Effect of Lipid Oxidation on the Functional and Nutritional Properties of Washed Chicken Myofibrils Stored at Different Water Activities†

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Effects of water activity (a_w) on the functional and nutritional properties of freeze-dried chicken breast myofibrils during storage at 20 °C were investigated. Control myofibrils and myofibrils with 0.6 μmol of added methyl linoleate (ML)/g of protein were stored for 21 days at a_w's between 0.11 and 0.85. Control and ML treatments were 67% and 34% soluble, respectively, after freeze-drying. Monolayer values determined from moisture sorption isotherms in the control and ML treatment were 6.5 and 9.1 g of H_2O/g of fat-free solids, respectively. Lipid oxidation was lowest in myofibrils stored at relative humidities near the monolayer value. Protein solubility decreased more rapidly during storage at high a_w's. Solubility and gel strength were lower in ML treatments than in controls stored at the same a_w. Protein digestibility and quality were not influenced by treatment or storage time.

Lipid oxidation has been cited as one of the major deteriorative reactions in foods leading to alterations in protein functional properties, texture, rehydration characteristics, browning, nutritional values, and off-flavors (Schaich, 1980). Chicken meat is particularly susceptible to lipid oxidation as the fat contains a high proportion of unsaturated fatty acids. The development of rancid flavors in raw chicken and warmed-over flavor in cooked products has been studied extensively in frozen products, freeze-dried products, chicken surimi, and many other further processed products (Pearson and Gray, 1983; Dawson et al., 1988).

Sikorski (1978) reported that lipid–protein interactions may alter the functional properties of meat and cause deleterious changes in final product quality. Oxidation of amino acids and decreases in nutritional quality have been reported in intermediate moisture meat systems (Obanu et al., 1980) and freeze-dried beef (Dvorak, 1968). Smith (1987) attributed some of the functional and biochemical changes in the proteins of deboned turkey to lipid oxidation during storage. Lipid–protein interactions caused toughening in freeze-dried meat products (El-Gharbawi and Dugan, 1965; Love and Pearson, 1971) and changed the functionality of offal protein isolates (Areas, 1986).

Several model systems have been used to study the reactions between meat proteins and oxidizing lipids (Buttkus, 1967; Shenouda and Pigot, 1974; Jarenback and Liljemark, 1975; Nakhost and Karel, 1983, 1984). Protein cross-linking, protein denaturation, polypeptide chain scission, enzyme inactivation, and amino acid destruction in the presence of oxidizing lipid have been reported (Funes and Karel, 1981; Funes et al., 1982; Cheftel et al., 1985). Schaich (1980) reviewed the free-radical mechanism involved in protein–lipid interactions. Interactions vary with water activity (a_w), temperature, pH, extent of oxidation, and type of protein and lipid involved (Schaich, 1980; Erickson, 1982). Interactions also occur between protein and secondary lipid oxidation products, such as aldehydes. Malondialdehyde–protein interactions have been implicated as the cause of a variety of undesirable changes in meat systems (Buttkus, 1967).

Although the biochemical and nutritional changes caused by oxidizing lipids have been investigated in several protein systems, very few studies have examined the influence of lipid oxidation on the functional, chemical, and nutritional properties of meat proteins and their interrelationships. The goal of this study was to better understand the influence of lipid oxidation on protein functional and nutritional properties in meat products.

The objectives of this study were (1) to evaluate the effect of freeze-drying on washed chicken myofibril solubility and lipid stability, (2) to determine the influence of fat content on the adsorption isotherm of the lyophilized proteins and relate monolayer moisture content to lipid stability during storage, and (3) to investigate the effect of a_w and storage time on the functional, chemical, and nutritional properties of washed chicken myofibrils.

MATERIALS AND METHODS

Model System. Fresh broiler breasts were obtained from a local processor. Skin, visible connective tissue, and bone were removed and myofibrils isolated in 0.1 M KCl and 0.05 M potassium phosphate buffer, pH 7.0, as described by Smith (1987). The final pellet was washed two times in 10 volumes of distilled water followed by centrifugation at 6000*g* to precipitate the protein. Protein content in the final pellet was determined by Kjeldahl (AOAC, 1980).

Methyl linoleate (ML) was obtained from Sigma Chemical Co. (St. Louis, MO) and stored at −24 °C until used. Oxidized ML was prepared for the first experiment by bubbling air through the sample for 24 h at 20 °C. The extent of oxidation in fresh and oxidized ML was determined by a conjugated diene assay (Bolland and Koch, 1945). The washed myofibrils were freeze-dried with and without 0.6 μmol of ML/g of protein. Methyl linoleate was added to the washed myofibrils by blending for 90 s at high speed in a Waring blender. The resulting samples were frozen in liquid nitrogen and freeze-dried for 24 or 48 h.

The freeze-dried protein was divided into subsamples and incubated for 3 weeks in the dark at 20 °C in desiccators containing saturated salt solutions. In the first experiment, proteins were stored at five a_w's between 0.11 and 0.85, while in

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the second experiment a_w's of 0.43 and 0.85 were used. Saturated salt solutions used for humidity control were lithium chloride (a_w = 0.11), potassium acetate (a_w = 0.22), potassium carbonate (a_w = 0.43), cobalt chloride (a_w = 0.65), and potassium chloride (a_w = 0.85). Preliminary experiments indicated that samples equilibrated to constant weight within 5 days. Stored proteins were evaluated at weekly intervals.

Chemical Tests. Moisture content of the freeze-dried proteins was determined in duplicate at each storage interval (AOAC, 1984). Brunauer–Emmet–Teller (BET) monolayer values were calculated from adsorption isotherms of proteins stored at a_w's of 0.43 and below (Labuza, 1984). Lipid oxidation was measured in duplicate by using the distillation thiobarbituric acid (TBA) method (Tarladgis et al., 1960) except that 100 ppm antioxidant (Tenox 6, Eastman Kodak, Rochester, NY) was added prior to sample homogenization to prevent oxidation during the assay procedure. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of the chicken proteins was performed on 10% acrylamide resolving gels as described by Smith and Brekke (1985).

Functional Tests. Protein solubility was measured in duplicate according to the method of Morr et al. (1985) with some modifications. The lyophilized proteins were homogenized for 4 s with 0.6 M KCl and 0.05 M potassium phosphate buffer, pH 7.0, with a Polytron homogenizer (Model PT-10/35, Brinkman Instruments, Westbury, NY) and then stirred for 24 h in a 4 °C cold room prior to centrifugation at 20000*g*. The protein content of the supernatants was determined by Kjeldahl (AOAC, 1984).

Gels were prepared in triplicate by pipeting 10 mL of 4% (w/w) protein in 0.6 M KCl and 0.05 M potassium phosphate buffer, pH 6.0, into 16 × 125 mm screw-cap test tubes. The tubes were centrifuged at 200*g* for 10 min to remove air bubbles and heated for 10 min in a water bath at 80 °C. Gels were cooled in an ice–water bath for 30 min and equilibrated to room temperature for 3 h prior to back extrusion testing (Smith et al., 1987). Water-holding capacity was determined in triplicate by centrifuging 1.5 g of gel in a filter paper thimble at 30000*g* for 15 min (Jaurequi et al., 1981). Expressible moisture was reported as a percentage of weight lost by the sample.

Nutritional Tests. Protein digestibility was determined by a multienzyme technique as described by Hsu et al. (1977). The proteases, porcine pancreatic trypsin (Type IX, 17260 BAEE units/mg of protein), bovine chymotrypsin (Type II, 53 units/mg of protein), and porcine peptidase (115 units/g of powder), were purchased from Sigma. In this method the decrease in pH at the end of a 10-min incubation at 37 °C was used as an index of proteolysis, and ANRC reference casein served as a reference protein with known digestibility. Digestibility was described as apparent because differences in protein buffering ability may prevent direct comparisons between different proteins.

Protein quality was measured with *Tetrahymena thermophila* (strain 30008, ATCC, Rockville, MD) as described by Stott and Smith (1966) and Shorrock (1977). The samples used for protein digestibility measurements were allowed to undergo proteolysis for 3 h. After the 3-h digestion, the digests were adjusted to pH 7.1 and diluted with distilled water to contain 1 mg of Kjeldahl nitrogen/mL. Preliminary tests demonstrated that *Tetrahymena* growth was proportional to protein quality and to nitrogen concentrations between 0.025 and 0.25 mg of N/mL. Protein quality was measured with 0.1 mg of N/mL concentrations since this concentration was in the linear phase of the assay for both the casein and myofibrils. Protein quality of the chicken protein was expressed relative to casein (casein = 100). Since the protein digests were soluble, *Tetrahymena* growth was assessed by measuring turbidity at 600 nm.

Statistical Analyses. All experiments were replicated three or four times using at least two preparations of myofibrillar proteins. Data collected during the freeze-drying experiment were analyzed by two-factor factorial testing. The remaining data were analyzed as a split plot model. The General Linear model included treatment, storage time, water activity, and their corresponding interactions as the error term. Mean separation was performed by using Tukey's test when significant ($p < 0.05$) main effects were observed.

Table I. Lipid Oxidation and Solubility of Washed Chicken Proteins at Different Stages of Preparation and Freeze-Drying[a]

treatment	control	+15% methyl linoleate	+15% oxidized methyl linoleate
	TBA No.		
fresh	0.20[a]	0.23[a]	1.01[a]
frozen	0.23[a]	0.31[a]	1.06[a]
freeze-dried for 24 h	0.29[a]	0.73[b]	1.69[b]
freeze-dried for 48 h	0.52[b]	1.30[c]	6.70[c]
	% Solubility in 0.6 M KCl, pH 7.0		
fresh	96.9[a]	96.9[a]	96.9[a]
frozen	82.3[b]	82.0[b]	79.6[b]
freeze-dried for 24 h	81.2[b]	81.9[b]	71.6[c]
freeze-dried for 48 h	67.2[c]	33.8[c]	17.8[d]

[a] Means in the same column within TBA number or solubility followed by a common superscript do not differ ($p < 0.05$).

RESULTS AND DISCUSSION

The composition of the control chicken protein after freeze-drying was 96% protein, 4% moisture, and 1% fat, while the ML-treated samples contained 82% protein, 14% fat, and 4% moisture. The pH of both preparations was 6.8. The extraction yield was 5.2 g of protein/100 g of chicken breast. Methyl linoleate was added to the washed proteins at a ratio of 1:5.9 (0.6 μmol of ML/g of protein) as this ratio of fat to protein is typical in chicken meat (USDA, 1979).

Effects of Freeze-Drying. The results in Table I show the influence of freezing and freeze-drying on protein solubility and lipid oxidation in the products. Freezing caused approximately a 14% decrease in protein solubility in all the treatments, but did not produce any significant change in TBA number. Freeze-drying for 48 h decreased the protein solubility by about 15%, 48%, and 62%, respectively, in the control, ML, and oxidized ML treatments. The largest decrease in protein solubility and increase in TBA number occurred during the last 24 h of freeze-drying. Several authors have reported that isolated salt-soluble meat proteins were easily denatured during frozen storage, although the mechanism has not been entirely elucidated (Matsumoto, 1980; Shenouda, 1980). It has been shown that lipid oxidation occurs readily during the isolation of chicken proteins due to cell disruption and concentration of phospholipids (Dawson et al., 1988). Insolubilization of the control was probably caused by dehydration or local increase in ionic strength causing denaturation of the chicken proteins (Matsumoto, 1980; Shenouda, 1980). Insolubilization of the ML treatment sample may be due to dehydration and reaction with free fatty acids. Insolubilization was accelerated as lipid oxidation increased in the oxidized ML treatment. The oxidized ML treatment sample was only half as soluble as the ML treatment sample, indicating that the presence of oxidized lipids was detrimental to protein solubility during freeze-drying.

Myofibrillar proteins are insolubilized by fatty acids (Sikorski et al., 1976). Egelandsdal et al. (1985) reported that bovine myosin aggregated in the presence of fatty acid salts. Purified myosin (Shenouda and Pigot, 1974) and actin (Shenouda and Pigot, 1976) bind lipids during extraction from muscle tissues. Jarenback and Liljemark (1975) found that linoleic acid hydroperoxides were 10 times more effective than linoleate in reducing the extractability of cod myofibrils.

Results indicate that proteins can be insolubilized by lipids and oxidized lipids; thus, precautions must be taken

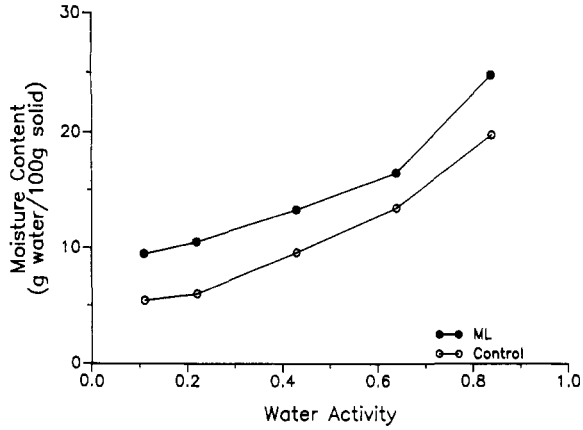

Figure 1. Adsorption isotherm of freeze-dried washed chicken breast proteins at 22 °C.

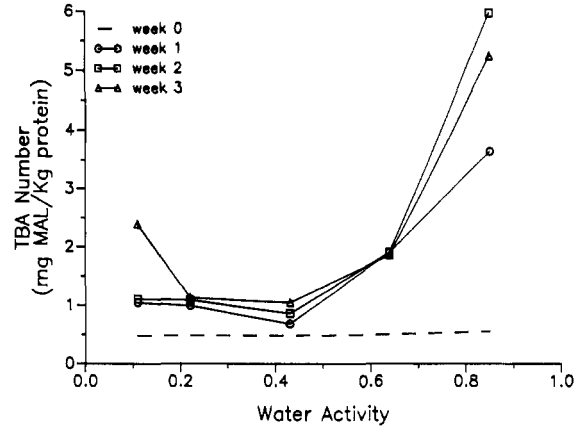

Figure 2. Effect of water activity and length of storage on the thiobarbituric acid (TBA) number of freeze-dried washed chicken breast protein. TBA number is expressed as milligrams of malonaldehyde and other TBA reactive substances per kilogram of protein. (Standard error of the means is ±0.97.)

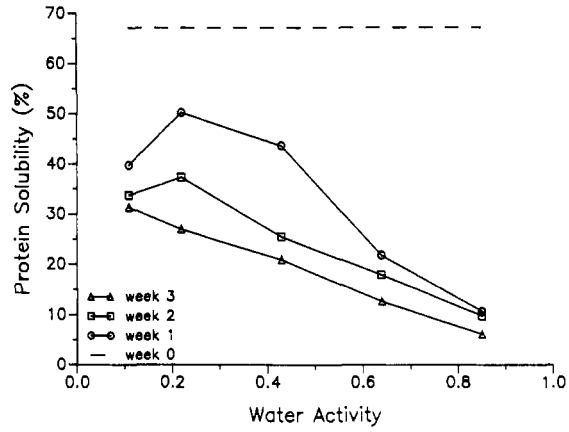

Figure 3. Effect of water activity and length of storage on the solubility of freeze-dried washed chicken breast proteins determined in 0.6 M KCl and 0.05 M potassium phosphate, pH 7.0. (Standard error of the mean is ±1.92.)

during the freeze-drying of muscle protein foods. Nonvolatile antioxidants (Moledina et al., 1977) and the use of cryoprotectants (Matsumoto and Arai, 1986; Maguruma et al., 1987) may be necessary to maintain protein functionality during freeze-drying.

Sorption Isotherm of Myofibrils. The adsorption isotherm for control and ML treatments measured at a_w's between 0.11 and 0.85 is shown in Figure 1. The isotherm is a type II curve which is characteristic for meat (Iglesias and Chirife, 1977; Saravacos and Stinchfield, 1965), muscle fibers (Mackenzie and Luyet, 1967), fish (Moschiar and Fardin, 1986), and fish protein concentrate (Rasekh et al., 1971). The calculated BET monolayer value (m_0) for the control was 6.5 g of water/100 g of total solids, while the ML treatment m_0 values were 7.7 g of water/100 g of total solids or 9.0 g of water/100 g of solids on a fat-free basis. Monolayer values decreased nonsignificantly during storage. Monolayer values calculated by using BET analysis from sorption isotherms of three raw fish species ranged from 6.5 to 8.8 g of H_2O/100 g of solids (Moschiar and Fardin, 1986). The m_0 values for the control and ML treatment sample correspond to an a_w of approximately 0.28 and 0.18, respectively. The monolayer value represents a theoretical moisture content where the protein binds one molecule of water per polar group (Kinsella and Fox, 1986). Theoretically, a dried food is least susceptible to lipid oxidation when stored at relative humidities corresponding to monolayer moisture content (Labuza, 1984). The ML treatment sample bound more water per gram of solid, when expressed on a total solids and fat-free basis, than the control at the same a_w. This observation may indicate a change in protein structure caused by lipid binding and subsequent unfolding and exposure of more peptide bonds and amino acid residues (Kinsella and Fox, 1986). O'Brien and Frazer (1966) reported that fatty acids exert a surfactant effect on proteins, leading to hydrophobic interactions and unfolding of the protein. Hermansson (1977) reported that heat-denatured proteins had higher sorption ability than the corresponding native proteins when stored at a_w's below 0.75.

Lipid Oxidation and Changes in Solubility of Stored Proteins. The influence of a_w on lipid oxidation in the control proteins is shown in Figure 2. The TBA values increased significantly with storage time at all a_w's, except at 0.22 and 0.43 a_w. These values were close to the a_w corresponding to the monolayer value of the freeze-dried proteins as determined from the sorption isotherm. Lipid oxidation increased most rapidly in proteins stored at a_w of 0.85. The TBA values in controls stored at 0.85 a_w decreased after 2 weeks of storage. The formation of secondary oxidation products that did not react with the TBA reagent may have caused the observed decrease in TBA values. The control was very susceptible to lipid oxidation even though it contained trace amounts of fat. Quaglia et al. (1988) reported that freeze-dried beef was more stable to oxidation at an a_w of 0.27 than at 0.156 or below.

Protein solubility decreased in the control at all a_w's as storage time increased (Figure 3). Protein solubility decreased 57% during the first week of storage at an a_w of 0.85. Solubility decreased more slowly in samples stored at 0.22 and 0.43 a_w, where lipid oxidation was lower. In general, protein insolubilization increased as lipid oxidation increased during storage. Protein insolubilization and complexing occurred rapidly at high and low a_w's in the presence of oxidized lipids.

At the same storage time, lipid oxidation was higher in ML treatment samples than in control proteins at all a_w's due to the presence of more TBA reactive substrates (Figures 2 and 4). The TBA values were lowest at 0.85 and 0.43 a_w's after 3 weeks of storage in the ML-treated proteins. Protein solubility of the ML-treated samples was lower than that of the controls at all a_w's when compared at the same storage time (Figures 3 and 5). Protein solubility of the ML-treated proteins decreased by approximately 15% and 23% at low and high a_w's, respectively, during the first week of storage. In general, protein solubility was highest in ML-treated samples stored at a_w's of 0.43 and below. Protein solubility

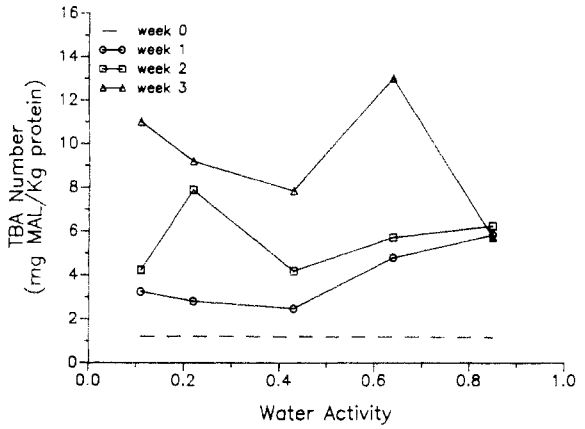

Figure 4. Effect of water activity and length of storage on the thiobarbituric acid (TBA) number of freeze-dried washed chicken breast protein containing 15% (w/w) methyl linoleate. TBA number is expressed as milligrams of malonaldehyde and other TBA reactive substances per kilogram of protein. (Standard error of the mean is ±0.97.)

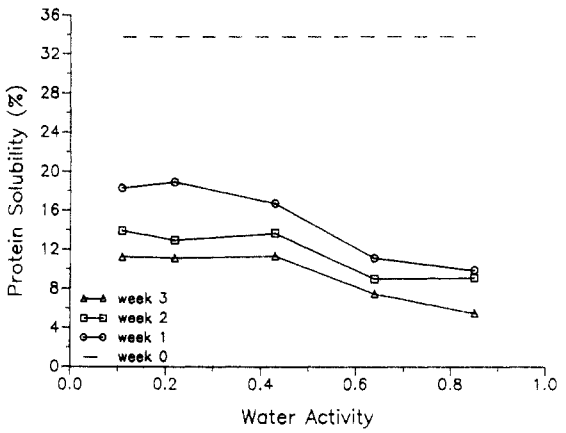

Figure 5. Effect of water activity and length of storage on the solubility of freeze-dried washed chicken breast proteins containing 15% (w/w) methyl linoleate. (Standard error of the mean is ±1.92.)

of the ML treatment samples ranged from 5% in a_w 0.85 to 11% in a_w 0.11 after 3 weeks of storage. The relative proportions of lipid and protein have been reported to influence the rate of lipid oxidation, although optimal concentrations vary with the system (Schaich, 1980).

Insolubilization can occur when proteins react with lipid free radicals, hydroperoxides, and secondary oxidation products, such as aldehydes and ketones (Schaich, 1980). The corresponding increases in protein insolubilization, TBA number, and browning observed in the washed myofibrils during storage suggest that aldehydes, such as malondialdehyde, may have reacted with the amino groups of the proteins to produce aldoximines, aldols, Maillard reaction products, and other addition compounds (Pokorny, 1981). The role of lipid hydroperoxides and free radicals in protein insolubilization during storage cannot be determined from these experiments. Results indicate that oxidation may be minimized if dried products are stored at relative humidities corresponding to their monolayer values.

Gel Properties. In the last experiment, freeze-dried chicken proteins were stored at a_w's of 0.43 and 0.85 for 3 weeks to evaluate the influence of lipid oxidation and protein insolubilization on work to penetrate a gel, water-holding ability, and protease digestibility. Water activities of 0.43 and 0.85 were used in this study as the proteins were most and least stable to oxidation and insolubilization, respectively, under these conditions.

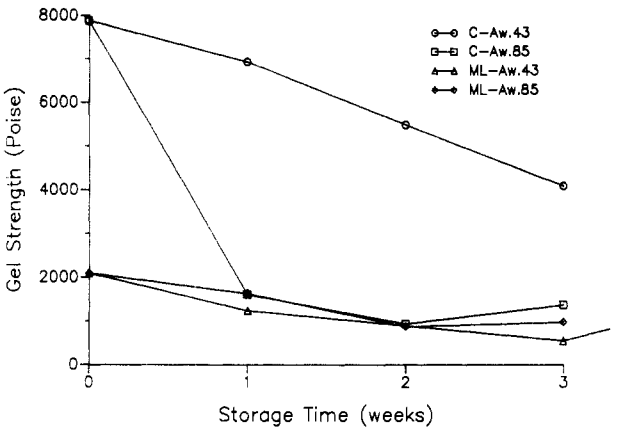

Figure 6. Viscosity index of freeze-dried washed chicken breast proteins as influenced by water activity and length of storage. (Standard error of the mean is ±382.1.)

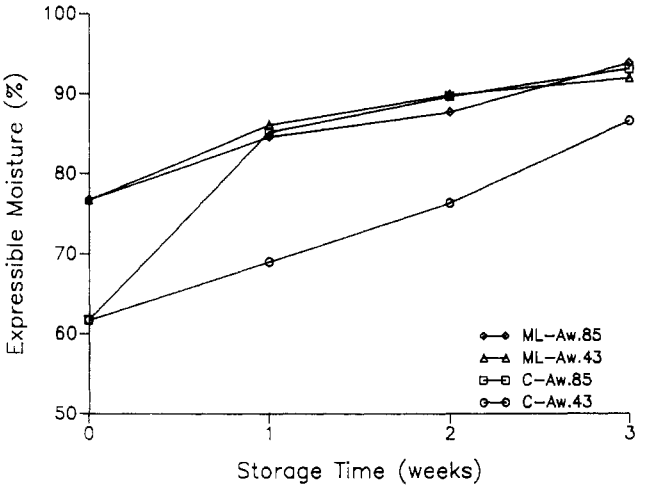

Figure 7. Water-holding capacity of freeze-dried washed chicken breast proteins as influenced by water activity and length of storage. (Standard error of the mean is ±3.13.)

Heat-induced gels prepared from ML-treated proteins were much weaker (Figure 6) and exhibited higher expressible moisture (Figure 7) than gels prepared from control proteins prior to storage. Work required to penetrate the gels decreased and expressible moisture increased significantly in all samples during storage, with the largest changes observed in the control samples. Work to penetrate the gels decreased by approximately 1000 P and expressible moisture increased approximately 15% in both ML-treated samples during storage. Storage a_w had no significant effect on gel strength or expressible moisture of the ML-treated samples. Work to penetrate control gels decreased by 3900 and 6500 P when the proteins were stored for 3 weeks at 0.43 and 0.85 a_w, respectively. Expressible moisture in the control stored at 0.85 a_w was 5% higher than the same sample stored at 0.43 a_w after 3 weeks of storage.

These results are in contrast to those of Egelandsdal et al. (1985), who reported an increase in shear modulus when myosin was equilibrated with octanoic and decanoic acids at various molar ratios, although dodecanoic acid did not increase shear modulus unless incubated with myosin for 15 h prior to gel formation. Whiting (1987) reported that added dodecanoic acid increased the gel strength and water-holding capacity of cooked meat batters, while tetradecanoic and decanoic acids had no influence on these functional properties. The influence of fatty acid salts on muscle protein gelation may be chain length specific.

Nutritional and Chemical Properties. Apparent protein digestibility and quality were assessed by in vitro techniques to determine if the losses in protein functionality were associated with a corresponding loss in nutritive value. Initial apparent protein digestibilities were 94.6% for control and 95.1% for ML-treated samples compared to 91.4% for ANRC casein. Digestibility of control and ML-treated myofibrils did not change ($P > 0.05$) during 3 weeks of storage at either a_w. Protein quality of the myofibrils was lower ($P < 0.05$; control = 67 and ML = 72) than the protein quality of the reference casein. However, there was no difference in protein quality due to either ML treatment or 3-week storage at 0.43 or 0.85 a_w. These results indicate that any cross-linking or amino acid modifications which occurred in the freeze-dried proteins were insufficient to reduce protease susceptibility or protein utilization. Damodoran and Kinsella (1981) reported that proteins with large numbers of hydrophobic residues bound sufficient fatty acids to reduce protein digestibility, whereas less hydrophobic proteins bound less fatty acids with no decrease in protein digestibility. Chung et al. (1986) reported a 20% loss of lysine residues before changes were observed in protease digestibility, suggesting that a certain amount of protein modification occurred before it was detected by the assay. Similarly, other researchers have reported that very small amounts of protein cross-linking cause large decreases in solubility with minimal changes in nutritional properties (Obanu et al., 1980).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed on the proteins stored at 0.43 and 0.85 a_w for 0 and 3 weeks. Changes in band intensity occurred, but no changes were seen in the electrophoretic protein patterns on the resolving gels, indicating that protein scission did not occur during storage at either a_w. Most of the myofibrillar proteins contain sulfur amino acids. Proteins containing sulfur amino acids tend to cross-link at all a_w's, while proteins with no sulfur might undergo scission at low a_w's (Zirlin and Karel, 1969; Karel, 1977; Funes et al., 1982). Large aggregates of proteins were trapped in the stacking gel of all treatments after 3 weeks of storage, indicating that large high molecular weight complexes were formed which were not disrupted by SDS and mercaptoethanol.

CONCLUSIONS

Results of this study indicate the functional properties of washed chicken myofibrils can be altered by reactions with lipids during freeze-drying and storage. Increases in lipid oxidation were correlated with decreases in protein solubility, gel strength, and water-holding capacity. Maintenance of protein functional properties was best achieved by minimizing lipid oxidation during drying and storage. Lipid oxidation was minimized by storing the dried proteins at water activities near their monolayer moisture content. Although large changes in protein functional properties were observed due to protein-oxidized lipid interactions, only small changes were observed in protein digestibility and quality.

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