Food Structure

Volume 5 | Number 1 Article 5

1986

Freeze Texturation of Proteins: Effect of the Alkali, Acid and Freezing Treatments on Texture Formation

Frisco I. Consolacion

Paul Jelen

FOOD MICROSTRUCTURE, Vol. 5 (1986), pp. 33-39
SEM, Inc., AMF O'Hare (Chicago), IL 60666-0507 U.S.A.

0730-5419/86$1.00+.05

FREEZE TEXTURIZATION OF PROTEINS: EFFECT OF THE ALKALI, ACID AND FREEZING TREATMENTS ON TEXTURE FORMATION

Frisco I. Consolacion and Paul Jelen

Department of Food Science
University of Alberta
Edmonton, Alberta, T6G 2P5
Canada

Abstract

The effects of alkali extraction, isoelectric precipitation, and rate of ice crystal formation on protein interaction in the freeze texturization process were studied. The protein isolate was obtained from mechanically deboned poultry meat residues by extraction with NaOH at pH 10.5 and precipitation by HCl at pH 5.0. The presence of large molecular weight subunits in the protein isolate was revealed by SDS-polyacrylamide gel electrophoresis. Scanning electron microscopy demonstrated the nature of the fiber formation and protein cross-linking as affected by freezing at -25°C or -196°C. The transformation of the level of protein organization from the lumpy or granulated nature of the unfrozen sample to the highly spongy and randomly oriented protein mass (-196°C) or to the highly organized, parallel sheets of interconnected, unidirectionally frozen protein (-25°C) was illustrated.

Initial paper received March 28 1985
Manuscript received April 14 1986
Direct inquiries to P. Jelen
Telephone number: 403 432 2480 /3236

KEY WORDS: Freeze texturization, unidirectional freezing, protein structures, fiber formation, protein cross-linking, texture formation, Scanning Electron Microscopy.

Introduction

The primary aim of food protein texturization is to simulate the texture of meat or other structured foods. The three main processes employed in protein texturization are spinning, extrusion and freeze texturization. The recent work of Lawrence (1981) contains an extensive review of these protein texturization techniques.

A variety of protein materials from conventional (e.g. milk, fish, meat and egg) and non-conventional (e.g. beans, blood, offal and meat deboning residues) sources can be used as raw materials for the production of texturized food products. However, according to Hartman (1978) there are some common molecular structure requirements for a protein to be suitable for fiber formation such as high molecular weight (greater than 10,000), long linear chain length, high degree of linear symmetry, absence of high ratio of bulky side chains and high degree of polarity. These structural requirements are necessary for development of orientation and crystallization among the molecular chains.

The basic principle involved in any protein texturization process is to convert the native, non-fibrous protein into a fibrous form; this may be accomplished by a series of treatments that change intramolecular stereochemistry and develop intermolecular structural arrangement of polypeptides in the protein chains. Unfolding of the native globular protein followed by reorganizing into a more aligned and cross-linked state imparts a higher degree of physical strength in the resulting fiber (Huang and Rha, 1974). This transformation of the native globular protein into edible fibrous protein can be achieved by a strong alkali treatment which dissolves and denatures the protein resulting in a solution of random coils (Shen and Morr, 1979; Kelley and Pressey, 1966; Kinsella, 1978) to be processed by a suitable texturization technique. Further, protein polymerization via disulfide bond formation is also favoured under the alkaline condition (Fukushima, 1980; Kelley and Pressey, 1966). It appears that the main differences between extrusion, spinning and freeze texturization lie in the extent to which the conformational changes

in the protein molecule are achieved. The phenomenon of protein fiberization during texturization was demonstrated by microscopic techniques such as light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Kazemzadeh *et al.*, 1982; Taranto *et al.*, 1978a, 1978b; Cegla *et al.*, 1978; Taranto and Rhee, 1978; Aguilera *et al.*, 1976; Maurice *et al.*, 1976; Cumming *et al.*, 1972).

In the production of freeze-texturized soy protein known as "Kori-tofu", unfolding of the globular proteins (2,7,11,15S components; molecular weights ranging from 32,000 to 320,000 daltons) is accomplished by heat treatment (Hashizume *et al.*, 1971; Wolf, 1970). This process exposes the masked sulfhydryl groups needed for the disulfide bond formation (Fukushima, 1980). This stable covalent bond plays an important role in the texture of the product (Saio *et al.*, 1971; Saio *et al.*, 1969; Saio and Watanabe, 1978). The calcium coagulated, heat denatured soy protein is frozen at -10°C and stored at -1°C to -3°C for about 3 weeks to allow further polymerization of the proteins via disulfide, hydrogen, ionic and hydrophobic bonds (Hashizume *et al.*, 1971; Fukushima, 1980). Hydrogen bond formation should be enhanced at this low temperature in view of the negative enthalpy that characterizes this process (Taborsky, 1979). These chemical reactions take place in the concentrated unfrozen liquid part of the material (Hashizume *et al.*, 1971).

In practice, the soy protein curd or solution is frozen in such a way that heat removal takes place in all directions, resulting in a spongy product that lacks the fibrous orientation characteristic of meat structure. In contrast, a parallel oriented protein mass which is retained after freeze drying can be obtained following the freeze-alignment process introduced by Lugay and Kim (1981). The technique is to freeze the protein solution in a manner and at a rate such that elongated ice crystals are generated unidirectionally. Hence, the proteins and other solutes are concentrated in the spaces between the ice crystals forming distinctly aligned parallel zones. Lawrence and Jelen (1982) investigated the effects of pH, total solids and freezing rate on the texturization of proteins extracted from mechanically deboned poultry meat residues. In a review of the various freeze texturization processes, Lillford (1985) highlighted the following critical steps: separation of phases on freezing (ice and concentrated solute or suspension), orientation of ice crystals and hence passive orientation of the concentrated phases of fibrous final structures, and fixation of the structure by formation of new chemical bonds.

Freeze texturization of proteins is still poorly understood and little information on the exact nature of the events occurring in the process is available. The study reported herein was conducted with the following objectives: a) to determine the molecular weight distribution in the alkali extracted, acid-precipitated chicken protein before subjecting it to the freeze texturization process; b) to determine the effect of freezing rate on the structural orientation of the freeze-texturized protein mass, and c) to illustrate, by scanning electron microscopy, the events leading to texture formation in freeze texturization of alkali solubilized poultry proteins.

Materials and Methods

Sample Preparation

Mechanically deboned poultry meat residues were obtained from a local poultry processing plant. The bone residues were collected during the deboning operation and immediately extracted following the procedure described earlier (Lawrence, 1981; Lawrence and Jelen, 1982). Briefly, the procedure consisted of mixing the ground bones with sufficient 20% NaOH to obtain pH 10.5, centrifuging (27,300 x g), acidifying the supernatant to pH 5.0, and centrifuging again (2,520 x g) in the same centrifuge (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Instrument, U.S.A.). About 150 grams of the fresh protein isolate (containing 9% protein by microKjeldahl) was frozen in an aluminum dish (7.0 cm x 3.3 cm) fitted at the center of a styrofoam block (17 cm x 17 cm x 10 cm) to insulate all sides except the top portion of the dish (Figure 1). This freezing method was adopted from Lugay and Kim (1981) and was used to achieve unidirectional freezing of the protein mass. The samples were frozen at -25°C and held at this temperature for 48 hours. After freezing

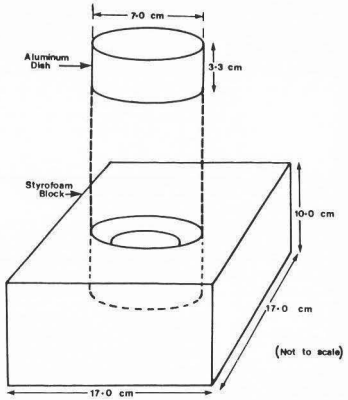


Figure 1. Schematic representation of the freezing mould used in the unidirectional freezing technique.

some samples were freeze dried while others were heat set by autoclaving at 121°C, 1.034 bar for 15 minutes and then cooled at room temperature. Some samples were frozen by direct immersion of the uninsulated dish in liquid nitrogen to illustrate the effect of the freezing rate on the textural pattern formed by non-directional freezing. The frozen sample was thawed in 95% ethyl alcohol for stabilization and dehydration. Some freeze texturized samples were freeze dried in order to fix the structure to see whether any possible alteration might have been caused by stabilizing the protein using ethyl alcohol.

Different sample preparations were used in order to demonstrate fiber formation and cross-linking in the acid-precipitated protein isolate. The protein isolate was washed with 500 ppm of EDTA, followed by washing with deionized water, to remove the extraneous, water-soluble materials that could have masked the protein fibers. To find out whether fiberization and cross-linking was present in the acid-precipitated protein, a fresh sample was analyzed by electron microscopy after immersion in 95% ethyl alcohol to stabilize and dehydrate the protein.

Microscopy

Texturized protein samples, fixed by freeze drying or heat setting, were fractured into about 0.5 cm^3 and then dried by the critical point drying technique with liquid CO_2 for microscopic analysis. The samples were mounted on aluminum stubs with silver conducting paint and sputter coated with gold to a thickness of 150 Å. Scanning electron microscopic examination was performed with a Cambridge Stereoscan 250 at 25 kV.

Determination of Molecular Weight

The molecular weight distribution of the protein in the isolate was determined using sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein isolate was purified following the method described by Parsons and Lawrie (1972) wherein the extract was homogenized in three volumes of 0.1 N HCl in 75% ethyl alcohol and then centrifuged at 30,000 x g for 30 minutes. The supernatant was discarded and the pellet was resuspended in five volumes of acetone. The suspension was left for 3 hours and centrifuged again. The acetone extraction was repeated and the pellets were then dried at 50°C and finely ground. The protein content of the dried protein powder was 95%, as determined by the microKjeldahl method. The protein powder was dissolved in 1 ml of sample buffer containing 0.05M Tris-HCl (pH 6.8), 1% SDS, 0.01% bromophenol blue, 30% glycerol and 20 µl of 2-mercaptoethanol. The buffer was heated in a boiling water bath for 3 minutes prior to use.

The proteins were resolved in acrylamide gels using a vertical slab electrophoresis (Electrophoresis Cell Model 220) following the procedure outlined by Bio-Rad Laboratories (1977), based on the design described by Akroyd (1967) and Laemmli (1970). A more detailed description of the method had been reported by Consolacion (1986).

The chemicals used in the SDS-PAGE were obtained from Sigma Chemical Company (U.S.A.). The standard proteins (Pharmacia Fine Chemicals, Uppsala, Sweden) used in this study as standards were carbonic anhydrase (29,000), egg albumin (45,000), bovine albumin (66,000), phosphorylase B (97,400), ß-galactosidase (116,000) and myosin (205,000).

Electrophoresis was carried out at 30 mA for 3-4 hours or until the tracking dye reached the bottom of the gel. After the electrophoretic run, the gel was stained in a solution of 45% methanol, 10% acetic acid and 0.35% Coomasie Blue R-250 for 12 hours and then destained in a solution of 10% acetic acid and 30% methanol. The mobilities of each protein subunit were calculated and the molecular weights estimated from the standard curve, obtained by plotting the log molecular weight versus mobilities of the standard proteins (Weber and Osborn, 1969).

Results and Discussion

The alkali treatment of the mechanically deboned poultry meat residues solubilizes both the myofibrillar and sarcoplasmic components of the muscle proteins. Upon acidification of the alkali extract from pH 10.5 to 5.0, the myofibrillar proteins are mainly precipitated while the sarcoplasmic proteins remain in the supernatant solution (Lawrence, 1981; Young, 1976; Young, 1975). A negligible amount of collagen is extracted under these conditions (Lawrence, 1981).

The electrophoretograms of the resolved, acid-precipitated protein and the corresponding molecular weights of the major bands are shown in Figure 2. The numerous bands indicate a wide range of protein subunits present in the isolate. Quite evident is the presence of the dark bands which indicate high protein concentrations, two of which should correspond to myosin and actin with molecular weights of 220,000 and 46,000-47,000 daltons, respectively (Hofmann and Hamm, 1978). In a similar study using poultry deboning residues, Kijowski and Niewiarowicz (1985) suggested that the presence of numerous protein bands in the electrophoretogram was due to the occurrence of proteins not only from meat residues but also from the skin and bone marrow. The majority of the salt soluble proteins recovered in the bands were of the molecular weight 17-21 x 10^3 and 38-47 x 10^3 daltons, from which they suggested the presence of the following proteins: actin, tropomyosin, the light chains of myosin and troponin and the degraded products of the heavy chains of myosin, possibly due to enzymic breakdown resulting from liberation of lysozomal enzymes by the deboning process.

The occurrence of quite large molecular

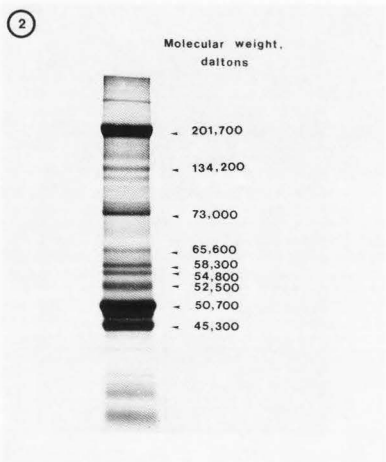
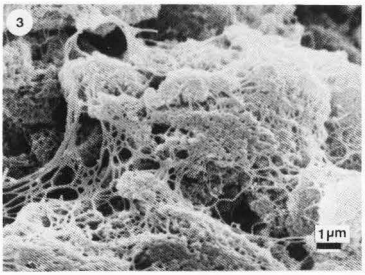


Figure 2. SDS-polyacrylamide electrophoretograms of protein extracted from mechanically deboned poultry meat residues at pH 10.5 and acid-precipitated at pH 5.0.



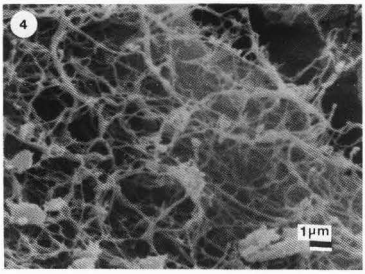


Figure 3. Fiber formation in fresh, acid-precipitated protein isolate.

Figure 4. Fiber formation and cross-linking in freeze-texturized, freeze dried protein.

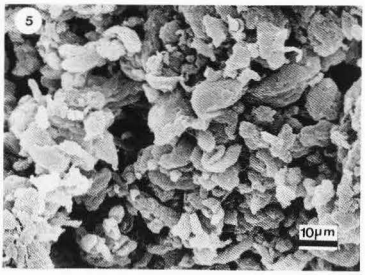
weight components in our protein isolate may be indicative of protein-protein interactions during the alkali extraction. Protein cross-linking is an important requirement for fiber formation and texturization (Hartman, 1978; Kelley and Pressey, 1966). The tensile strength of the fibers increases with increasing subunit chain length until a limiting plateau is reached at approximately 200 residues resulting in sub-unit chain molecular weight of ca. 22,000 daltons (Shen and Morr, 1979). Much larger sub-units do not cause an increase in the tensile strength and even can be detrimental to fiber formation (Shen and Morr, 1979; Huang and Rha, 1974).

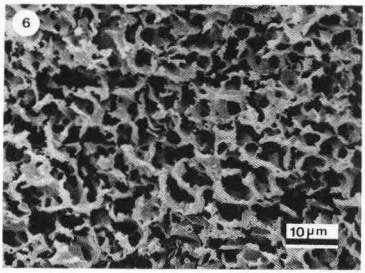
The presence of protein interactions leading to fiber formation and extended cross-linking was clearly demonstrated in the acid-precipitated, unfrozen protein isolate, as shown in Figure 3. These results strongly depict the occurrence of protein interactions during the acidification of the alkali extract from pH 10.5 to 5.0; needed for the isoelectric precipitation. In our study, the protein samples used for electrophoresis were dissolved in the sample buffer containing 2-mercaptoethanol which showed relatively higher solubility of the protein than in the absence of this reducing agent. Kelley and Pressey (1966) postulated that alkaline conditions favor disulfide bond formation while acidification brings many polypeptide chains close together, favoring hydrogen and ionic bonding. The formation of the disulfide bonds in our protein sample seemed possible, considering the sulfhydryl groups present in the fresh protein isolate (Consolacion, 1986).

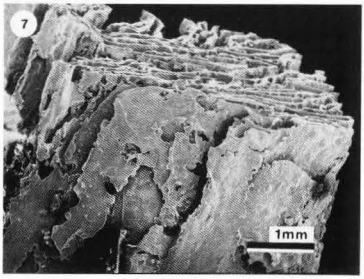
A network similar to that in the unfrozen protein was also observed in the freeze texturized, freeze dried protein as shown in Figure 4. It appears that fiber formation and extended cross-linking following the alkali and acid treatments could be preserved and probably promoted during freeze texturization. Hashizume *et al.* (1971) postulated two events that could take place during this process. First, ice crystal formation during freezing brings the protein close together thereby concentrating the fibers between the parallel-oriented ice crystals. Second, further protein-protein interactions occur at the early stages of the freezing process in the unfrozen concentrated solution. The high degree of protein polymerization as a result of the freeze texturization was confirmed by the poor solubility of the thawed sample (prior to heat setting) in the sample buffer for the SDS-PAGE. Rhee *et al.* (1981) also employed the SDS-PAGE technique to determine the molecular weight distribution of the extruder-textured protein and indicated that

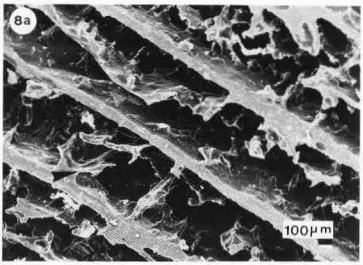
non-covalent forces (hydrogen, ionic, and hydrophobic forces) and sulfhydryl-disulfide interchange reactions occur during extrusion texturization.

The macroscopic transformation of the protein organization as affected by the freezing technique is illustrated in Figures 5-8. Figure 5 shows the lumpiness or granulated nature of the unfrozen, acid-precipitated protein isolate. Rapid freezing of this sample by direct immersion in liquid nitrogen resulted in a highly porous, and randomly oriented protein mass (Figure 6). The small voids represent the area formerly occupied by the numerous ice nuclei formed by the









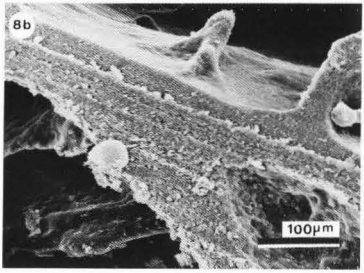


Figure 5. Unfrozen, acid-precipitated protein isolate in the untexturized state.

Figure 6. Acid-precipitated protein frozen in liquid nitrogen showing random orientation of the protein sheet structures and high degree of porosity.

Figure 7. Unidirectionally, freeze-texturized protein showing parallel sheet-like structures.

Figure 8. Unidirectionally, freeze-texturized and heat-set protein: (a) interconnected parallel sheets (arrow), and (b) a single sheet showing homogeneity.

rapid freezing (Fennema, 1973). In contrast, unidirectional freezing resulted in a very different sheet-like parallel orientation of the fibrous protein mass on the macrolevel (Figures 7 and 8). Similar results were shown by Lugay and Kim (1981) in freeze texturizing soy protein and by Lawrence and Jelen (1982) for poultry protein extracts. As these authors also noted, the sheets of proteins are not completely independent of each other and are joined at sufficient

locations to provide a cohesive fibrous mass (Figure 8a).

A closer analysis of the cross-section of protein sheet structures (Figure 8b) reveals the homogeneity of the protein mass. There is a distinct contrast in appearance of the freeze texturized samples from that of the original material (Figure 5), demonstrating the transformation of the level of organization of proteins as a result of freezing. The fiberization and extended cross-linking and the mechanical compression by the ice crystals had provided a considerable amount of cohesive forces that enabled the texturized protein to maintain its structural integrity during thawing at room temperature and subsequent heat setting.

There is no doubt that the freezing process causes protein concentration, however, the mechanical force exerted by the growing ice crystals may not be enough to align and stretch the randomly oriented protein fibers. There appears to be two levels of orientation exhibited by the freeze texturized protein, namely the distinctly parallel oriented protein sheets and the randomly arranged and totally entangled fibers compressed in these protein sheets, a product characteristic that would differentiate the freeze-alignment process from the other methods for protein texturization.

Conclusions

The slowly growing literature on freeze texturization appears to emphasize the occurrence of protein-protein interactions in the unfrozen liquid part of the protein solution during frozen storage (Hashizume *et al.*, 1971). However, only a few workers demonstrated possible protein polymerization during the pretreatments undergone by the protein material; in our view, this is an important event prior to the texturization. In our study, the alkali extraction and isoelectric precipitation of proteins provided favorable conditions for fiber formation and cross-linking of the freshly extracted proteins. This protein cross-linking was preserved and probably promoted as a result of freeze concentration. The retention of the structural integrity of the freeze-texturized protein during thawing at room temperature and heat setting provided tentative evidence of the strong chemical bonds formed during protein cross-linking and the compressive force exerted by ice crystals. The parallel, interconnected protein mass, characteristic of the unidirectional freezing technique, was clearly illustrated.

Acknowledgement

This research was funded in part by the National Sciences and Engineering Research Council of Canada. We are indebted to Mr. George Braybrook from the Department of Entomology, University of Alberta, who assisted us with electron microscopy procedures.

References

Aguilera JM, Kosikowski FV, Hood LF. (1976). Ultrastructural changes occurring during thermoplastic extrusion of soybean grits. J. Food Sci. 41:1209-1213.

Akryod P. (1967). Acrylamide gel slab electrophoresis in a simple glass cell for improved resolution and comparison of serum proteins. Anal. Chem. 19:399-410.

Bio-Rad Laboratories. (1977). Model 220 and 221 Vertical Slab Electrophoresis Cell Instruction Manual. Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario. 5.

Cegla GFM, Taranto MV, Bell KR, Rhee KC. (1978). Microscopic structure of texturized cottonseed flour blends. J. Food Sci. 43:775-779.

Consolacion FI. (1986). An investigation of the freeze texturization mechanism in food proteins. Ph.D. Dissertation. University of Alberta, Edmonton, Alberta.

Cumming DB, Stanley DW, DeMan JM. (1972). Texture-structure relationship in texturized soy protein. II. Textural properties and ultrastructure of an extruded soybean product. Inst. Can. Sci. Technol. Aliment. 5:124-128.

Fennema OR. (1973). Water and ice. In: Low Temperature Preservation of Foods and Living Matter. O. Fennema, W.D. Powrie, E.H. March (eds.). Marcel Dekker. New York. 3-67.

Fukushima D. (1980). Deteriorative changes of proteins during soybean food processing and their use in foods. In: Chemical Deterioration of Proteins. J.R. Whitaker, M. Fujimaki (eds.), ACS Sym. Ser. 123. Am. Chem. Soc., Washington, D.C., 211-240.

Hartman WE. (1978). Texturization through spinning. J. Texture Stud. 9:125-134.

Hashizume K, Kakiuchi K, Koyama E, Watanabae T. (1971). Denaturation of soybean protein by freezing. Part I. Agr. Biol. Chem. 35:449-459.

Hofmann K, Hamm RH. (1978). Sulfhydryl and disulfide groups in meats. Adv. Food Res. 24:2-111.

Huang FF, Rha C. (1974). Protein structures and protein fibers - a review. Polym. Eng. Sci. 14:81-91.

Kazemzadeh M, Aguilera JM, Rhee KC. (1982). Use of microscopy in the study of vegetable protein texturization. Food Technol. 36:111-118.

Kelley JJ, Pressey R. (1966). Studies with soybean protein and fiber formation. Cereal Chem. 43:195-206.

Kijowski J, Niewiarowicz A. (1985). A method of protein extraction from chicken bone residue and the chemical and electrophoretic characteristics of the extract. J. Food Technol. 20:43-49.

Kinsella JE. (1978). Texturized proteins: Fabrication, flavoring, and nutrition. In: CRC Critical Reviews in Food Science and Nutrition. Chemical Rubber Company, Cleveland. 10:147-207.

Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680-685.

Lawrence RA. (1981). Freeze-induced texturization of protein recovered by alkaline extraction from mechanically deboned poultry residues. M.Sc. Thesis. University of Alberta, Edmonton, Alberta, Canada.

Lawrence RA, Jelen P. (1982). Freeze-induced fiber formation in protein extracts from residues of mechanically separated poultry. Food Microstructure 1:91-97.

Lillford PJ. (1985). Freeze-texturing and other aspects of the effects of freezing on food quality. In: Properties of Water in Foods, D. Simatos, J.L. Multon (eds.). Martinus Nijhof Publ., Dodrecht, The Netherlands, 543-552.

Lugay JC, Kim MK. (1981). Freeze alignment: A novel method for protein texturization. In: Utilization of Protein Sources, D.W. Stanley, E.D. Murray, D.H. Lees (eds.). Food and Nutrition Press Inc., Westport, Conn., 177-187.

Maurice TJ, Burgess LD, Stanley DW. (1976). Texture-structure relationship in texturized soy protein. III. Textural evaluation of extruded products. Can. Inst. Food Sci. Technol. J. 9:173-176.

Parsons AL, Lawrie RA. (1972). Quantitative identification of soya protein in fresh and heated meat products. J. Food Technol. 7:455-462.

Rhee KC, Kuo CK, Lusas CW. (1981). Texturization. In: Protein Functionality in Foods. ACS Symp. Series 147. J.P. Cherry (ed.). Am. Chem. Soc., Washington, D.C., 51-88.

Saio K, Watanabe T. (1978). Differences in functional properties of 7S and 11S soybean properties. J. Texture Stud. 98:135-157.

Saio K, Kajikawa M, Watanabe T. (1971). Food processing characteristics of soybean proteins. Part II. Effect of sulfhydryl groups in physical properties of tofu gel. Agr. Biol. Chem. 35:890-897.

Saio K, Kamiya M, Watanabe T. (1969). Food processing characteristics of soybean 11S and 7S proteins. Part I. Effect of difference of protein components among soybean varieties on formation of tofu gel. Agr. Biol. Chem. 33: 1301-1308.

Shen JL, Morr CV. (1979). Physicochemical aspects of texturization: fiber formation from globular proteins. J. Am. Oil Chemists' Soc. 56:63A-71A.

Taborsky G. (1979). Protein alterations at low temperatures: an overview. In: Proteins at Low Temperature, O. Fennema (ed.), ACS Sym. Ser. 180. Am. Chem. Soc., Washington, D.C., 1-25.

Taranto MV, Rhee KC. (1978). Ultrastructural changes in defatted soy flour induced by non-extrusion texturization. J. Food Sci. 43:1274-1278.

Taranto MV, Cegla GF, Bell KB, Rhee KC. (1978a). Textured cottonseed and soy flours: a microscopic analysis. J. Food Sci. 43:767-771.

Taranto MV, Cegla GF, Rhee KC. (1978b). Morphological, ultrastructural and rheological evaluation of soy and cottonseed flours textured by extrusion and non-extrusion processing. J. Food Sci. 43:973-979, 984.

Weber, K., Osborn, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.

Wolf WJ. (1970). Scanning electron microscopy of soybean protein bodies. J. Am. Oil Chem. Soc. 147:107-108.

Young, LL. (1975). Aqueous extraction of protein isolate from mechanically deboned poultry meat. J. Food Sci. 40:1115-1118.

Young LL. (1976). Composition and properties of an animal protein isolate prepared from bone residues. J. Food Sci. 41:606-608.