

## Physical and Chemical Changes in Spun Soy Protein Fibers during Storage

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### ABSTRACT

Prolonged storage of textured soy fibers induces changes at the molecular level. Solubility in buffers, urea, and mercaptoethanol indicates that the protein structure is held together by hydrogen and hydrophobic bonds as well as disulfide bonds. Determination of sulfhydryl and disulfide groups shows that at elevated temperature the number of sulfhydryl groups decreases in time due to oxidation. EDTA and ascorbic acid will retard the aging of soya fibers by preventing the catalytic oxidation of sulfhydryl groups. Disc electrophoresis measurements revealed a fast-moving peak which disappears in aged fibers. The same peak can be restored by splitting of disulfide bonds with 2-mercaptoethanol. Changes of appearance occurring in aged fiber were correlated to loss of water retention capacity.

Textured vegetable proteins, soy protein in particular, are being increasingly used as extenders and analogs of meat products. One way to achieve texture is to spin a solution of soy protein into fiber (1,2,3). The soy protein fibers can be used to extend meat products or, with flavoring and processing, to resemble existing meat products. There are now on the market "chicken" chunks, "turkey" loaf, "bacon," and other analogs of traditional meat products made from textured soya protein.

The mechanism involved in the formation of fiber from solutions of soya protein has been reported (4). Yet no study related to storage changes has been published. Freshly manufactured spun protein fibers have a cream-white color, are tender and elastic. Prolonged storage, before formulation into the end product, affects the appearance; the fibers turn into a gray color, are tough and inextensible. Since processing of fibers may be delayed, it is important to determine the chemical and physical changes which occur during storage and suggest possible preventive action.

## MATERIALS AND METHODS

Fresh-spun soy fiber was obtained in frozen form from Worthington Foods, Worthington, Ohio, a subsidiary of Miles Laboratories, Inc. Moisture content was 68%, protein 30%, pH 4.5 to 5.0.

### Storage Conditions

Pieces of spun fiber were closed in 80-ml. cylindrical glass bottles, then stored at 22°, 4°, and -20°C.

### Chemical Treatment of the Spun Fiber

Fifty grams of fresh spun fiber was soaked overnight at room temperature in 100 ml. of: a) 1% NaCl and 0.01% EDTA at pH 5.0; and b) 1% NaCl and 0.1% ascorbic acid. The fibers were hand-squeezed, then stored in sealed glass bottles at room temperature.

### Solutions

Standard buffer (SB) prepared by mixing 0.0325M  $K_2HPO_4$  with 0.0026M  $KH_2PO_4$  to a pH of 7.6, then adding NaCl to 0.4M, urea 8M, in standard buffer.

### Determination of Sulfhydryl Groups

The procedure of Ellman (5) was followed. The sample was dissolved in the 8M urea-SB solution to a concentration of 1% protein. All samples were read against two blanks, one containing the fiber solvent with Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid), the other a fiber solution without added reagent.

### Determination of the Disulfide (SS) Groups

Determination of the SS groups was made by the method of Robyt et al. (6). The samples were dissolved in the 8M urea-SB solution.

### Determination of Protein Solubility

Spun soy fibers were suspended in SB, in SB buffer containing 0.1M  $\beta$ -mercaptoethanol, and SB-8M urea solutions to a concentration of 1% protein. The suspensions were gently shaken at 23°C. for 10 min. After centrifugation, the amount of solubilized protein was measured by Kjeldahl nitrogen determination using a factor of 6.25. All solutions containing urea were extensively dialyzed at 4°C. vs. the standard buffer prior to N determination.

### Disc Electrophoresis

Electrophoretic analyses were conducted in a disc electrophoresis apparatus, Model 1200 (Canalco, Rockville, Md.). The 5% gel solution was made by dissolving 50 g. acrylamide, 0.7 g. N,N'-methylene-biscarylamide, 240 g. urea, and 0.5 ml. N,N,N',N'-tetramethylene diamine to 1 liter of 0.3M *tris*-HCl buffer, pH 9.0. Gel formation was catalyzed by adding 0.7 mg. ammonium persulfate per 1 ml. gel solution.

The electrode buffer was *tris*-glycine buffer containing 4M urea, pH 8.3.

Aniline blue-black was used for the staining. Tracings were made on a Photovolt

TABLE I. SOLUBILITY OF FRESHLY MADE SPUN SOY FIBER

Solvent	Amount Soluble %
SB <sup>a</sup>	6.0
SB + 0.1M m.e. <sup>b</sup>	12.5
SB + 1.5M urea	18.1
SB + 0.1M m.e. + 1.5M urea	39.1
SB + 3M urea	41.5
SB + 0.1M m.e. + 3M urea	91.8
SB + 8M urea	88.0
SB + 0.1M m.e. + 8M urea	96.6

<sup>a</sup>SB = standard buffer, pH 7.6,  $\mu = 0.5$ .

<sup>b</sup>m.e. =  $\beta$ -mercaptoethanol.

Densicord Densitometer (Photovolt Corp., New York, N.Y.).

Samples containing 4 mg. protein per ml. were prepared in 8M urea-SB with or without 0.1M  $\beta$ -mercaptoethanol. About 0.05 ml. of sample solution was layered directly on the top of the polymerized gel. The time for each run was 40 min. at 2 ma. per tube at the beginning and 5 ma. per tube after the front migrated into the gel.

#### Determination of Water Retention by Spun Fibers

Triplicate 25 g. amounts of fibers were finely blended with 100 ml. water in a Waring Blender. The homogenous slurry was transferred to a funnel containing one layer of Whatman No. 1 filter paper. The funnel was inserted in a 100-ml. graduate cylinder and the slurry allowed to filter at room temperature. The filtrate volumes were recorded at 5, 10, 20, and 30 min.

## RESULTS AND DISCUSSION

The solubilities of freshly made spun fibers in various solvents are given in Table I.

The standard buffer solubilized 6% of the protein.

The solubility increased by addition to the buffer of  $\beta$ -mercaptoethanol and/or urea. Increasing the concentration of urea from 1.5M to 8M increased the solubility of the protein from 18.1 to 88%. The solubilizing effect of  $\beta$ -mercaptoethanol, a known SS cleaving agent, was enhanced by urea. These data, in agreement with the results of Kelley and Pressey (4) suggest that the protein structure of spun soy fibers is held together by hydrogen and hydrophobic as well as SS bonds.

Direct evidence for the presence of SS bonds in spun soy fibers was obtained by the free sulfhydryl (SH) groups analysis. The freshly made spun soy fiber contained 46.8  $\mu$ moles of SH group per g. protein when the determination was made in the presence of 8M urea and 0.01% EDTA. In the absence of EDTA only 35.6  $\mu$ moles of SH groups were determined per g. of protein. Both values were short of the 72  $\mu$ moles of half-cystine groups determined by the amino acid autoanalyzer, which accounts for the presence of SS bonds in the fresh fiber. The difference between the SH groups determined in the presence of EDTA versus the

TABLE II. METAL CONTENT OF SPUN SOY FIBER<sup>a</sup>

Metal	Amount p.p.m.
Calcium	200
Magnesium	54
Iron	33
Zinc	14
Copper	6
Manganese	2

<sup>a</sup>The cations were determined by atomic absorption.

determination in the absence of EDTA is probably due to the presence of metal mercaptides formed by the free SH groups and the various cations which are present in spun soy fiber (Table II).

In Fig. 1, the changes which occur in the amounts of free SH groups during storage of the fiber are presented. Little change is noticed in the fiber stored at low temperature ( $-20^{\circ}\text{C}$ ). At higher temperatures the number of free SH groups of spun soy fiber decreases with time, while the number of SS bonds increases. The stability of antioxidant-treated fiber and EDTA-treated fiber was greater than that of untreated fiber, under the same storage conditions. At elevated temperature storage, namely,  $38^{\circ}\text{C}$ . for 24 hr. the untreated, ascorbic acid-treated, and EDTA-treated fiber contained 27.1, 52.8, and 42.6  $\mu\text{moles}$  of SH per g. protein,

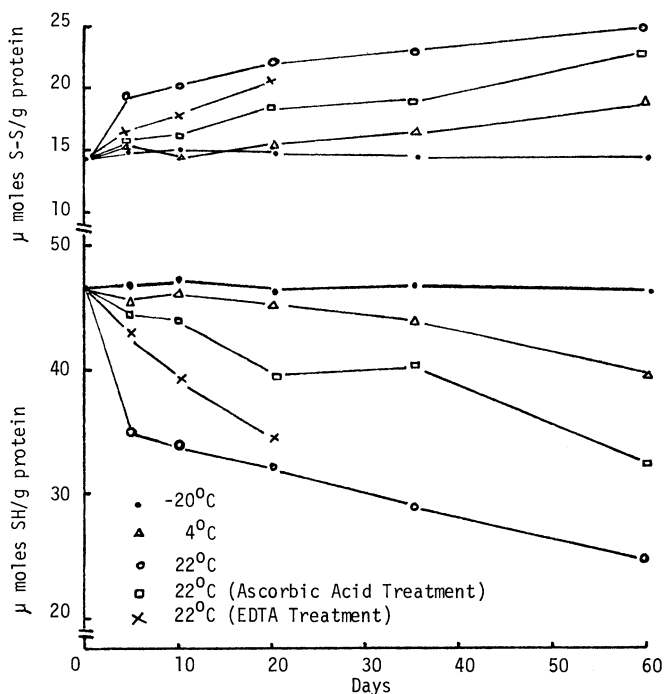


Fig. 1. Change in free SH groups and SS bonds of spun soy fiber during storage.

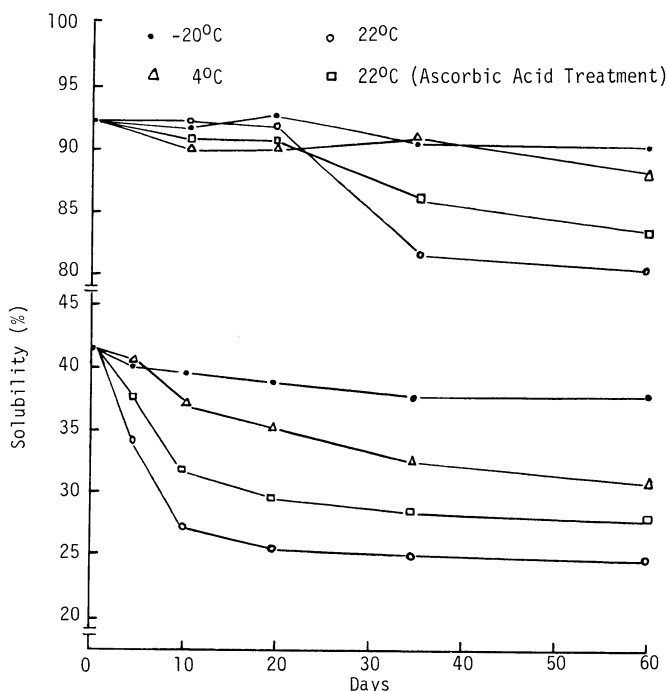


Fig. 2. Solubility of storage spun soy fiber. Top, solubility in SB + 3M urea + 0.1M  $\beta$ -mercaptoethanol; bottom, solubility in SB + 3M urea.

respectively. It is apparent that storage decreases the SH group content due to oxidation.

The effect of minute amounts of copper and iron on strongly catalyzing the oxidation of SH groups to the SS has been amply proved (7). The effect of EDTA in reducing the loss of free SH groups during storage is probably due to complexing the catalyzing metal ion.

Similar changes were observed on the solubility of the stored fiber (Fig. 2). The solubility decrease may be due to the aggregation of protein molecules by SS cross-linking. The solubility determined in the presence of  $\beta$ -mercaptoethanol was nearly the same as for the frozen control for the first 20 days of storage. Above this storage time mercaptoethanol did little to improve solubility, probably because of the prevalence of hydrophobic bonds.

Evidence that SS cross-linking plays an important role in the aging of spun soy protein was obtained from the disc electrophoresis determinations (Fig. 3). As the fiber ages and toughens, a fast mobility peak disappears from the electropherogram and changes can be noticed in the area of slow mobility (right of picture). The disappearing peak can be restored if mercaptoethanol is added to the sample solution, thus cleaving the SS linkages.

Storage of nonformulated soy fibers is accompanied by changes in appearance, such as loss of elasticity, a gray off-color, and toughness. These signs of fiber aging correlate to a decrease in free SH groups.

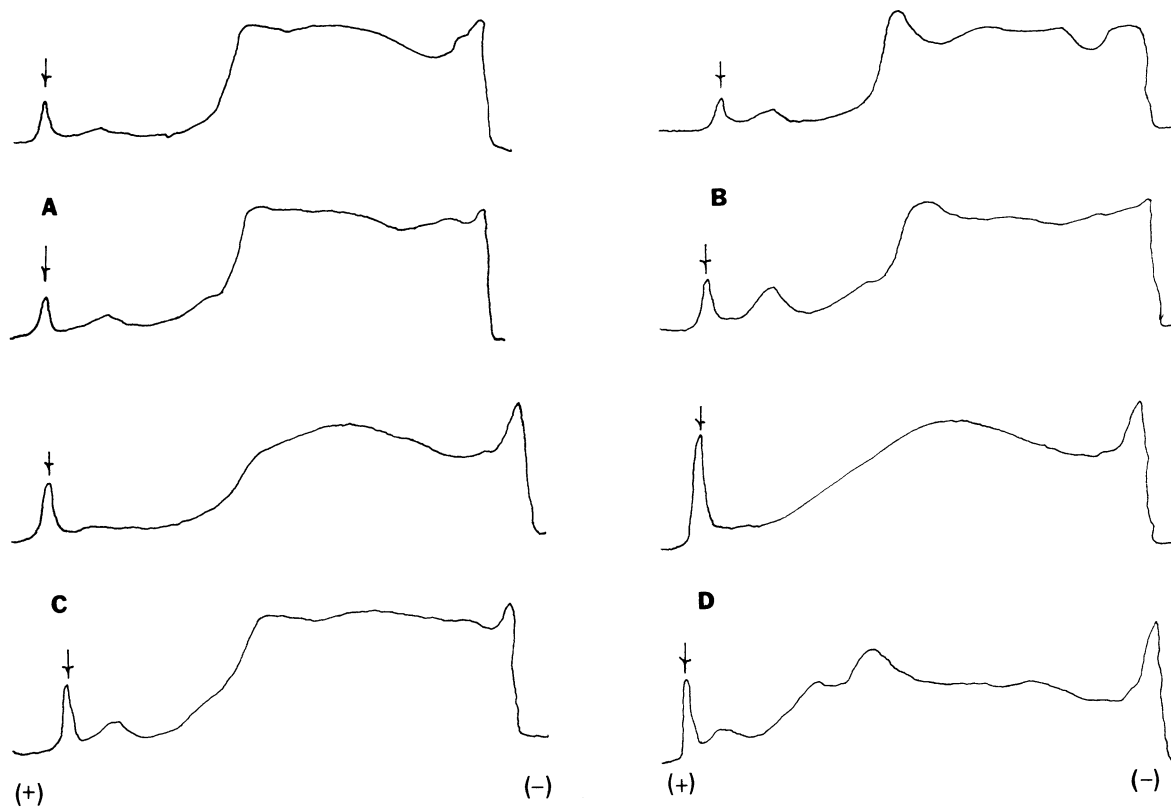


Fig. 3. Densitometric scans of proteins of spun soy fiber at 570 nm. Proteins were stained with aniline blue-black. Arrow indicates position of tracking dye. A = fresh fiber; B = fiber stored 2 months at  $-20^{\circ}\text{C}$ .; C = fiber stored 2 months at  $4^{\circ}\text{C}$ .; D = fiber stored 2 months at  $22^{\circ}\text{C}$ . Solvents: SB + 8M urea (top), and SB + 8M + 0.1M  $\beta$ -mercaptoethanol (bottom).

TABLE III. WATER RETENTION BY FIBERS AFTER STORAGE FOR 2 MONTHS

Storage Temperature °C.	Water Retention Capacity, %			
	5 min.	10 min.	20 min.	30 min.
-20	90	81	76	68
4	88	77	69	62
22	72	62	60	59
22 <sup>a</sup>	80	67	63	60

<sup>a</sup>Ascorbic acid treatment.

The data in Table III show that fibers aged at temperatures other than  $-20^{\circ}\text{C}$ . retain less water. The material stored at  $-20^{\circ}\text{C}$ ., the control, showed the same water retention volumes at 2 months as the freshly prepared fiber. Treatment with ascorbic acid did improve the water retention capacity by preventing aggregation of molecules within the fiber. Saio et al. (8,9) found that tofu made from soybean protein became harder and stronger as the number of SS groups increased. The fine structure of hard tofu appeared to be aggregated, whereas the soft tofu gel appeared homogenous.

Storage of unfrozen soy fibers which have not been formulated into an end product results in changes in elasticity, color, toughness, syneresis, and water absorption capacity. Aging of the fibers during storage correlates with a decrease of free SH groups and aggregation through SS formation. It seems most likely that the changes of appearance are due to the compacting of the protein aggregates and loss of water from the fibers.

#### Literature Cited

1. BOYER, R. A. U.S. Patent No. 2,682,466 (1954).
2. ANSON, M. L., and PADER, M. U.S. Patent No. 2,879,163 (1959).
3. WESTEEN, J. W., and KURAMOTO, S. U.S. Patent No. 3,118,959 (1964).
4. KELLEY, J. J., and PRESSEY, R. Studies with soybean protein and fiber formation. *Cereal Chem.* 43: 195 (1966).
5. ELLMAN, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82: 70 (1959).
6. ROBYT, J. F., ACKERMAN, R. J., and CHITTENDEN, C. G. Reaction of protein disulfide groups with Ellman's reagent: A case study of the number of sulfhydryl and disulfide groups in *Aspergillus oryzae*  $\alpha$ -amylase, papain and lysozyme. *Arch. Biochem. Biophys.* 147: 262 (1971).
7. CECIL, R., and McPHEE, J. R. The sulfur chemistry of proteins in advances in protein chemistry. Vol. XIV, p. 293. Academic Press: New York and London (1959).
8. SAIO, K., KAMIYA, M., and WATANABE, T. Food processing characteristics of soybean 11S and 7S proteins. Part I. Effect of difference of protein components among soybean varieties of formation of tofu-gel. *Agr. Biol. Chem. (Tokyo)* 33: 1308 (1969).
9. SAIO, K., KAJIKAWA, M., and WATANABE, T. Food processing characteristics of soybean proteins. Part II. Effect of sulfhydryl groups on physical properties of tofu-gel. *Agr. Biol. Chem. (Tokyo)* 35: 890 (1971).

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