MODIFIED SOYBEAN PROTEIN WITH HIGH WATER-HOLDING CAPACITY

S. OCHIAI-YANAGI, H. MIYAUCHI, K. SAIO, and T. WATANABE, National Food Research Institute, Ministry of Agriculture and Forestry, Shiohama, Koto-ku, Tokyo 135, Japan

Cereal Chem. 55(2): 157-167

ABSTRACT

Soybean protein was modified by various methods to develop high water-holding capacity. The most effective method tried was urea addition and heating in dry form at high temperature in vacuum. Mohammad *et al.* (1) phosphorylated wheat gluten to develop high water-holding capacity by a similar method. However, phosphorylation alone failed to impact water-holding capacity to soybean protein. The highest water-holding capacity was obtained when the added urea concentration was 0.5 *M* and heating temperature was 150°C. A 5% suspension of the modified protein formed a paste without heating. No water could be separated from the paste even when it was centrifuged at 160 × *g* for 5 min. A soft gel was formed when the modified protein was mixed with an eight-fold excess of water and heated. Texture of the gel was like Japanese traditional cakes 'Awayuki' and 'Kibi-Uirou'. The yield of product was about 60% of starting protein material.

Chemical modification of proteins to change their physical, chemical, and biological properties has been widely investigated to provide basic information and to expand their commercial utilization. This work is a trial of such investigations on soybean protein.

Phosphorylated gluten (1) and gluten sulfate (2) were reported to have high water-holding capacities. They absorbed 100–300 times their weight of water and formed firm, odorless, tasteless, and nontoxic gels. Phosphorylation or sulfation was claimed to be essential to gain these properties.

In this paper, various modifications of reported methods to phosphorylate protein and to increase water-holding capacity are examined on soybean protein. Differences between soybean protein and wheat gluten to increase water-holding capacity and the mechanism are discussed.

MATERIALS AND METHODS

Starting Protein Materials

Soybean protein. Defatted soybean flour was stirred in a five-fold weight of warm water (40°C) for 40 min, and centrifuged at 10,000 × *g* for 30 min. The supernatant was filtered through 3 layers of cheesecloth, and extracted protein was precipitated by adjusting the pH of the solution to 4.8 with 1 *N* HCl. The precipitate was collected by centrifugation at 1,000 × *g* for 10 min, and washed with water three times by centrifugation.

Wheat gluten. Wheat flour was mixed with 0.6 times of water and kneaded for 15 min with a farinograph. Kneaded dough was soaked in cold water for 1 hr, and kneaded again by hand for 2 hr in water. Remaining insoluble material was used as wet gluten.

Preparation of Modified Protein

The method of Mohammad *et al.* (1) was followed essentially. Two parts of water was added to 3 parts of acid-precipitated soybean protein or wet wheat

0009-0352/78/000020$03.00/0

gluten. Then urea (indicated quantities in each experiment), ortho-phosphoric acid, or other reagents (if indicated) were added, making the ratio of protein to total water 1:1.4. The slurry was mixed with a homo-mixer, and spread on a tray in a layer of approximately 5 mm thickness and dried by lyophilization. The dried material was ground in a coffee mill into flour, wrapped with aluminum foil, and hung in a flask which was immersed in heated oil. The flask was evacuated with a vacuum pump. The material in the flask was heated for 30 min at the temperature indicated in each experiment. After the heating, a ten-fold amount of water was added, and the pH was adjusted to 7.5 with 0.1 *N* NaOH. The same amount of water was added again to the suspension, and it was stirred for 20 min. The precipitate was collected by centrifugation at 1,000 × *g* for 10 min or by filtration with suction. The precipitate was washed twice with 70% acetone or alcohol and washed twice more with 95–99.5% acetone or alcohol. It was dried in air and kept in a desiccator under vacuum to dry thoroughly.

Measurement of Water-Holding Capacity

Water-holding capacity was measured with a tube devised at the National Institute of Agricultural Science, Japan (Fig. 1). Water was added to modified soybean protein (A g) making the total amount to 10 g. For example, 9.5 g of water was added to 0.5 g of modified protein when the concentration was desired to be 5%. The swollen sample was placed on water-permeable nylon cloth, and centrifuged at 160 × *g* for 5 min. The leakage water (B ml = B g) was measured in the calibrated tube, and the amount of water remained in the sample (C g) was calculated by difference. Thus water-holding capacity (gram water held per gram protein) was C/A.

Texture Measurement of Gel

Physical properties of gels were measured with a 'Texturometer' (General Foods Co., USA). Plunger was Lucite 17 mm in diameter, and platform was flat aluminum cup. Clearance was 2 mm, voltage was 0.5–15 V, and speed was 'low'. The shape of gel sample was a cylinder of about 1.4 cm radius and of 1.5 cm height. The textural parameters were calculated by the method of Friedman *et al.* (3).

Analytical Methods

Phosphorus. Nakamura's method (4) was used, which is a modification of Allen's method (5). Analyses were carried out after washing the samples with 0.5 *M* NaCl twice to remove the weakly bound phosphorus.

Nitrogen. Micro-Kjeldahl method (6) was used.

RESULTS

As shown in Fig. 2A, for modified protein the apparent amounts of water held per gram of protein changed greatly with sample concentration of the measurement. It was larger when more water than capable of being held by the modified protein was added. For example, for No. 5 in Fig. 2A, the apparent water-holding capacity was 13.3 g at 7% sample and 29.4 g at 2%. In Fig. 2B percentage of retained water to added water by protein samples No. 4 or No. 5

became 100% at a 7% sample concentration. However, complete holding of added water required a protein concentration of 30% or more for protein with treatment No. 1 or No. 2. Water-holding capacities (g/g protein) at relatively high concentration of samples, therefore, are not meaningful unless an excess of water is used in the test. On the other hand, because the samples of low protein concentration were not pastes or gels but just aqueous suspension, they were too

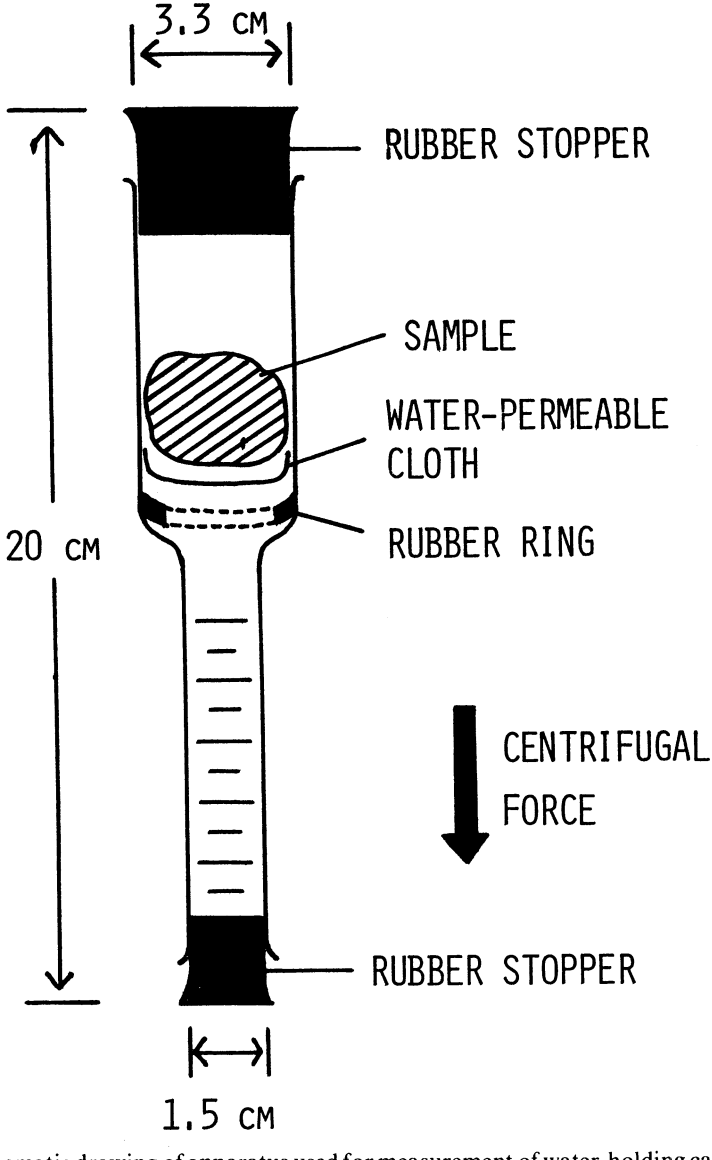

Fig. 1. Schematic drawing of apparatus used for measurement of water-holding capacity.

far from the condition of practical use. Therefore, apparent water-holding capacities at various concentration of proteins were shown here. It is to be emphasized that treatment with urea alone gave as much water-holding capacity as treatment with both urea and phosphoric acid. Phosphorylation did not have a direct relation to water-holding capacity of modified soybean protein as measured by these procedures. Hydrochloric acid may be more effective than phosphoric acid (Fig. 2, No. 2 and 3), suggesting that the role of phosphoric acid in this procedure might be only to make the mixture acidic.

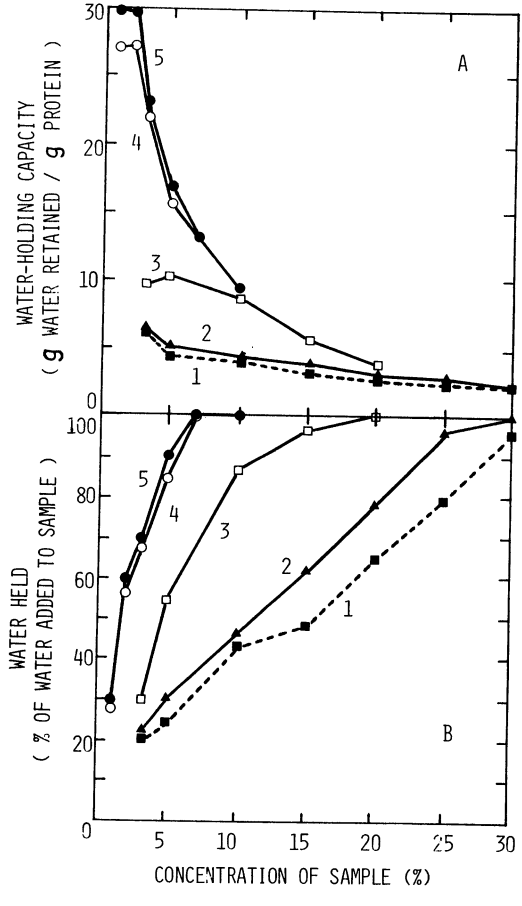

Fig. 2. A. Water-holding capacity of modified soybean protein by variations of Mohammad's method (see **Materials and Methods**). 1. No reagent, 2. phosphoric acid (1.5 *N* final), 3. HCl (1.5 *N* final), 4. urea (0.5 *M* final), 5. phosphoric acid (1.5 *N* final) and urea (0.5 *M* final) were used. Heating temperatures were 150°C. Concentration of Sample means the sample concentration just before measurement of water-holding capacity. B. Amounts of water held (percent of water added before measurement), calculated from the same experiments as A.

Use of phosphoric acid to make modified proteins clearly increased phosphorus contents of the products (Table I), indicating that phosphorylation of soybean protein occurred by this method.

TABLE I
Phosphorus and Nitrogen Content of Modified Soybean Proteins

Reagent[a]	P μg/mg sample[b]	N mg/mg sample	P/N × 10^{-2}
None	3.3	0.14	2.4
Phosphoric acid and urea	7.3	0.12	6.1
Phosphoric acid	14.2	0.14	10.2
Urea	1.5	0.14	1.1

[a]Modified soybean proteins by Mohammad's method using reagents indicated.
[b]A few percent higher than precise dry weight after keeping at 110°C.

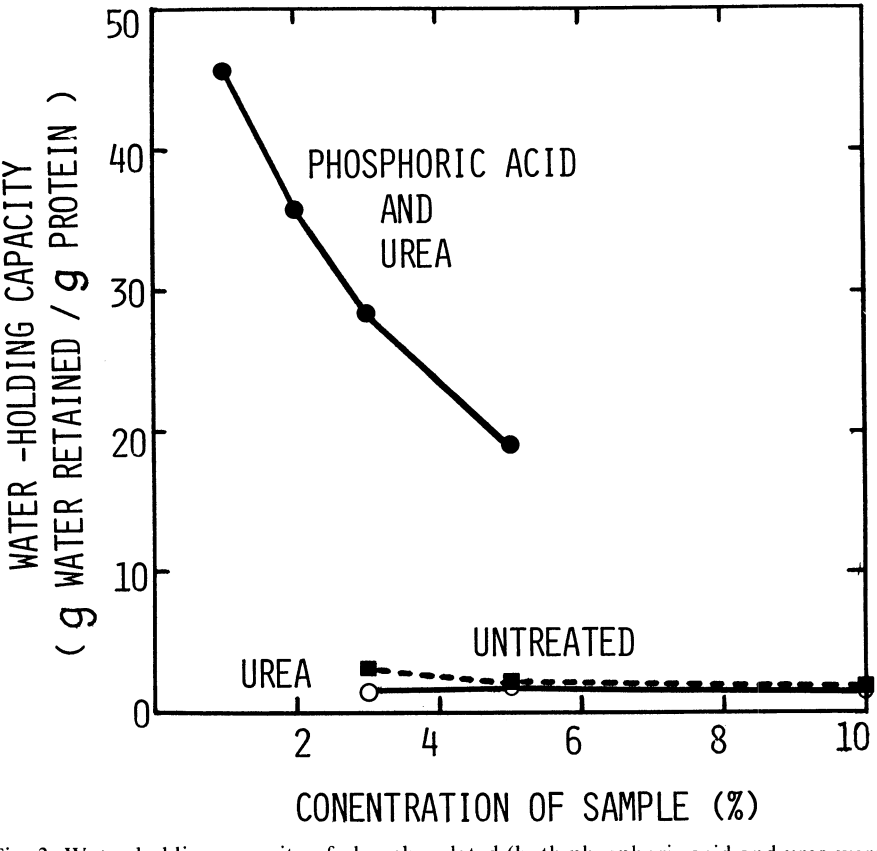

Fig. 3. Water-holding capacity of phosphorylated (both phosphoric acid and urea were used) and urea (only urea was used) treated gluten.

Since Mohammad *et al.* (1) described that phosphorylation or sulfation was essential to increase water-holding capacity of wheat gluten, the procedure was repeated for gluten. Figure 3 shows the striking difference for gluten as compared to results of soybean protein. Phosphorylated gluten showed high water-holding capacity as mentioned before, but the use of urea alone did not increase water-holding capacity of gluten at all.

Optimum conditions to make modified soybean protein with high water-holding capacity, using only urea as a reagent, were examined. As seen in Fig. 4, the most effective concentration of urea was around 0.5 *M* (to the mixture of water and protein, urea was added making the final concentration to 0.5 *M*). Optimum heating temperature in vacuum condition was 150–155°C (Fig. 5). The ratio of acid precipitated soybean protein to water of 1–1.4 gave the best results when urea or other reagents were mixed. As the dry weight of acid-

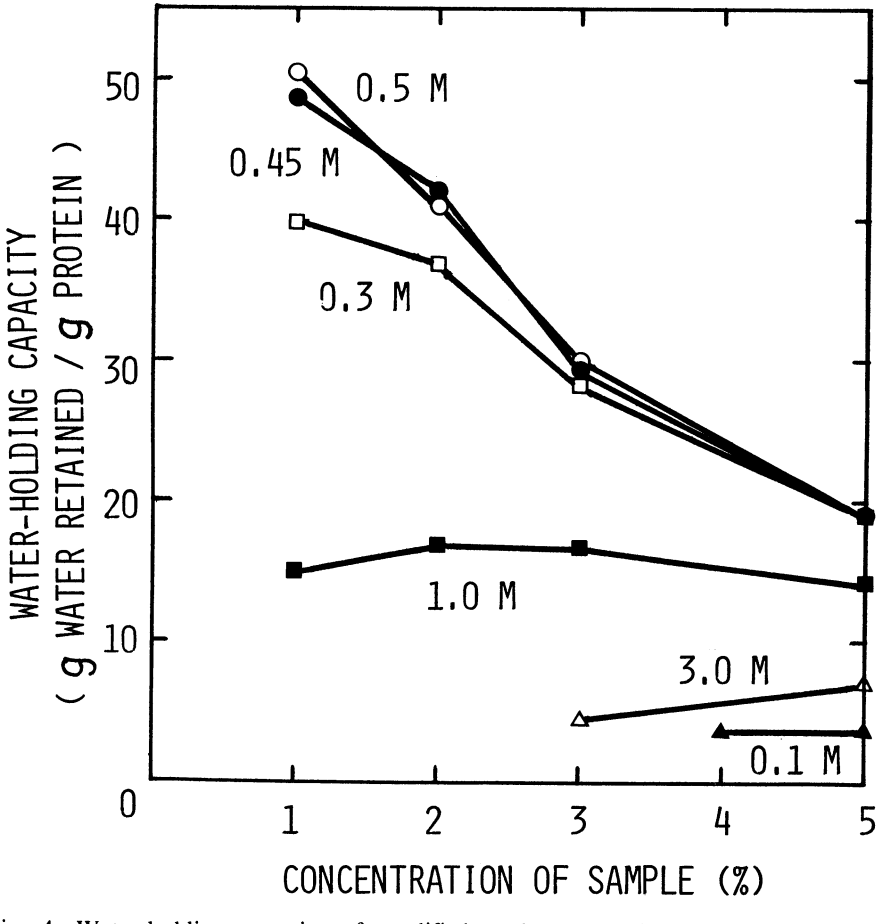

Fig. 4. Water-holding capacity of modified soybean protein made with various concentrations of urea. Heating temperature in vacuum was 150°C for all experiments.

precipitated and centrifugally separated soybean protein was about 60% of its weight, concentration of the protein in the mixture was $0.6/(1 + 1.4) = 25\%$. More dilute protein mixtures need a longer time to dry and more concentrated ones were difficult to mix uniformly. Lyophilization was the best way of drying to obtain clean, light color, good odor and texture of the product, as compared with an air or vacuum oven, or spray dryer. Modified protein prepared this way was yellowish light brown and had no taste or odor.

Yield of the final product was about 60% of starting acid-precipitated protein on a dry weight. Considerable amounts of protein were recovered from the water and organic solvent used for washing in the procedure, if the pH of the waste solution was adjusted to 4.8.

A soft gel was formed when 1 part of the modified protein was kneaded with 8 parts of water, packed in a saran tube and heated in a water bath at 70–100°C for 30 min. A texturometer pattern of the gel was compared with those of other

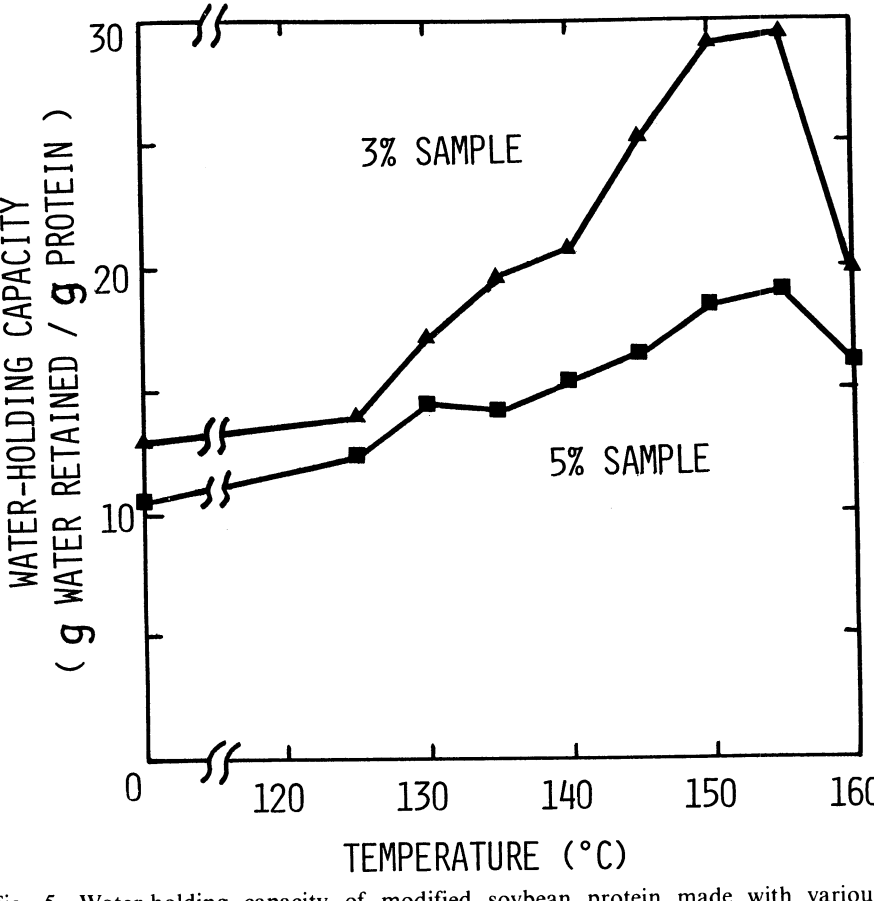

Fig. 5. Water-holding capacity of modified soybean protein made with various temperatures. All samples were made with 0.5 *M* urea. Water-holding capacity was measured with the samples of 3 and 5% concentrations.

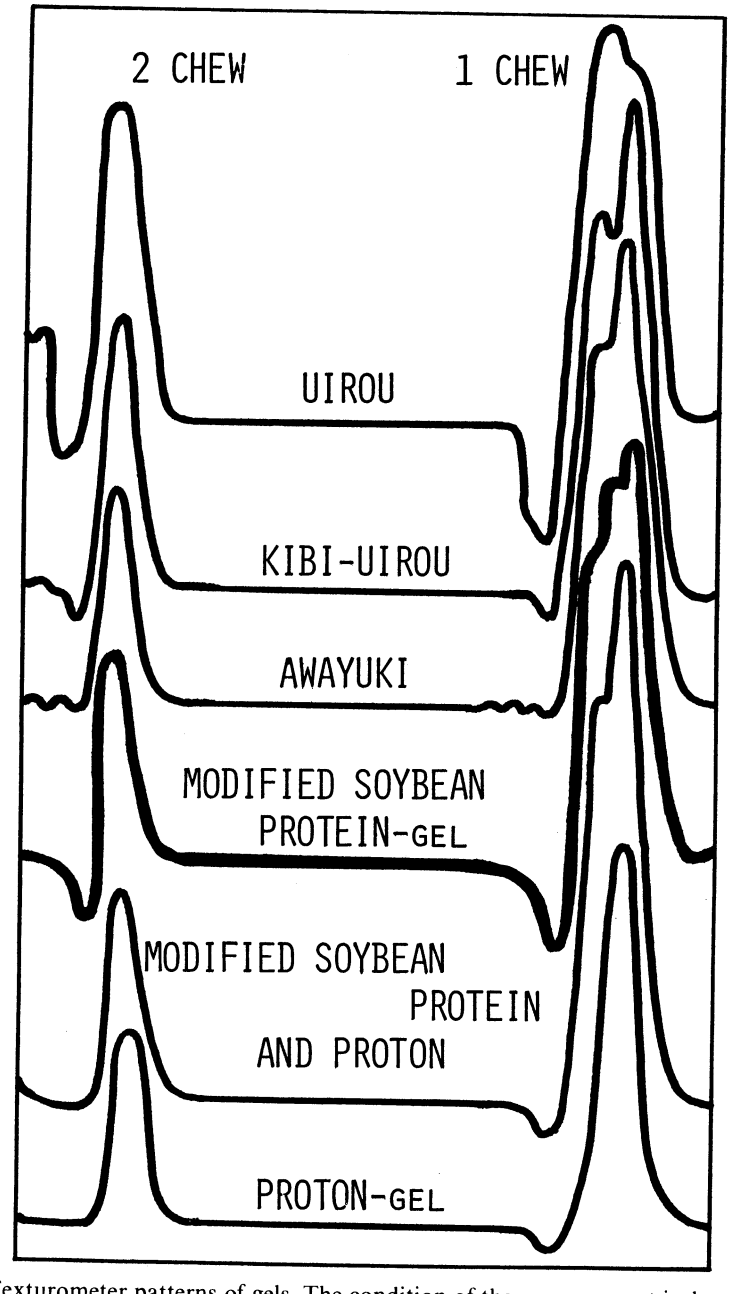

Fig. 6. Texturometer patterns of gels. The condition of the measurement is described in the **Materials and Methods**. 'PROTON' is spray dried soy milk. 'Uirou' and 'Kibi-Uirou' are Japanese traditional cakes made mainly from boiled rice flour and millet seed flour, respectively. 'Awayuki' is also Japanese traditional cake made by gelatinizing egg white, agar, and sugar. See **Results** for further explanation.

products (Fig. 6). PROTON-gel was made from 1 part of spray dried soy milk called 'PROTON' (NIHON TANPAKU Co. = Japan Protein Co.) and 3 parts of water. A mixed gel of modified soybean protein and PROTON was made from 3 parts of the former, 8 parts of the latter and 48 parts of water. (This ratio was equivalent to the mixture of half of plain modified protein gel and half of plain PROTON gel.) 'Uirou' and 'Kibi-Uirou' are Japanese traditional cakes made mainly from boiled rice flour and millet seed flour, respectively. 'Awayuki' is also a Japanese traditional cake made by gelatinizing egg white, agar, and sugar. Calculated parameters from the texturometer patterns are presented in Table II. A clear difference was seen in hardness; modified soybean protein gel was considerably softer than other gels. But other parameters—cohesiveness, elasticity (springiness), adhesiveness, and peak pattern were very similar to some one of other gels, especially to Kibi-Uirou and Awayuki. Gel made from spray dried soy milk PROTON had a distinctly different texture than modified protein gel.

Effects of other reagents on water-holding capacity of soybean protein modified by Mohammad's method were studied (Table III). Instead of phosphoric acid and urea, each reagent listed was added. The concentration of modified proteins, which held water completely after they were centrifuged (see **Materials and Methods**), are listed. No reagents tested, equaled or surpassed urea. As seen in Table III, about 1 part of dried acid-precipitated soybean protein could hold 3 parts of water (*i.e.*, a paste of 25% protein did not release any water when centrifuged). All reagents examined increased water-holding capacity of soybean protein, excepting calcium chloride. As has been described before and shown in Table III, less than 5% aqueous paste of the modified protein with 0.5 *M* urea by the method of Mohammad's could hold all added water (95% water). Alcohol treatment had a significant effect on water-holding capacity and the product showed a clear white color. But the alcohol treated protein took longer time to absorb added water and seemed to have very low adhesiveness or cohesiveness.

TABLE II
Mechanical Characteristics of Gels, Calculated from Texturometer Patterns[a]

Gels	Hardness	Cohesiveness	Elasticity	Adhesiveness	Area of Peak / Height of Peak
Uirou	3.20	0.42	2.00	1.50	0.83
Kibi-Uirou	2.40	0.27	1.76	0.62	0.84
Awayuki	2.48	0.28	1.76	0.65	0.83
Modified Soybean Protein	0.69	0.26	1.74	0.80	0.87
Modified Soybean Protein and PROTON	2.88	0.30	2.30	0.65	0.68
PROTON	5.04	0.35	2.40	0.47	0.50

[a]Calculated by the method of Friedman *et al.* (6).

DISCUSSION

Phosphorylated or sulfated wheat gluten was reported to have extremely high water-holding capacity, and groups in gluten molecules which react with phosphoric acid to produce stable products were assumed to be aliphatic hydroxyl groups (1, 2). Phosphorylation alone, however, did not increase water-holding capacity of soybean protein, but urea treatment according to the procedure of Mohammad *et al.* (1) was effective. We tried phosphorylation of soybean protein by other methods (7–9). Kainuma's method (9) which was developed to phosphorylate starch, failed to phosphorylate soybean protein, judging from assays of phosphorus contents and Sephadex-G column chromatography (Modified proteins were dissolved in 0.5 *M* NaCl and chromatographed through Sephadex-G 75 or 200 gel in 0.5 *M* NaCl in 2 × 100 cm column. Phosphorus which was covalently bound to protein eluted with the protein, but free phosphorus eluted separately.) Phosphorylated proteins by the method of Ferrel's (7) or Mayer's (8) were water soluble.

The reason phosphoric acid increased water-holding capacity of gluten by Mohammad's method but not to soybean protein is unclear. Soybean protein used here was acid-precipitated at pH 4.8 while the pH of gluten was 6.8. Phosphoric acid might be required to lower the pH of the gluten and urea reaction mixture. Phosphate or sulfate groups attached to gluten molecule might be needed as hydrophilic groups to react with water molecules, which might be sufficient in the unmodified molecules of soybean protein.

Protein denatured by urea probably exists as random coils, but the transition is often incomplete even at the highest attainable urea concentrations (10). The optimum urea concentration used in this paper, 0.5 *M*, is much lower than that which is necessary to completely denature proteins. Still, after partial disruption

TABLE III
Percent of Modified Soybean Proteins which Hold Whole Water[a]

Reagent used for Preparation[b]	Concentration[a] (%)	Reagent used for Preparation[b]	Concentration[a] (%)
Untreated	26	HCl (pH 0.5)	23
Urea 0.5 *M*	4.5	(pH 0.9)	16
Alcohol 30%	10	(pH 1.8)	17
40%	7	NaCl (μ[d] = 0.1)	11
50%	8	(μ = 0.5)	10
65%	12	KCl (μ = 0.1)	10
NaOH (pH 9.5[c])	11	(μ = 0.5)	10
(pH 12.7)	10	$CaCl_2$ (μ = 0.1)	25
		(μ = 0.5)	28

[a]Samples of these concentration (%) did not release any water when they were centrifuged at 160 × *g* for 5 min.
[b]Acid-precipitated soybean protein was mixed with the indicated reagents, instead of phosphoric acid and urea in the method of Mohammad *et al.* (1). 'Untreated' was prepared without any other treatment than drying after acid-precipitation of soybean protein.
[c]Mixture of acid-precipitated soybean protein with water adjusted to pH 9.5 with NaOH.
[d]Ionic strength.

of native protein structure by urea, aggregation or entanglement might occur during drying and heating, which results in a large network of protein molecules. Small amounts of cyanate present in urea are known to react with amino and sulfhydryl groups of proteins (11), and urea tends to decompose with the formation of cyanate ion at elevated temperatures (10). Some other derivatives of urea might be derived by heating or by reaction with contaminating urease in soybean protein. Experiments using an equivalent molar concentration of guanidine hydrochloride, which closely resembles urea in structure and in the manner of denaturation of proteins (10), did not increase water-holding capacity at all. (Data not shown.)

Reitz *et al.* (2) reported that their gluten sulfate was nontoxic in animal experiments on oral administration or subcutaneous injection, and nonantigenic. They suggested its use in therapeutic jellies, ointments, and other pharmaceutical preparations, and as a thickening agent and emulsifying agent in ice cream or other foods. We expect that the modified soybean protein here is also nontoxic, though careful examination for food safety should be carried out by various advanced analytical techniques. It was interesting that gelatinization occurred on heating the modified soybean protein at lower concentrations than other proteins, and that the texture was quite like the Japanese traditional cakes.

Literature Cited

1. MOHAMMAD, A., MECHAM, D. K., and OLCOTT, H. S. Gel-forming phosphorylated derivative of wheat gluten. J. Agric. and Food Chem. 2: 136 (1954).
2. REITZ, H. C., FERREL, R. E., and OLCOTT, H. S. Gel-forming derivative of wheat gluten. Ind. Eng. Chem. 36: 1149 (1944).
3. FRIEDMAN, H. H., WHITNEY, J. E., and SZCZESNIAK, A. S. The texturometer—a new instrument for objective texture measurement. J. Food Sci. 28: 390 (1963).
4. NAKAMURA, M. Colorimetric determination of phosphorus. J. Agric. Chem. Soc. Japan 24: 1 (1950).
5. ALLEN, R. J. L. The estimation of phosphorus. Biochem. J. 34: 858 (1940).
6. BURRIS, R. H., and WILSON, P. W. Methods for measurement of nitrogen fixation. Methods in Enzymol. IV. 355 (1957).
7. FERREL, R. E., OLCOTT, H. S., and FRAENKEL-CONRAT, H. Phosphorylation of proteins with phosphoric acid containing excess phosphorus pentoxide. J. Am. Chem. Soc. 70: 2101 (1948).
8. MAYER, M., and HEIDELBERGER, M. Physical, chemical, and immunological properties of phosphorylated crystalline horse serum albumin. J. Am. Chem. Soc. 68: 18 (1946).
9. KAINUMA, K., ODA, T., and SUZUKI, S. Studies on the phosphate derivative of starch. II. Determination of reaction conditions of phosphorus pentoxide with starch. J. Technol. Soc. Starch 16: 60 (1968).
10. TANFORD, C. Protein denaturation. Advances in Protein Chem. 23: 121 (1968).
11. STARK, G. R., STEIN, W. H., and MOORE, S. Reactions of cyanate present in aqueous urea with amino acids and proteins. J. Biol. Chem. 235: 3177 (1960).

[Received June 2, 1977. Accepted August 26, 1977]