Preparation of a New Protein Food Material by Freezing

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Reflecting the expected shortage of protein resources in relation to world population growth, an increasing attention has been paid to the direct utilization for food of defatted soybean, a by-product of oil-extraction industry that has so far been used mostly for feedstuff. New techniques for that purpose were developed in the United States more than 10 years ago. One of them is to prepare a fibre-like product by extracting protein from defatted soybean and forcing it jet into the protein-coagulating bath through a large number of small pores, after the alkali treatment. The other method is to prepare a textured protein by heating defatted soybean with small amount of water under a high pressure, and forcing it out to the air, making it foaming. The products thus prepared become a meat-like food when appropriate color, flavor and taste are added, and can substitute partly for meat products. However, these methods require complicated techniques and the products are not so superior in their usability to food.

On the other hand, in Japan soybean has been used for centuries to prepare fermented foods, like Miso and Natto, and also to produce Tofu (soybean curd), Aburaage (fried tofu) and Kori-tofu (dried tofu) by taking advantage of characteristics of protein. Methods of preparing these traditional foods have been improved and selected for many years so that they are regarded as of rational and superior methods of food processing.

At the National Food Research Institute, the study[1] has been carried out to elucidate the freezing denaturation of protein which occurs during the cold storage of Kori-tofu, one of the traditional foods. In the course of this study, a new method to produce textured protein from soybean protein was developed[2,3]. This new method adopts the freezing process of the traditional method of producing Kori-tofu to change protein into textured product, i.e., the application of freezing denaturation.

Freezing denaturation of soybean protein

Before explaining the new method for preparing the textured protein, the freezing denaturation of soybean protein, that plays an important role in the new method will be described.

Tofu is a very soft and fragile gel food. When it is kept frozen for 2–3 weeks, the gel changes into spongy Kori-tofu due to the freezing denaturation of protein. Apart from this, when protein dissolved in water is frozen, it shows a spongy texture because the protein becomes insoluble. Thus the simple freezing treatment can change protein gel or dissolved protein into the textured protein with completely different properties. The mechanism of the freezing denaturation causing such a change is as follows:

When protein gels or solutions are frozen at the temperature of about −5°C, at which the freezing denaturation is easy to occur, most of the water becomes ice crystals, but a part of the water still remains unfrozen, containing various substances. Protein is also contained in the unfrozen water at a concentrated condition. As a result, protein molecules, which were spaced each other at a certain distance, come to be close enough to cause intermolecular reactions. Inter-molecular disulfide bonds are formed by the sulfhydryl-disulfide interchange reaction between molecules, and furthermore hydrogen bonds and hydrophobic bonds are formed, thus giving strong intermolecular bonds. These bonds are not destroyed by the thawing of ice, and the spaces occupied by ice crystals remain as pores of spongy texture (Fig. 1).

The mechanism of the above freezing denaturation experimentally elucidated is shown

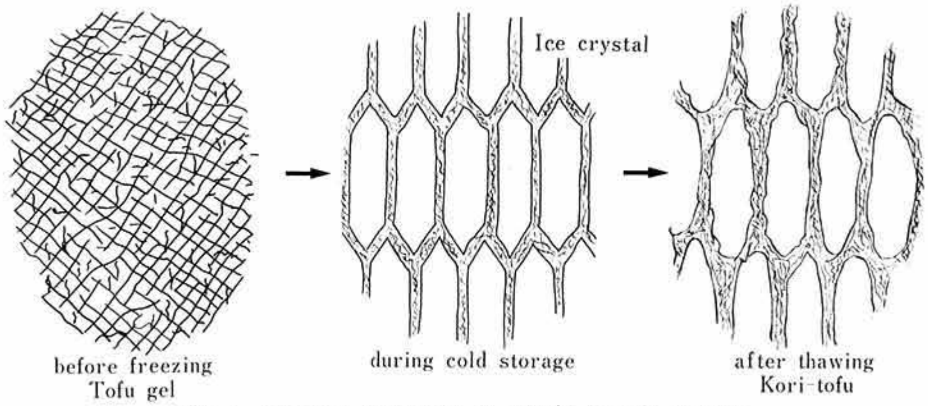

Fig. 1. Changes in texture of protein by cold storage

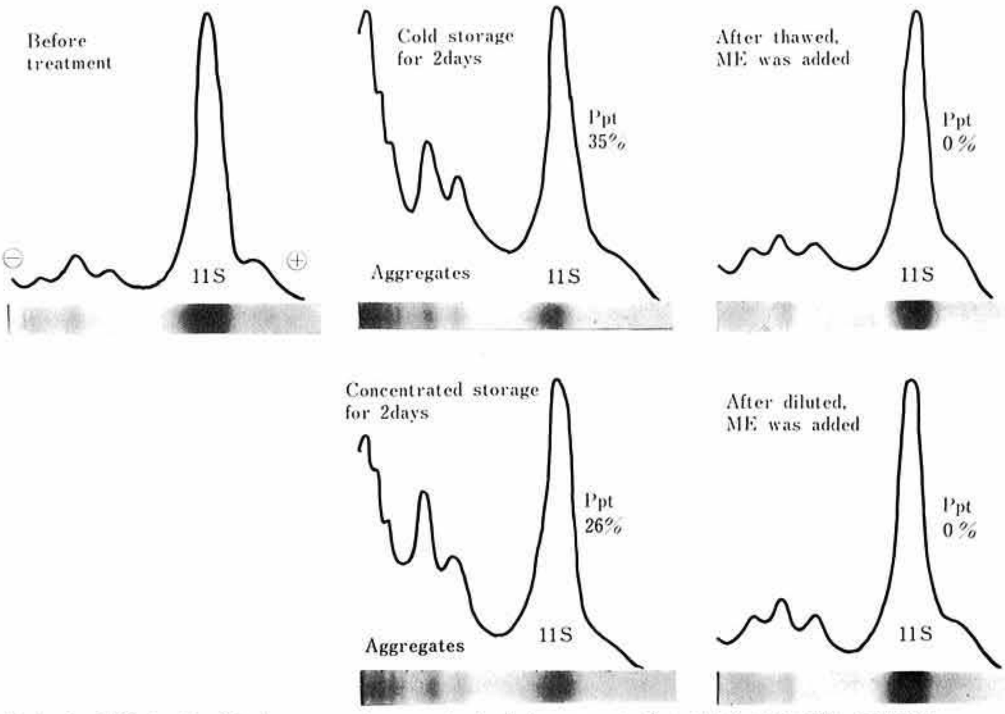

Plate 1. Effect of cold storage and concentrated storage on disc electrophoretic patterns of 11S protein.

Note: ME=0.01 M Mercapto ethanol
Ppt=Precipitate

in Plate 1. A major component of soybean protein, 11S protein, was dissolved in water and divided into two plots. In one plot, it was frozen while in another plot it was concentrated. When it was thawed or diluted with water, a part of the protein in each plots was found to have been insolubilized, as shown by the marked increase of aggregates which were in small amount before the treatments in the disc electrophoretic patterns of supernatants. When mercaptoethanol, a cleavage reagent of disulfide bonds, was added, however, the insoluble portions disappear completely, giving the electrophoretic patterns similar to that of original protein. Thus, the freezing plot and the concentrating plot showed an identical behavior, indicating that the freezing denaturation of protein depends on the concentration effect caused by freezing.

A new protein material obtained by freezing

When the heated soybean protein solution was frozen, the protein was insolubilized, and colorless, translucent and spongy protein was formed. This product is more soft and has higher water-absorbing capacity and water-holding capacity, as compared to the product such as Kori-tofu which was coagulated by using calcium salt and then frozen, and therefore can be used as a superior protein food material. It has an appropriate strength and tolerant to a certain tensile stress (Plate 2). Its inner structure shown in Plate 3 varys with sections. A section at a right angle to the growth direction of ice crystals shows a network, indicating that the protein was concentrated between ice crystals to form film-like gel, while the section made along the growth direction of ice crystals shows a film-like structure with complicated folds. As these structures make up a spongy appearance, it is called spongy protein.

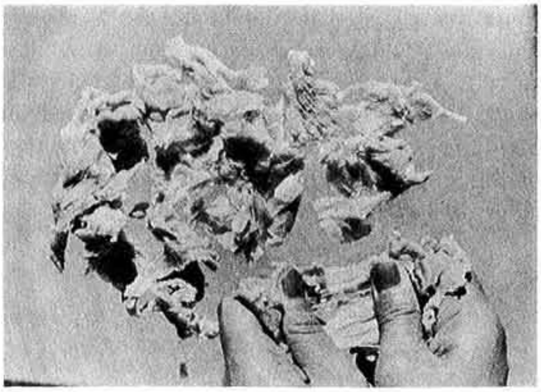

Plate 2. New textured protein formed by freezing

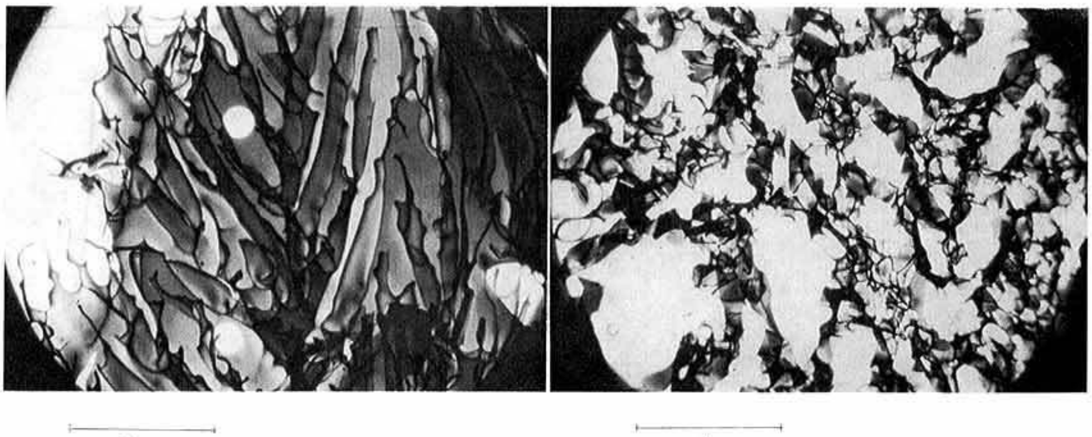

Plate 3. Structure of new textured protein formed by freezing

Conditions of preparing spongy protein

As the spongy protein is formed by the chemical reactions between protein molecules, a pre-heating treatment is made to facilitate the chemical reactions. The treatment is run for a short period (100°C, within 1 min.) not to reduce the sulfhydryl group of protein, which is necessary for the inter-molecular reaction, by oxidation.

Ratio of insoluble protein formed by freezing is about 60% even at a good condition. A result of adding $CaCl_2$, a protein-coagulating agent, before freezing to increase the ratio of insolubilization is shown in Fig. 2. In case when $CaCl_2$ was added to cooled protein after heating at varying concentrations, and the mixtures were kept frozen at −5°C for 3 days, the ratio of insolubilization increased with the increase of $CaCl_2$ added, but beyond the $CaCl_2$ concentration of 3.0×10^{-4} mol/g of protein the produced spongy protein was of poor quality for protein food material, being hard with less water-absorbing capacity. It was concluded that high yields and good quality can be obtained at the $CaCl_2$ concentration of about 2.5×10^{-4} mol/g of protein.

As the spongy protein is characterized by the spongy structure of protein gel formed between ice crystals, its texture must vary depending upon the size of ice crystals. At the freezing temperature of −5°C, large ice-crystals were produced and which caused very coarse textures. A rapid freezing at −70°C produced very minute ice-crystals, leaving very small pores after thawing, and hence the product was incomplete. At the temperature of −20°C, relatively small ice-crystals were produced which left small pores with a complete spongy texture. The texture obtained by a −5°C freezing was hard with strength, whereas that obtained at −70°C was soft and very fragile. Thus, textures with different properties can easily be obtained by adopting different temperatures for freezing. The temperature of about −20°C is considered to

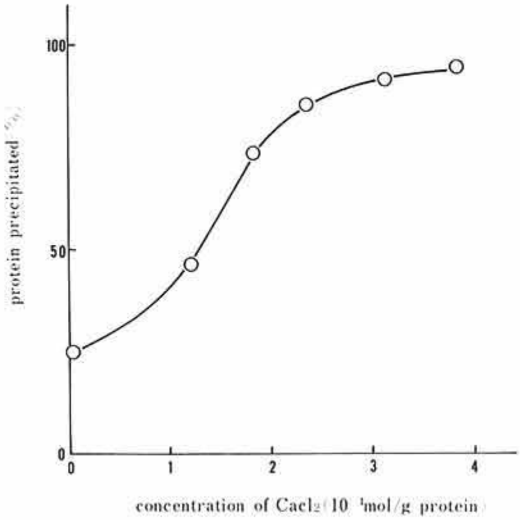

Fig. 2. Insolubilization of soybean protein solution in relation to $CaCl_2$ concentration during cold storage.
Soybean protein solutions were frozen at −20°C and then stored at −5°C for 3 days.

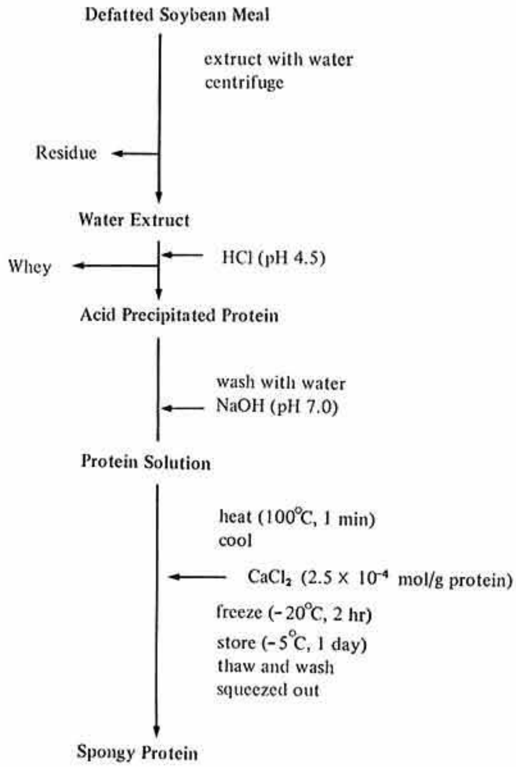

Fig. 3. Scheme of preparation of spongy protein

be appropriate for obtaining the spongy protein to be used as a substitute for animal meat.

The procedure of manufacturing spongy protein is shown in Fig. 3. Protein extracted from defatted soybean is dissolved in water (pH 7.0) at the concentration of 5–10% with dilute alkali. The solution is heated at 100°C for 1 min, and immediately cooled to the temperature lower than 20°C. $CaCl_2$ is added at the rate of 2.5×10^{-4} mol/g of protein; the solution turns to be white-turbid. Immediately, it is transferred to trays, frozen at −20°C, and stored at −2°C for 1 days in a refrigerator. Then, it is thawed, washed with water, and squeezed to remove water. The spongy protein is obtained at the yield ratio higher than 95%.

Characteristics of spongy protein and problems involved

Because the method used for traditional foods in Japan is employed, the procedure of manufacturing is simple. Costly facilities and apparatus, or sophisticated techniques are not required. The product has a soft, spongy texture, and gives no adverse effect when mixed to meat products. These are the characteristics of the spongy protein.

However, there are some problems. As the protein extracted from defatted soybean has to be used as the material instead of the defatted soybean itself, the cost of production increases. Another problem is a difficulty of drying, because the product hardens when it is dried by the usual drying methods such as heated air drying.

References

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