

Myosin Cross-Linking in Freeze-Dried Meat

H.-J. KIM, V. A. LOVERIDGE, and I. A. TAUB

ABSTRACT

Myosin cross-linking in freeze-dried meat has been investigated. Extraction of proteins and electrophoretic separation of the extracted proteins were used to monitor the cross-linking in freeze-dried meat. The extractability of proteins decreases upon heating and the loss of solubility of myosin is evident. Treating the freeze-dried meat with N- α -acetyl-L-lysine or L-lysine-HCl prevents this loss. The decrease in extractability of myosin is accompanied by the development of brown color. The cross-linking is attributed to a Maillard-type reaction and is suggested to be responsible for textural changes in freeze-dried meat. Treating the meat with hydrolyzed vegetable protein is proposed as a practical method of preventing such cross-linking in freeze-dried meat.

INTRODUCTION

IT HAS BEEN RECOGNIZED that textural change is associated with dehydrated meat (Harper and Tappel, 1957; Connell, 1962; Hultin, 1976). Air-drying the meat at elevated temperature leads to moisture loss and shrinkage of muscle fibers (Wang et al., 1953). In addition to this heat effect, the increased salt concentration in the nondehydrated part of the muscle is believed to promote the denaturation of the muscle proteins. Freeze-drying the meat avoids such protein denaturation, as demonstrated by Wang et al. (1954). Unfortunately, textural changes can take place subsequent to the freeze-drying (Connell, 1962). These changes are accelerated when the freeze-dried meat is exposed to heat. For example, when freeze-dried muscle tissue is heated for 24 hr at 80°C, 65% of the original extractability of structural proteins is lost (MacKenzie and Luyet, 1967). It has been suggested, but never directly demonstrated, that cross-linking between neighboring myosin molecules is responsible for the freeze-dried meat becoming less soluble and tougher upon storage and less juicy upon rehydration (Connell, 1962; MacKenzie and Luyet, 1967).

Regier and Tappel (1956) suggested that the nonoxidative active carbonyl-amine browning reaction is the most important deteriorative mechanism in the storage of freeze-dried meat. They demonstrated a negative correlation between browning and rehydratability in the freeze-dried meat. It is the purpose of this paper to demonstrate that such browning reaction is responsible for the cross-linking of myosin in the freeze-dried meat.

MATERIALS & METHODS

THE CHANGES IN THE PROFILE of extractable sarcoplasmic and myofibrillar proteins upon exposure to accelerated storage conditions were followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were extracted from the freeze-dried meat samples with either an 8M guanidine-HCl solution containing 10 mM dithiothreitol (DTT) or a 6M urea solution containing 2% SDS and 10 mM DTT. The total amount of proteins in

Authors Kim, Loveridge, and Taub are affiliated with the Food Engineering Laboratory, U.S. Army Natick Research & Development Center, Natick, MA 01760. Author Kim is a visiting scientist from the Dept. of Radiology, Harvard Medical School, Boston, MA 02115.

the 8M guanidine-HCl extract was determined by a dye-binding method (Bradford, 1976). The extent of browning reaction was determined on the basis of the water-soluble peptide pigment released upon pronase digestion.

Preparation of freeze-dried meat samples

Pieces (15g) cut from center portions of raw beef round (fat content, 5.3%) were freeze-dried (Stokes freeze-drier; platen temperature, 52°C) overnight. The water content of the freeze-dried meat was 2%. The freeze-dried meat pieces were sealed individually in cans under vacuum.

CHCl₃/MeOH extraction of freeze-dried meat

In some experiments the lipid material from the freeze-dried meat was removed by CHCl₃/MeOH extraction. Pulverized freeze-dried meat (2g) was gently stirred in 200 mL CHCl₃/MeOH (2:1) overnight. The supernatant was decanted, and the residual CHCl₃/MeOH removed from the meat by evaporation in the hood. The sample was sealed in cans under vacuum in 0.5g aliquots.

Treatment of meat with N- α -acetyl-L-lysine or L-lysine-HCl

Pulverized freeze-dried meat (0.4g) was reconstituted in 4 mL 1.5M N- α -acetyl-L-lysine solution for 24 hr at 4°C. Excess solution was removed from the meat sample by blotting with filter paper. The sample was freeze-dried and sealed under vacuum, as was an untreated control sample. In a similar experiment the freeze-dried meat was reconstituted with 1.2M L-lysine-HCl solution.

Water extraction and glucose treatment of meat

In some experiments water-soluble components of the meat were removed by extensive extraction with water. Raw meat pieces (15g) sliced into 2 mm thickness were stirred constantly in several changes of 300 mL water until no carbohydrate was detectable in the extract by phenol-sulfuric acid method (Dubois et al., 1956). The almost colorless meat was either freeze-dried and vacuum sealed or subjected to further treatment.

In one experiment, excess water was removed from the water-extracted meat by blotting with filter paper, and 4g meat pieces were stirred gently in 10 mL 1M α -D-glucose (Sigma Chemical Company) solution overnight at room temperature. Excess solution was removed and the meat was freeze-dried and vacuum sealed. In another experiment, the water-extracted meat was freeze-dried, and a 0.5-g portion of the freeze-dried and pulverized meat was soaked in 5 mL 0.1M α -D-glucose solution overnight at 4°C. In a control experiment the freeze-dried meat was soaked in water. After removal of the excess solution, the meat was freeze-dried and vacuum sealed.

Treatment of meat with HVP

Raw meat pieces (10g) sliced into 2 mm thickness with the sliced surface perpendicular to the fiber axis was soaked overnight at 4°C in 10 mL liquid hydrolyzed vegetable protein (Hercules HVP E-610: total solids, 37.0%; equivalent protein, 14.0%) diluted 10-fold with water. After removing excess solution with filter paper, the HVP-treated meat was freeze-dried, vacuum sealed, and heated at 100°C for 10 and 30 min. In a control experiment, the meat was soaked in water and freeze-dried.

Heat treatment

For 100°C experiments, the freeze-dried meat amples, sealed in cans, were heated in boiling water for either 10 or 30 min. For lower temperature experiments, samples were stored in a 52°C oven for either 4 or 9 days.

—Continued on next page

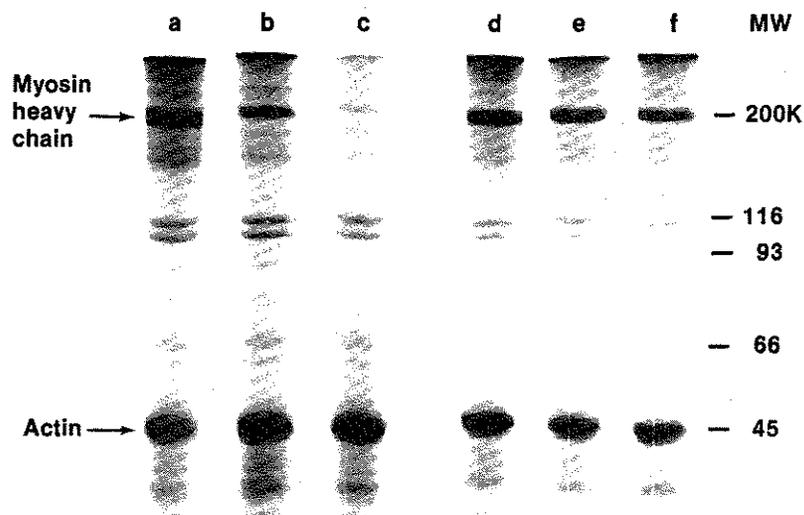


Fig. 1—SDS-PAGE pattern of proteins extracted with 8M guanidine·HCl-10 mM DTT solution from freeze-dried meat. The gels in a, b, c correspond to untreated samples and those in d, e, f correspond to samples treated with N- α -acetyl-L-lysine. The samples in a and d were not heated. The samples in b and e were heated at 100°C for 10 min, and those in c and f for 30 min.

Protein extraction

After the sample was withdrawn from the boiling water (100°C experiment) or from the oven (52°C experiment), the can was opened and the meat sample pulverized into a fine powder by hand. Visible connective tissue was removed. In some experiments 20 mg aliquots of the meat powder were extracted with 4 mL 8M guanidine·HCl-10 mM DTT solution by gently stirring for 3 hr or overnight. Unheated samples were extracted in the same way. The extract was filtered through Whatman 4 filter paper, and 1 mL aliquot of the filtrate was dialyzed against 6M urea-10 mM DTT solution for SDS-PAGE. In other experiments 20 mg aliquots were extracted with 4 mL 6M urea-2% SDS-10 mM DTT solution by gently stirring overnight. The latter extraction method did not require the dialysis step, and therefore was used routinely.

SDS-PAGE

Tube gels containing 7.5% polyacrylamide were made according to the method of Laemmli (1970). The protein extract was mixed with an equal volume of sample buffer containing 0.0625M Tris-HCl (pH 6.8), 3% SDS, 10% glycerol, and 5% 2-mercaptoethanol, and heated for 2 min in boiling water. The amount of total protein loaded per gel varied from 50-100 μ g. Electrophoresis was carried out with a constant current of 2 mA/gel for 3 hr. The gels were stained with 0.05% Coomassie brilliant blue R250 solution. The gel was destained with 7.5% acetic acid solution and the intensity of the protein bands determined by densitometric scanning at 595 nm with a Varian model 634 spectrophotometer-gel scanner system. To measure the myosin-to-actin ratio, a densitogram with broad peaks was obtained by either decreasing the scanning rate or increasing the chart speed. The areas under the actin and myosin peaks were measured by cutting out the chart paper under the peaks and weighing. The two extraction methods gave similar protein band intensities. Bio-Rad high molecular weight standard proteins were used as molecular weight markers.

Protein analysis

The amount of protein extracted with 8M guanidine·HCl-10 mM DTT solution was measured by the dye-binding method of Bradford (1976). The protein extract was filtered through a Schleicher & Schuell nitrocellulose filter (5.0 μ m) and diluted 10-fold with the extraction solution for assaying. Bio-Rad protein assay kit II was used. 8M guanidine·HCl does not interfere with the protein analysis, but 6M urea-2% SDS does.

Measurement of browning reaction

The intensity of brown color of the meat samples was determined after pronase digestion. An aliquot (50 mg) of the water-extracted, glucose-added, freeze-dried meat, stored at 52°C for 9 days, was suspended in 2 mL 0.1M NH₄HCO₃ solution (pH 8.0) in a test tube. One mg pronase (Calbiochem-Behring) was added to the solution

and the tube then incubated at 37°C for 24 hr, with occasional stirring. CHCl₃ (1ml) was then added to remove lipid material from the suspension. The solution was mixed in a vortex mixer and allowed to stand overnight at room temperature. Absorbance of the clear aqueous phase was measured at 420 nm. A control experiment was done by keeping the glucose-added meat sample at 25°C for 9 days. Another set of control experiments was done using water-extracted, freeze-dried meat without added glucose.

RESULTS & DISCUSSION

FIG. 1 clearly shows that when the freeze-dried meat was heated at 100°C the amount of extractable myosin heavy chain (MW ~200,000 daltons) in the meat decreased (gels a, b, c). The decrease in the intensity of myosin heavy chain in gels b and c without the concomitant appearance of smaller protein bands suggests that myosin heavy chain became unextractable and the amount of total extractable protein decreased upon heating. However, the loss of myosin extractability was prevented by treating the meat with N- α -acetyl-L-lysine (Fig. 1, gels d, e, f). The rationale for using N- α -acetyl-L-lysine in order to prevent the loss of myosin extractability is discussed below.

The densitometric scans of gels a, b, and c from Fig. 1 are shown in Fig. 2. In addition to the decrease in myosin heavy chain, loss of other high molecular weight proteins toward the top of the gel occurred. It is clear, however, that the decrease in the myosin heavy chain extractability was the major effect of heating the freeze-dried meat. Since the amount of extracted actin was unchanged, it was used as the internal standard. The change in myosin-to-actin ratio upon heating the freeze-dried meat, treated and untreated with N- α -acetyl-L-lysine, is shown in Fig. 3. The myosin-to-actin ratio in the meat not treated with N- α -acetyl-L-lysine, but heated at 100°C for 10 and 30 min, decreased to 32% and 3%, respectively, when compared to that of the sample not heated. When the meat was treated with N- α -acetyl-L-lysine, the decrease in the myosin-to-actin ratio upon heating was prevented.

For samples untreated with N- α -acetyl-L-lysine and stored in a 52°C oven for 4 days, results similar to gel b in Fig. 1 and densitogram b in Fig. 2 were obtained. The myosin-to-actin ratio decreased to 24% after 4 days at 52°C.

Fig. 4 shows that the amount of total protein extracted with 8M guanidine·HCl-10 mM DTT from the freeze-dried meat untreated with N- α -acetyl-L-lysine decreased upon heating at 100°C, consistent with the electrophoretic results. From 20 mg aliquot of the freeze-dried meat that was not heated, 13.4 mg protein was extracted with 8M

Fig. 2—Densitograms of gels a, b, c from Fig. 1. Peaks denoted by A and M represent actin and myosin heavy chain, respectively. The peak position at the far right of each densitogram corresponds to the material that did not enter the running gel.

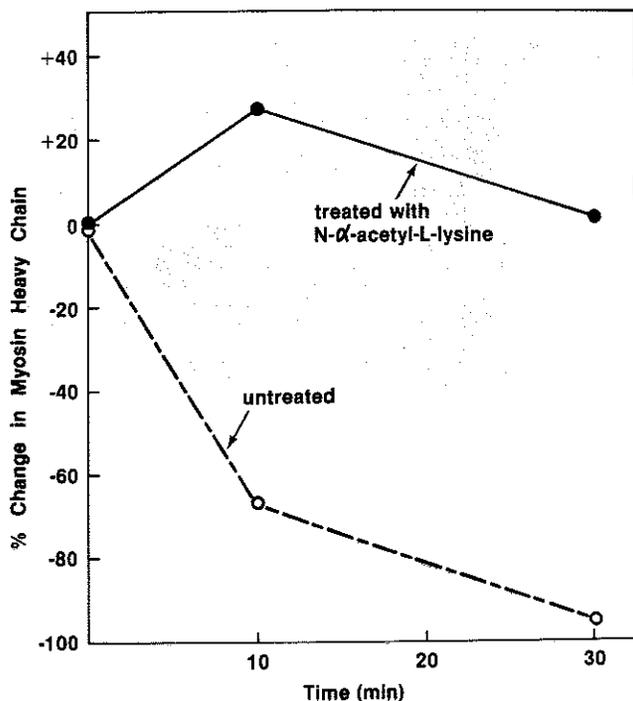
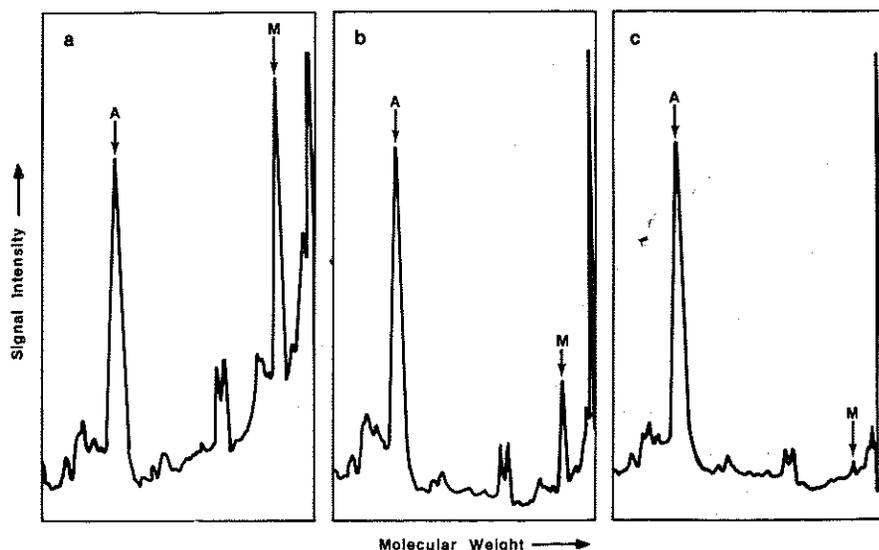


Fig. 3—Effect of *N*- α -acetyl-L-lysine on the amount of myosin heavy chain extracted with 8M guanidine-HCl-10 mM DTT solution from freeze-dried meat heated at 100°C for 10 and 30 min. Untreated samples \circ — \circ ; samples treated with *N*- α -acetyl-L-lysine \bullet — \bullet .

guanidine-HCl-10 mM DTT solution. The decrease in extractable protein was 24% and 40% after 10 and 30 min of heating at 100°C, respectively. Since approximately 33% of the muscle protein (sarcolemmal and myofibrillar) is myosin heavy chain, a 97% decrease in extractable myosin heavy chain after heating for 30 min would correspond to a 32% decrease in total protein extractability. The observed 40% decrease was somewhat higher; but this difference might be due to the insolubilization of other high molecular weight proteins described above. Nevertheless, it is clear that myosin heavy chain is the major protein associated with the decreased extractability. Treating the meat with *N*- α -acetyl-L-lysine prevented the decrease in total extractable protein upon heating.

It has been suggested that myosin is involved in the tex-

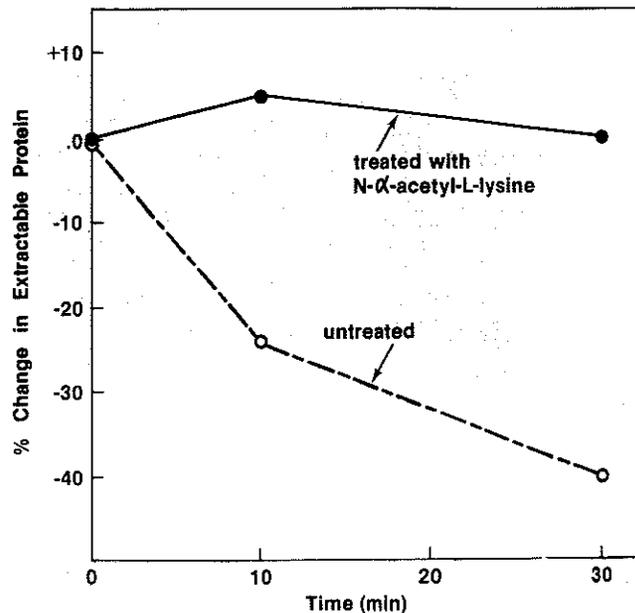


Fig. 4—Effect of *N*- α -acetyl-L-lysine on the amount of total protein extracted with 8M guanidine-HCl-10 mM DTT solution from freeze-dried meat heated at 100°C for 10 and 30 min. Untreated samples \circ — \circ ; samples treated with *N*- α -acetyl-L-lysine \bullet — \bullet .

tural changes in freeze-dried meat (Connell, 1962; MacKenzie and Luyet, 1967). Our data demonstrate that changes in myosin are primarily responsible for the loss of protein extractability. We also observed a correlation between myosin extractability and rehydratability of the freeze-dried meat (unpublished data).

The fact that myosin heavy chain becomes unextractable in either 8M guanidine-HCl or 6M urea-2% SDS solution excludes noncovalent association between myosin heavy chains as the mechanism for the decrease in extractability. Chemical modifications in the side groups of the myosin heavy chain involving a small increase in molecular weight is also excluded as an explanation for the decrease in myosin heavy chain band in the SDS-PAGE after guanidine-HCl or urea-SDS extraction. We conclude, therefore, that the decreased extractability of myosin heavy chain is due to covalent cross-linking between myosin heavy chain mole-

—Continued on next page

MYOSIN CROSS-LINKING IN FREEZE-DRIED MEAT...

cules. To investigate the nature of the covalent cross-linking, a series of elucidative experiments was performed.

When the freeze-dried meat heated at 100°C for 30 min was extracted with 8M guanidine·HCl solution with and without the reducing agent DTT present, the same loss of myosin extractability was observed, indicating that covalent bonds other than disulfide bonds are responsible for the cross-linking of myosin.

Furthermore, the loss of myosin extractability was not prevented by first extracting lipids with chloroform/methanol (2:1) before heating the freeze-dried meat (Fig. 5). Thus cross-linking of myosin is not mediated by lipid peroxides formed by the autoxidation of membrane lipids.

On the other hand, as mentioned above, the loss of myosin extractability was prevented by treating the meat with N- α -acetyl-L-lysine (Fig. 1, gels d, e, f, and Fig. 3). In fact, after the meat treated with N- α -acetyl-L-lysine was heated for 10 min at 100°C, the extractable myosin heavy chain increased slightly (Fig. 3). This increase might be due to the release of myosin upon initial heating that occurred concurrently with protein cross-linking. As expected, the amount of extractable protein also did not decrease upon heating the meat treated with N- α -acetyl-L-lysine (Fig. 4).

When the meat was treated with 1.2M L-lysine·HCl solution, results similar to those obtained with samples treated with N- α -acetyl-L-lysine (Fig. 1, gels d, e, f, and Fig. 3 and 4) were obtained. In fact, L-lysine·HCl appeared to be approximately twice as effective as N- α -acetyl-L-lysine in preventing the loss of myosin extractability. Approximately 70% of myosin heavy chain remained extractable when the freeze-dried meat was treated with a 0.2M L-lysine·HCl

solution and heated for 30 min at 100°C compared with 28% when the treatment was with a 0.2M N- α -acetyl-L-lysine solution. It seems likely that the amino group, which is present in a ratio of 2:1 in L-lysine·HCl compared with

N- α -acetyl-L-lysine, exerted the preventive effect. This observation strongly suggests that the ϵ -amino groups in the myosin heavy chain are involved in the cross-linking of myosin (see below for detailed discussion).

The loss of myosin extractability was also prevented by first extensively extracting water-soluble components from the meat using water. As shown in the control gel of Fig. 6, myosin heavy chain remained extractable when the water-extracted, freeze-dried meat was heated at 100°C for 30 min (compare with gel c, Fig. 1). It is reasonable to assume that there are molecular components in the water-soluble fraction of meat that can act as linkers for the cross-linking of myosin heavy chains. Clark and Tannenbaum (1974) suggested that glucose is directly involved in the cross-linking between peptide chains in the insulin-glucose system. They associated the cross-linking with extensive condensation of sugar residues. As shown in Fig. 6, when the water-extracted meat was treated with 1M glucose solution and then freeze-dried and heated, myosin heavy chain became cross-linked. On the other hand, when the treatment was with 1M sucrose solution, myosin heavy chain was unaffected (the result was similar to the control in Fig. 6). The amount of total extractable protein measured by the dye-binding method was unchanged upon heating the water-extracted, freeze-dried meat, but it decreased by 48% upon treatment with 1M glucose solution; it did not decrease upon treatment with 1M sucrose solution.

When the glucose-treated meat was stored at 52°C for 9 days, browning of the meat was evident to the naked eye. Fig. 7 shows the development of brown color measured by the concentration of the limit peptide pigment released by pronase digestion. No browning was observed either in the control sample without added glucose or in the glucose-treated meat kept at 25°C for 9 days. Another control experiment with sucrose treatment of the water-extracted meat showed no browning after the same heat treatment.

From the above results it appears that reducing sugars such as glucose mediate cross-linking between myosin heavy

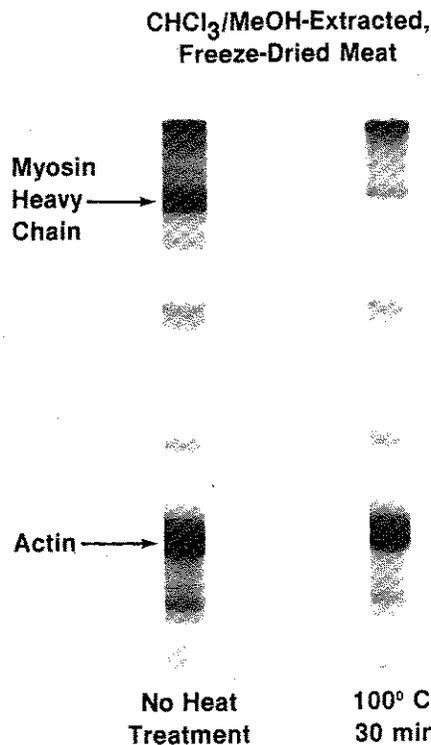


Fig. 5—SDS-PAGE pattern of proteins extracted with 6M urea-2% SDS-10 mM DTT solution from CHCl₃/MeOH-extracted, freeze-dried meat with (right) and without (left) heating at 100°C for 30 min.

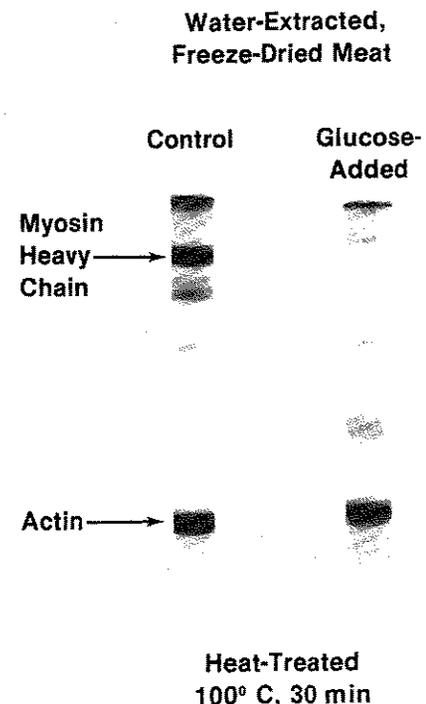


Fig. 6—Effect of added glucose on the cross-linking of myosin heavy chain in water-extracted, freeze-dried meat.

chain molecules with accompanying development of brown color through a Maillard-type reaction. This hypothesis is consistent with the suggested involvement of ϵ -amino groups in the protein molecules. Presumably the amino groups in N- α -acetyl-L-lysine and L-lysine·HCl compete with the ϵ -amino groups of the myosin heavy chain for reaction with reducing sugars. Cross-linking of myosin heavy chains is thus prevented.

Regier and Tappel (1956) pointed out that active carbonyl-amine browning is the main deteriorative reaction during the storage of freeze-dried meat. They suggested that a correlation exists between the decrease in glucose and amino groups and the loss of protein extractability and rehydratability. Our data confirm their conclusion that a

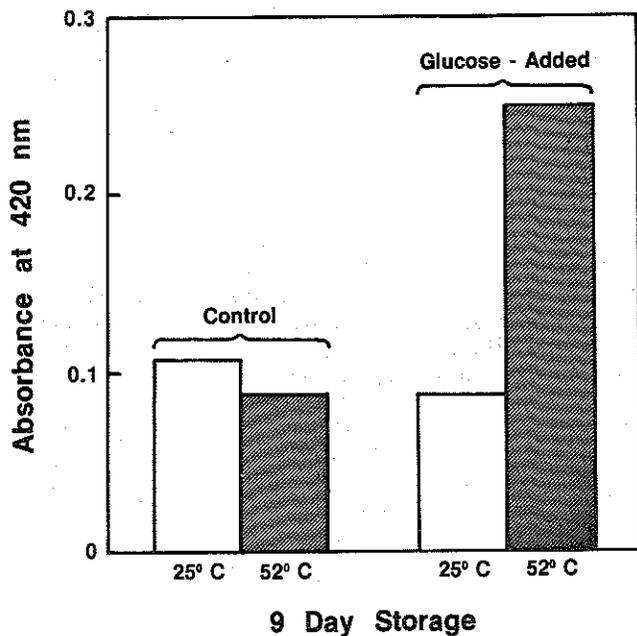


Fig. 7—Effect of added glucose on browning of water-extracted, freeze-dried meat.

Maillard-type browning reaction, not oxidative deterioration, is the primary cause for textural changes in the storage of freeze-dried meat. Furthermore, our data indicate that the browning reaction is associated with the cross-linking of myosin heavy chain molecules, which is the primary cause of decreased protein extractability.

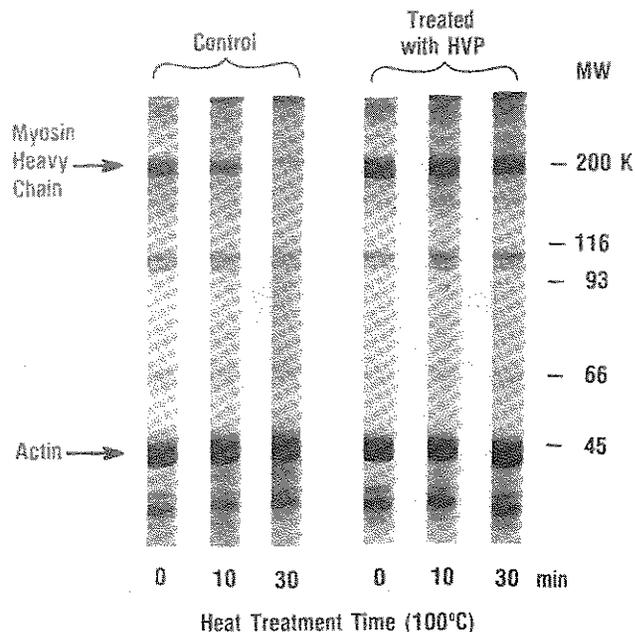
Mohammad et al. (1949) suggested that browning of bovine serum albumin-glucose solution leads to the cross-linking of the protein. Clark and Tannenbaum (1974) investigated the structure of the limit peptide pigments resulting from pronase digestion of the cross-linked insulin chains that contain condensed glucose. Recently, covalent cross-linking of ovalbumin through Maillard reaction with glucose in a freeze-dried state was demonstrated by gel electrophoresis (Watanabe et al., 1980) and by gel filtration (Kato et al., 1981).

The formation of ϵ -(γ -glutamyl)lysine and ϵ -(β -aspartyl)lysine isopeptide bonds upon heating chicken muscle at low water content has been reported, and its nutritional implication has been discussed (Hurrell et al., 1976; Otterburn et al., 1977). The relationship between the extent of isopeptide bond formation and the textural parameters has not been studied. Based upon the results presented in Fig. 6, it seems unlikely that, in the accelerated storage of freeze-dried meat, isopeptide bond formation is responsible for the loss of extractability of proteins to any significant extent. If isopeptide bond formation is as important as browning for the loss of myosin extractability, there would be a significant decrease in the extractable myosin heavy chain when the water-extracted, freeze-dried meat is heated at 100°C for 30 min. Fig. 6, left gel, shows that no significant loss of myosin extractability occurred.

The myosin molecule contains two heavy chains (MW ~200,000 daltons each). The N-terminal portions of the heavy chains form the globular head with four light chains and the remaining regions of the heavy chain form a long α -helical coiled coil, known as the rod. It has been suggested recently that the molecular surface of the myosin rod contains alternate clusters of positive and negative charge with a periodicity of 28 residues (Parry, 1981; McLachlan and Karn, 1982). Thus a strong electrostatic attraction between neighboring myosin rods is possible when the stagger is an odd multiple of 14 residues. This unique arrangement of

—Continued on page 708

Fig. 8—Prevention of the loss of myosin extractability with HVP treatment. Freeze-dried meat treated with diluted HVP (right) and untreated control (left) was heated at 100°C for 10 and 30 min. Proteins extracted with 6M urea-2% SDS-10 mM DTT solution were separated by SDS-PAGE.



the myosin rods may predispose the myosin heavy chains from neighboring myosin molecules to cross-linking mediated by reducing sugars. The structure of the cross-link should be a subject of further investigation.

The fact that myosin cross-linking can be prevented by treatment with lysine has great practical significance. One can make freeze-dried meat products that can be subjected to severe storage conditions without textural deterioration. As a practical approach to introducing lysine and other amino acids, sliced raw meat was treated with HVP diluted 10-fold (final concentration, 1.4% equivalent protein) before freeze-drying. The loss of myosin extractability was prevented as shown in Fig. 8. The 10-fold dilution reduced the problem associated with the strong flavor of HVP. Prevention of myosin cross-linking by treatment with HVP may also prevent the decrease in the bioavailability of the proteins (Ford, 1965; Tanaka et al., 1977), thus improving the nutritional quality as well as the textural quality of the freeze-dried meat.

REFERENCES

- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248.
- Clark, A.V. and Tannenbaum, S.R. 1974. Isolation and characterization of pigments from protein-carbonyl browning systems. Models for two insulin-glucose pigments. *J. Agr. Food Chem.* 22: 1089.
- Connell, J.J. 1962. The effects of freeze-drying and subsequent storage on the proteins of flesh foods. In "Freeze-Drying of Foods," p. 50. National Research Council, Washington, DC.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350.
- Ford, J.E. 1965. A microbiological method for assessing the nutritional value of proteins. 4. Analysis of enzymically digested food proteins by Sephadex-gel filtration. *Brit. J. Nutr.* 19: 277.
- Harper, J.C. and Tappel, A.L. 1957. Freeze-drying of food products. *Adv. Food Res.* 7: 171.
- Hultin, H.O. 1976. Characteristics of muscle tissue. Ch. 13. In "Principles of Food Science. Part I. Food Chemistry," (Ed.) O.R. Fennema, p. 577. Marcel Dekker, Inc., New York, NY.
- Hurrell, R.F., Carpenter, K.J., Sinclair, W.J., Otterburn, M.S., and Asquith, R.S. 1976. Mechanism of heat damage in proteins. 7. The significance of lysine-containing isopeptides and of lanthionine in heated proteins. *Br. J. Nutr.* 35: 383.
- Kato, Y., Watanabe, K., and Sato, Y. 1981. Effect of Maillard reaction on some physical properties of ovalbumin. *J. Food Sci.* 46: 1835.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680.
- MacKenzie, A.P. and Luyet, B.J. 1967. Freeze-drying and protein denaturation in muscle tissue; losses in protein solubility. *Nature* 215: 83.
- McLachlan, A.D. and Karn, J. 1982. Periodic charge distribution in the myosin rod amino acid sequence match cross-bridge spacings in muscle. *Nature* 299: 226.
- Mohammad, A., Fraenkel-Conrat, H., and Olcott, H.S. 1949. The browning reaction of proteins with glucose. *Arch. Biochem.* 24: 157.
- Otterburn, M., Healy, M., and Sinclair, W. 1977. The formation, isolation and importance of isopeptides in heated proteins. Ch. 17. In "Protein Cross-linking," (Ed.) M. Friedman, Part B, p. 239. Plenum Press, New York, NY.
- Parry, D.A.D. 1981. Structure of rabbit skeletal myosin. *J. Mol. Biol.* 153: 459.
- Regier, L.W. and Tappel, A.L. 1956. Freeze-dried meat. 3. Nonoxidative deterioration of freeze-dried beef. *Food Res.* 21: 630.
- Tanaka, M., Kimiagar, M., Lee, T.-C., and Chichester, C.O. 1977. Effect of Maillard browning reaction on nutritional quality of protein. Ch. 22. In "Protein Cross-linking," (Ed.) M. Friedman, Part B, p. 321. Plenum Press, New York, NY.
- Wang, H., Andrews, F., Rasch, E., Doty, D.M., and Kraybill, H.R. 1953. A histological and histochemical study of beef dehydration. 1. Rate of dehydration and structural changes in raw and cooked meat. *Food Res.* 18: 351.
- Wang, H., Auerbach, E., Bates, V., Doty, D.M., and Kraybill, H.R. 1954. A histological and histochemical study of beef dehydration. 4. Characteristics of muscle tissue dehydrated by freeze-drying techniques. *Food Res.* 19: 543.
- Watanabe, K., Sato, Y., and Kato, Y. 1980. Chemical and conformational changes of ovalbumin due to the Maillard reaction. *J. Food Proc. & Pres.* 3: 263.

Ms received 10/11/83; revised 2/14/84; accepted 2/20/84.

This paper reports research undertaken at the US Army Natick Research & Development Center and has been assigned No. TP-2257 in the series of papers approved for publication. The findings in this paper are not to be construed as an official Department of the Army position.