum albumin (Sigma) as standard.

Lipid Oxidation. Thiobarbituric acid-reactive substances (TBARS) produced from lipid oxidation in myofibril pellets (5% protein) were determined using the extraction method described by Witte et al. (1970). TBARS were measured immediately after myofibril isolation. Absorbances at 530 nm were read with a Milton Roy spectrophotometer. TBARS values were expressed as micrograms of malonaldehyde per gram of sample.

Carbonyls. Carbonyls of myofibrillar proteins were measured essentially according to the method described by Levine (1984). Myofibril samples (6 mg/mL protein) were incubated with DNPH reagent for 30 min at room temperature (21−23 °C). Absorbances at 387 and 400 nm were measured. Carbonyl content (nanomoles per milligram of protein) was calculated from the differential absorptivity ($Abs_{387} - Abs_{400}$) using an extinction coefficient of $6.9 \times 10^2\ M^{-1}\ cm^{-1}$.

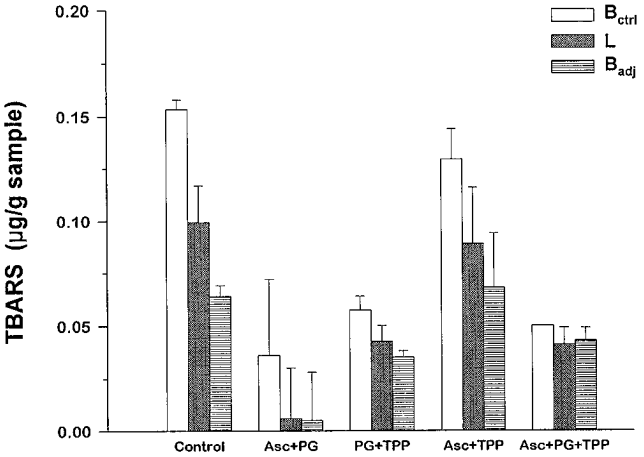

Figure 1. TBARS concentrations of myofibril samples prepared from three groups of chicken muscle (B_{ctrl}, L, and B_{adj}) in the presence or absence (control) of combined antioxidants [5 mM propyl gallate (PG), 0.2% ascorbate (Asc), and 0.2% sodium tripolyphosphate (TPP)].

Dynamic Rheological Measurements. Fresh myofibril pellets were suspended in 50 mM sodium phosphate buffer (pH 6.0) and subsequently stored at 0 °C for 12 h. The buffer contained predetermined amounts of NaCl to produce a final NaCl concentration of 0.6 M and a protein concentration of 20 mg/mL. Nondestructive, oscillatory measurements of the protein suspensions during thermal gelation were performed using a Bohlin VOR rheometer (Bohlin Instruments, Inc., Cranbury, NJ) as described by Xiong (1993). Thermal sol-to-gel transformation was induced by heating the protein suspension from 20 to 73 °C at 1 °C/min. Gelation was monitored by shearing the sample placed between two parallel plates (upper plate, 30 mm diameter) at a fixed frequency of 100 mHz with a maximum strain of 0.02. Rheological properties of the myofibrillar gelling system were described in terms of storage modulus (G', the elastic component) and loss modulus (G'', the viscous component).

Statistical Analysis. Data were analyzed using the general linear model procedure of the Statistix 3.5 software package (Statistix 3.5, Analytical Software Inc., St. Paul, MN). Analysis of variance (ANOVA) was conducted to determine the significance of the main effects (muscle type, antioxidants). Significant ($P < 0.05$) differences between means were identified using the least significant difference procedure (Snedecor and Cochran, 1989).

RESULTS

Oxidation. The extent of lipid oxidation in fresh myofibril pellets as measured by TBARS is shown in Figure 1. Isolation of myofibrils in the absence of antioxidants resulted in the formation of TBARS during preparation. For each gram of breast muscle (B_{ctrl}) myofibril pellet, approximately 0.15 μg of TBARS was generated, assuming a zero TBARS content in fresh muscle prior to myofibril preparation. No significant difference ($P > 0.05$) in TBARS content was found between B_{ctrl} and leg (L) muscle myofibril samples, whereas both had slightly higher ($P < 0.05$) TBARS values than adjusted breast muscle (B_{adj}) myofibril pellet.

Addition of combined antioxidants [propyl gallate and ascorbate, propyl gallate and tripolyphosphate, or a combination of all three] to the washing media significantly decreased ($P < 0.05$) TBARS concentrations in all myofibril samples (B_{ctrl}, B_{adj}, and L). No significant differences ($P > 0.05$) in TBARS values were observed between samples prepared with these antioxidant combinations. The antioxidant buffer containing combined ascorbate and tripolyphosphate, however, was not ef-

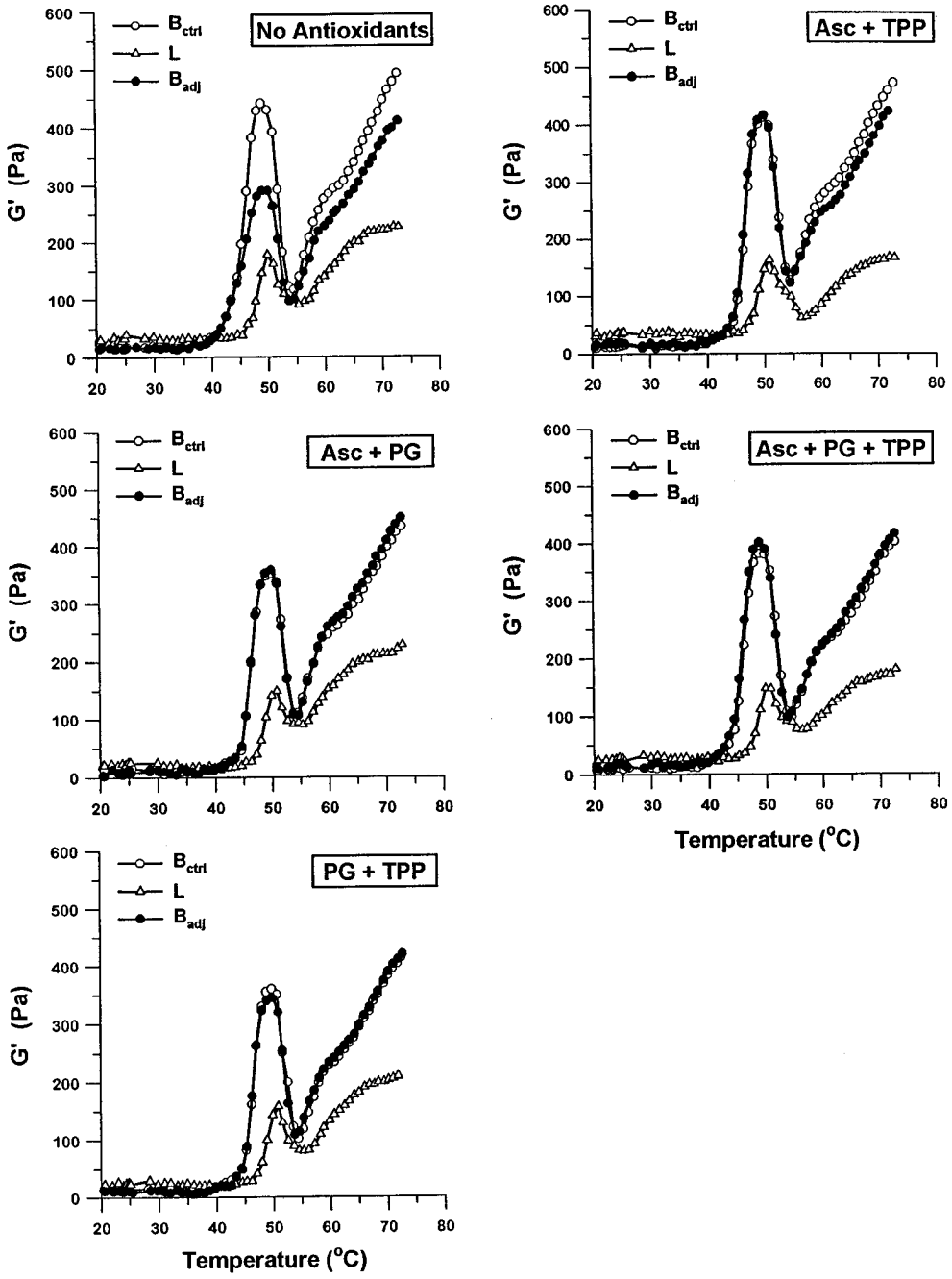

Figure 2. Changes in storage modulus (G') of chicken muscle myofibrillar suspensions during thermal gelation. Myofibrils were isolated in the presence or absence (control) of combined antioxidants [5 mM propyl gallate (PG), 0.2% ascorbate (Asc), and 0.2% sodium tripolyphosphate (TPP)] and were suspended (20 mg/mL protein) in a 50 mM phosphate buffer (pH 6.0) containing 0.6 M NaCl for gelation.

fective in inhibiting lipid oxidation during myofibril preparation. The TBARS value of B_{ctrl} myofibril pellet prepared with this antioxidant combination was essentially equal ($P > 0.05$) to that of the B_{ctrl} sample prepared without antioxidants and higher ($P < 0.05$) than that of the B_{ctrl} samples prepared in the presence of other antioxidant combinations. B_{ctrl} and L myofibril pellets prepared using antioxidant buffer containing ascorbate and tripolyphosphate had slightly higher ($P < 0.05$) TBARS values than did the B_{adj} myofibril pellet prepared under the same condition.

Protein carbonyls of myofibril samples as an indicator of the extent of protein oxidation were also determined. The results were variable and no significant differences ($P > 0.05$) in carbonyl content of myofibrillar proteins were observed among all samples which had a mean carbonyl value of 1.5 nmol/mg of protein (results not shown).

Dynamic Viscoelastic Properties. All myofibril suspensions formed gels during heating from 20 to 73 °C and exhibited similar changes in storage modulus (G') (Figure 2) and loss modulus (G'') (results not shown) despite the differences in magnitude. During the sol → gel transformation, both B_{ctrl} and B_{adj} myofibrils produced a viscoelastic peak with maximum G' values around 50 °C and maximum G'' values around 49 °C. L myofibrils produced G' and G'' peaks at slightly higher temperatures (51 and 50 °C, respectively). A sharp decline in G', after reaching a peak maximum, was observed for all myofibril samples. These viscoelastic patterns were essentially identical to those previously reported for dynamic rheological properties of chicken

Table 1. Shear Storage Modulus (G') at Peak and at the End of Heating and Loss Modulus (G'') at Peak for Myofibrillar Suspensions during Thermal Gelation[a]

	G' at peak (Pa)			final G' (Pa)			G'' at peak (Pa)		
washing medium	B_{ctrl}	L	B_{adj}	B_{ctrl}	L	B_{adj}	B_{ctrl}	L	B_{adj}
control	366.7[a]	154.0[c]	305.3[b]	472.3[a]	224.3[c]	419.0[b]	96.2[a]	42.2[c]	77.3[b]
Asc + PG	305.0[a]	131.4[b]	310.0[a]	367.0[a]	193.0[b]	404.5[a]	77.0[a]	34.8[b]	78.8[a]
PG + TPP	368.5[a]	161.0[b]	351.0[a]	412.5[a]	200.5[b]	415.5[a]	92.8[a]	44.1[b]	87.3[a]
Asc + TPP	423.0[a]	168.0[b]	420.0[a]	501.5[a]	186.5[c]	468.5[b]	110.0[a]	47.5[b]	104.6[a]
Asc + PG + TPP	343.5[a]	161.0[b]	330.0[a]	**338.5**[a]	179.5[b]	330.0[a]	88.2[a]	46.8[b]	81.7[a]

[a] Myofibrils were isolated from three groups of chicken muscle (B_{ctrl}, L, and B_{adj}) in the presence or absence (control) of combined antioxidants [5 mM propyl gallate (PG), 0.2% ascorbate (Asc), and 0.2% tripolyphosphate (TPP)]. Data are means of two replicated experiments. Means within the same row and same parameter group bearing different letters differ significantly ($P < 0.05$). Boldfaced data are significantly different ($P < 0.05$) from nonbolded data in the same column.

myofibrillar proteins (Xiong and Blanchard, 1994) and similar to those of bovine myofibrillar proteins (Culioli et al., 1993).

The magnitudes of G' at peak (50−51 °C) and at the end of heating (73 °C), as well as G'' at peak (49−50 °C), were different among muscle groups and varied with antioxidant treatments. Without antioxidants in the isolation buffer, the resultant B_{adj} myofibrils exhibited G' and G'' values lower ($P < 0.05$) than those of B_{ctrl} myofibrils (Figure 2; Table 1), suggesting that added iron and fat had deleterious effects on viscoelastic properties of myofibrillar proteins. These values, however, were much higher ($P < 0.05$) than those of L myofibrils. The G' at peak, final G', and G'' at peak of B_{adj} myofibrils were 98%, 87%, and 83% higher than L myofibrils, respectively. Incorporation of antioxidants during myofibril preparation increased G' and G'' of B_{adj} myofibrils to a level comparable to G' and $G''$of the B_{ctrl} counterpart (Figure 2; Table 1). There were no significant differences ($P > 0.05$) in G' and G'' values between B_{ctrl} and B_{adj} myofibrils prepared under the same antioxidative condition, except that B_{adj} myofibrils had a slightly lower ($P < 0.05$) final G' value than B_{ctrl} myofibrils when isolated using buffer containing ascorbate and tripolyphosphate. Leg myofibrils consistently produced low ($P < 0.05$) G' and G'' values, compared with breast myofibrils, even in the presence of antioxidants.

DISCUSSION

It has been well documented that chicken or turkey leg muscle is more susceptible to lipid oxidation than breast muscle due to the higher total lipid content of leg muscle (which is more than twice that in the breast muscle) (Marion and Forsythe, 1964; Pikul et al., 1984, 1985; Salih et al., 1989). In this study, however, L myofibril pellet did not show higher TBARS value than B_{ctrl} myofibril pellet even when prepared without antioxidants. The results may be explained by the fact that washing removed most of the water-soluble constituents as well as some lipids in the muscle tissues and, hence, changed the proportions of prooxidants and antioxidants in the resulting pellet. Chicken breast muscle contains higher amounts of water-soluble endogenous antioxidants, especially anserine and carnosine, than does leg muscle, while the opposite is true for lipid-soluble antioxidants such as α-tocopherol (Chan and Decker, 1994). The preparation of myofibrils entailed an extensive homogenization to disrupt cell structures. This mechanical process would facilitate interactions of lipids with myofibrillar proteins to form emulsion-like suspensions, hence making it difficult to remove all of the lipids, especially the membrane lipids. Thus, some lipid-soluble antioxidants would probably stay with protein via emulsion, while water-soluble antioxidants were diluted and washed away. The protective effect of lipid-soluble antioxidants in leg myofibril samples would compensate for the higher concentration of prooxidants in leg muscle. Another unexpected result was that addition of fat and inorganic iron to breast muscle resulted in a decreased TBARS formation in myofibril pellet. This again could be explained by the lipid-soluble antioxidants present in the chicken body fat since it contains a rather large amount of tocopherol, i.e., 1.07 mg/100 g of fat, of which 58% is α-tocopherol (Kanenatsu et al., 1983). Thus, the addition of chicken body fat may actually protect the myofibril pellet from lipid oxidation through the same mechanism described above.

Propyl gallate is a well-known antioxidant that acts as an effective free radical scavenger to inhibit lipid oxidation (Kelleher et al., 1992; Wan et al., 1993). In this study, antioxidant buffers containing propyl gallate inhibited lipid oxidation substantially during myofibril preparation, while the buffer containing solely water-soluble ascorbate and tripolyphosphate did not. Propyl gallate is only marginally soluble in water and tends to stay at the water−air or water−lipid interface when incorporated in the myofibril system. The distribution of propyl gallate and its known potency as a strong free radical scavenger probably explain its effectiveness in inhibiting lipid oxidation in the present study. On the other hand, both ascorbate and tripolyphosphate are water-soluble antioxidants and act as reducing agent and metal chelator, respectively; neither acts as a true free radical scavenger. Doba et al. (1985) reported that in the absence of lipid-soluble antioxidants, ascorbate was a good antioxidant for peroxidations initiated in the aqueous phase but did not trap peroxyl radicals in the lipid phase. Shahidi et al. (1987) showed that a combination of 0.05% ascorbate and 0.3% tripolyphosphate inhibited lipid oxidation in cooked pork during storage. However, it was not clear whether other muscle endogenous antioxidants were also involved since cooked pork is a complex oxidative/antioxidative system. In fact, ascorbate is known to be a dual-function agent, capable of both inhibiting and promoting lipid and myoglobin oxidation depending on its concentration and environment (Yin et al., 1993). Our results suggested that lipid oxidation occurring during chicken myofibril preparation was most likely via a free radical mechanism. In the presence of propyl gallate, initiation of free radicals might also have occurred, but the generated free radicals would soon be terminated by this antioxidant.

Carbonyl contents of myofibrillar proteins were found not to be affected by lipid oxidation in myofibril samples. The increase in protein carbonyls is only one of the

biochemical changes that may occur during protein oxidation. There are certain cases in which oxidative changes of protein can take place without forming carbonyl derivatives, such as oxidation of histidine (Uchida and Stadtman, 1992) and the loss of thiol groups (Takenaka et al., 1991). Protein oxidation could have occurred in some of the myofibril samples, even when these oxidative changes were not detected by the carbonyl method.

Lipid oxidation in the myofibril pellet did not seem to correlate with rheological properties of myofibrillar proteins. With a low TBARS value, B_{adj} myofibril pellets prepared without antioxidants had decreased G' and G'' values when compared with those of B_{ctrl} myofibrils. However, antioxidant treatments, which further lowered the TBARS formation, increased G' and G'' values of B_{adj} sample to the level of the B_{ctrl} sample. It is possible that some oxidative changes in B_{adj} proteins, which either did not involve carbonyl formation or generated carbonyls at an undetectable level, had occurred at the low level of lipid oxidation due to the addition of inorganic iron. Breast and leg myofibrillar proteins showed distinctive viscoelastic patterns during thermal gelation, which were consistent with previous findings (Xiong and Blanchard, 1994). Moreover, myofibrillar proteins from breast (white) muscle formed more elastic gels than their leg (red) muscle counterparts, whether or not they were treated with antioxidants. Thus, the differences in rheological properties between these two types of muscles could be accounted for mostly by intrinsic factors, such as isoforms of myofibrillar proteins (Asghar et al., 1984). The different extents of lipid oxidation of white and red myofibrils apparently did not have a major influence on their rheological properties, hence, their differences in gel-forming ability.

In conclusion, intrinsic factors, isomerization and polymorphism of myofibrillar proteins, are ostensibly responsible for most of the functional discrepancies observed between white and red myofibrillar proteins. Different distributions of prooxidants in these muscles do not seem to play a critical role in dictating functionality of isolated myofibrillar proteins. The exact mechanisms of protein oxidation involved in the preparation of myofibrillar proteins from different muscle types remain unclear. The results from this study suggest that in poultry processing muscle types should be differentiated in formulation to obtain standardized products. Also, careful control of processing factors to prevent oxidative changes will help to minimize the variations in functionality of processed poultry products.

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