Protein-Calcium-Phytic Acid Relationships in Soybean

Part III. Effect of Phytic Acid on Coagulative Reaction in Tofu-making

By Kyoko SAIO, Emiko KOYAMA*, Sadao YAMAZAKI*
and Tokuji WATANABE

Food Research Institute, Ministry of Agriculture and Forestry,

**Japan Tofu Association*

Received May 7, 1968

This paper constitutes the third in a research series undertaken to clarify the interactive relation among soybean meal protein, calcium and phytic acid, dealing with the practical subject closely related to tofu-making. The results may conclude that phytic acid, naturally present in soybean seeds or added to soybean milk, gave significant effect on physical properties of prepared tofu-gel. The higher content of phytic acid in soybean milk resulted in the slower coagulative reaction between soybean protein and calcium, which is favorable for tofu gel formation.

In the previous papers,^{1,2)} it has been made clear that phytic acid gives remarkable effects on solubility characteristics or affinity of calcium ion to soybean meal protein. The facts let us presume that the difference of inherent characteristics among soybean varieties for tofu-making may partly contributed to phytic acid content in soybean seeds. Moreover, it is considered that the addition of phytic acid to soybean milk during tofu-making may control the physical properties of tofu-gel prepared.

MATERIAL AND METHODS

Materials. The defatted soybean meal of Hawkeye (1966 crops) was used for preparation of cold insoluble fraction protein (CIF) after Wolf's method.³⁾ Whole beans of Hawkeye and Harosoy varieties (U. S. Soybeans, 1966 crops), Tokachi-nagaha variety (Hokkaido

Agricultural Station, 1967 crops, described hereafter as Tokachi) and Shirasaya variety (Saga Agricultural Station, 1967 crops) were used to see the suitability for tofu-making and the effect of phytic acid on gel formation. Phytic acid was purchased from Tokyo Kasei Co. Ltd., which was neutralized with NaOH to pH 6.7 before using.

Preparation of soybean milk. Fifty grams of soybean seeds swelled up by soaking in water overnight were ground to mash with approximately 200 ml of water by a home mixer (SM-220 of Sanyo Electric Co.), and filled up to 500 ml unless otherwise stated. The mash was heated for 5 min after boiling, being carefully stirred and added with a few drops of silicon Ks 66 antifoam. After replenishing any water lost by evaporation, the heated mash was filtered by a basket centrifuge for home use (JI63 of Fuji Electric Co.). In the case of CIF, the fresh CIF from 100 g of defatted meal was dissolved in 200 ml of water and heated. The yield of CIF protein was 6 to 7 g from 100 g of defatted meal, being affected by various factors during preparation.⁴⁾ The concentration of the CIF protein dissolved in 200 ml of water was almost the same as the soybean milk obtained by 1:10 meal:water extract.

1) K. Saio, E. Koyama and T. Watanabe, *Agr. Biol. Chem.*, **31**, 1195 (1967).

2) K. Saio, E. Koyama and T. Watanabe, *ibid.*, **32**, 448 (1968).

3) D. R. Briggs and W. J. Wolf, *Arch. Biochem. Biophys.*, **72**, 127 (1957).

4) W. J. Wolf and D. A. Sly, *Cereal Chem.*, **44**, 653 (1967).

Method for evaluating the suitability of soybean varieties for tofu. At first, the evaluation of suitability for tofu was followed by the method developed by Watanabe *et al.*⁵⁾ Calcium sulfate (30, 40, 50 or 60 mg) was placed in 50 ml of centrifuge tube, suspended in 2 ml of water and warmed at 70°C beforehand in a water bath. Stirring constantly the calcium sulfate suspension on a magnetic stirrer, 25 ml of soybean milk at 70°C was poured into each tube. The tubes were incubated in a water bath to keep their contents at 70°C for 10 min. After breaking the curd with a spatula, the contents were centrifuged for 10 min at 1000×g. The prepared tofu remaining on the bottom of tubes were taken out and their weights were measured. Then the hardness was determined by curd tension meter (Iio Electric Co.) and weighed after flattening on aluminum dish and drying at 105°C for 24 hr to determine the solid matter. The supernatant separated by centrifugation was submitted to measurement of volume and pH. An improved method in which calcium chloride was used for calcium sulfate and the wider range of coagulant was adopted, was developed for the evaluation. The method could reduce time and personal error by easiness to change calcium concentration and homogeneous reaction between protein and calcium because of high solubility of calcium chloride. To 10 ml of soybean milk, 1 ml of calcium chloride solution in different concentration was added with constant stirring. The mixture was warmed at 70°C for 10 min and centrifuged at 1000×g for 10 min. The supernatant was transferred to a vessel with cover and weighed accurately. The determination of nitrogen was done by micro-Kjeldahl method,⁵⁾ and phosphorus and calcium by Nakamura's method⁶⁾ and Kubo-Tsutsumi's method,⁷⁾ respectively.

RESULTS AND DISCUSSION

CIF was used to pursue the effect of phytic acid on tofu gel formation of soybean protein, because CIF contains smaller amount of phosphorus and is easily prepared without denatura-

tion. CIF precipitates in cold with less than 30% in total phosphorus in the whole water extract and contains less than 0.04 mg/l mg N, comparing with 0.1~0.15 mg/l mg N in acid-precipitated protein or calcium-precipitated protein. Figure 1 shows the effects of phytic acid on physical characteristics of tofu-gel from CIF. By the addition of phytic acid, the weight of tofu increased. As the increase of tofu weight means an increase of water holding capacity, the volume of supernatant decreased. Tofu prepared from CIF was exceedingly harder than usual tofu prepared in the same condition, but the hardness of tofu prepared from CIF remarkably decreased as the added amount of phytic acid increased. The formation of softer gel by addition of phytic acid may be caused by retarding coagulative reaction between soybean protein and calcium. It may be considered that calcium reacts faster on phytic acid than directly on soybean protein but the calcium phytate forms a sort of combination with protein.²⁾ The pH of supernatant slightly increased by the addition of phytic acid, because phytic acid may prevent from a decrease of pH caused by direct combination of calcium and protein. Figure 2 shows the amounts of nitrogen, phosphorus and calcium in prepared tofu. In Fig. 2, all of protein in soybean milk from CIF precipitated into tofu in the range of added phytic acid. Amounts of calcium and phosphorus transferred into tofu increased as added phytic acid increased. The fact that binding calcium and phosphorus per protein increased with increase in added phytic acid in an equilibrium system was confirmed by previous work using differential equilibrium dialysis.²⁾

According to the fact that the addition of phytic acid was effective for slower coagulation of CIF and maintenance of high water holding capacity in its tofu-gel, phytic acid naturally present in soybean may give some influences on binding affinity for calcium with protein in tofu-making. Shirasaya and Hawkye (both are preferred by tofu makers⁵⁾) and

5) T. Watanabe, C. Fukamachi, O. Nakayama, Y. Teramachi, K. Abe, S. Suruga and S. Miyanaga, *Report of the Food Research Institute*, **14B**, 6 (1960).

6) M. Nakamura, *Nippon Nogeikagaku Kaishi*, **3**, 244 (1950).

7) S. Kubo and C. Tsutsumi, *Report of the Food Research Institute*, **5**, 171 (1951).

FIG. 1. Effect of Phytic Acid on Coagulation of Tofu-gel.

N; 3.85 mg and P; 0.114 mg/ml of soybean milk used. 20 mg of CaSO_4 was added for coagulation.

●—● weight of tofu, ○—○ volume of supernatant, x—x hardness of tofu by curd tension meter, Δ—Δ pH of supernatant.

FIG. 2. Changes in Nitrogen, Phosphorus and Calcium Content of Tofu Prepared by Addition of Phytic Acid.

N; 3.85 mg, P; 0.114 mg/ml of soybean milk.

●—● N, ○—○ P, x—x Ca.

Tokachi and Harosoy (not preferred⁵⁾) were selected to investigate the correlation between

suitability of soybeans for tofu-making and their content of phytic acid. At first the four varieties were evaluated by usual means after Watanabe *et al.*⁵⁾ (Ex. 1). Nitrogen and phosphorus content in whole beans and soybean milk used in Ex. 1 are shown in the left and middle column, respectively, of Table I. Figure 3 shows the difference in weight of tofu prepared by different amounts of calcium sulfate added. And Fig. 4 shows the changes in hardness of tofu by different amounts of calcium sulfate. In the left column in Table II, weights of tofu and solid matter of the four varieties are given. In the course of our investigations, it was found that weight of tofu and its solid matter measured in this way were generally correlated to the suitability of soybean variety for tofu. The weight of tofu and solid matter in Table II were calculated at the points where tofu approximates to 88% of moisture and 20g of hardness within the range of calcium sulfate in this way. Together with the values in Table II, the evaluation

TABLE I. NITROGEN AND PHOSPHORUS IN WHOLE BEANS AND SOYBEAN MILK OF SOYBEAN VARIETIES TESTED

	Whole Beans			Soybean Milk					
	TN	TP(%)	TP/TN	TN	TP(mg/ml)	TP/TN	TN	TP(mg/ml)	TP/TN
Tokachi	5.74	0.598	0.104	4.24	0.522	0.123	5.06	0.586	0.116
Shirasaya	6.30	0.690	0.109	5.39	0.761	0.138	5.08	0.612	0.121
Hawkeye	6.15	0.528	0.086	4.93	0.457	0.093	4.98	0.446	0.090
Harosoy	6.51	0.521	0.080	5.18	0.413	0.080	4.90	0.401	0.082

FIG. 3. Changes in Weight of Tofu Prepared by Different Amount of CaSO_4 .

●—● Tokachi, ○—○ Shirasaya,
x—x Hawkeye, △—△ Harosoy.

was conducted by overall judging throughout laboratory method, as to the state of coagulation, allowance of added calcium sulfate and sensory properties of tofu. The state of coagulation was related to the rate of coagulation which was immeasurable because it proceeded in a few seconds but was comparable each other in observation of large number of samples. In Ex. 1, Shirasaya was found to be the best, followed by Hawkeye, Harosoy and Tokachi in that order. As protein content in soybean milk is one of the important factors for tofu-making, the protein concentration of

FIG. 4. Changes in Hardness of Tofu Prepared by Different Amount of CaSO_4 .

●—● Tokachi, ○—○ Shirasaya,
x—x Hawkeye, △—△ Harosoy.

TABLE II. WEIGHT OF PREPARED TOFU AND SOLID MATTER

	Ex. 1		Ex. 2	
	Tofu (g)	Solid matter (g)	Tofu (g)	Solid matter (g)
Tokachi	9.40	1.088	9.80	1.193
Shirasaya	11.40	1.311	9.40	1.062
Hawkeye	10.73	1.306	9.45	1.147
Harosoy	10.30	1.257	9.18	1.115

soybean milk among the four varieties were adjusted to be almost equivalent beforehand, and they were evaluated once more by the

usual way (Ex. 2). Figures 5 or 6 show the

FIG. 5. Changes in Weight of Tofu after Adjusting Protein Content Equivalent.

●—● Tokachi, ○--○ Shirasaya,
×---× Hawkeye, Δ---Δ Harosoy

FIG. 6. Changes in Hardness of Tofu after Adjusting Protein Content Equivalent.

●—● Tokachi, ○--○ Shirasaya,
×---× Hawkeye, Δ---Δ Harosoy

difference in weight or hardness of tofu prepared by different amounts of calcium sulfate. In the right column of Table II, weights of tofu and its solid matter obtained by Ex. 2 are shown. The difference among the varieties in Ex. 2 was smaller than that in Ex. 1 but the evaluation showed that Shirasaya ranked first followed by Tokachi, Hawkeye and Harosoy in that order, which is the same order in total-P/total-N (TP/TN) shown in Table I. It was noted that the rank in Ex. 1 seemed to coincide with the evaluation in the market, while that in Ex. 2 coincided with the order in TP/TN. The protein concentration in soybean milk was not always correlated to the evaluation of tofu⁸⁾ but the variety of low protein and high phosphorus content like Tokachi was ranked higher by adjusting the

FIG. 7. Relation between Hardness of Tofu and TP/TN in Soybean Seeds.

● Ex 1, ⊙ Ex 2; CaSO₄ 30 mg, ○ Ex 1, ⊙ Ex 2; 40 mg, × Ex 1, ⊗ Ex 2; 50 mg; Δ Ex 1, ⊕ Ex 2; 60 mg.

8) T. Watanabe, unpublished.

9) K. Saio and T. Watanabe, *J. Food Sci. Tech.*, 15, 290 (1968).

protein concentration in Ex. 2. Figure 7 shows the relation between hardness and TP/TN obtained in both experiments, indicating that the hardness of tofu is closely related to the amount of phosphorus.

In order to get more accurate information of coagulative reaction, soybean milk was coagulated by the new method using calcium chloride solution in wider range. Figure 8 shows the coagulative states of the four varieties. The difference of the curves among the varieties suggests the high correlation between phytic acid content and coagulative state, that is to say, the higher phytic acid content in soybean requires the higher amounts of calcium to start the coagulation, to have the maximum volume of tofu and also to reach the minimum volume of tofu. To make the effect of phytic acid clearer, addition of phytic acid to soybean milk of Harosoy, having smallest TP/TN, was evaluated. The difference of curves in Fig. 9 with added phytic acid was reasonably coincided with that in Fig. 8 and showed that phytic acid was effective on the slow coagulation of soybean protein with calcium. Table III in the results calculated from Figs. 8 and 9 to see the relation between calcium and phosphorus at the points where tofu showed the maximum volume or reached the minimum

TABLE III. RELATION BETWEEN Ca AND P AT THE POINTS WHERE TOFU IS AT MAX. OR MIN. VOLUME

	P	At max. volume		At min. volume	
		Ca	Ca/P (μ moles/10 ml)	Ca	Ca/P of milk)
Harosoy	129	40	0.310	68	0.528
Hawkeye	144	56	0.389	91	0.633
Tokachi	189	59	0.312	111	0.588
Shirasaya	197	62	0.314	111	0.564
Average			0.332		0.578
Harosoy 0	129	40	0.310	68	0.528
1	146	46	0.312	80	0.545
2	168	51	0.304	91	0.542
3	183	61	0.333	102	0.558
4	206	75	0.364	111	0.540
Average			0.341		0.542

FIG. 8. Inherent Characteristics of Different Soybean Varieties in Coagulation State by Calcium.

●—● Harosoy, ○—○ Hawkeye,
×—× Tokachi, △—△ Shirasaya.

FIG. 9. Changes in Coagulative State by Addition of Phytic Acid to Soybean Milk.

●—● Harosoy 0 (added P, 0; TN, 49.0 mg/10 ml)
○—○ Harosoy 1 (added P, 0.705; TN, 49.3 mg/10 ml)
×—× Harosoy 2 (added P, 1.41; TN, 49.3 mg/10 ml)
△—△ Harosoy 3 (added P, 1.91; TN, 49.7 mg/10 ml)
◎—◎ Harosoy 4 (added P, 2.65; TN, 49.7 mg/10 ml).

volume. The values of Ca/P were relatively equivalent in the both cases where phytic acid was present naturally and when it was added to soybean milk. At the point where tofu gel showed maximum volume and held maximum water content, Ca:P was about 1:3. And as Ca:P changed to 1:1.5, tofu gel

decreased in volume and water holding and then reached the maximum volume.

The authors previously reported the fine structure of tofu-gel observed under electron microscope.⁹⁾ The gel consists of network of protein granules, being $0.02\sim0.05\mu$, and oil droplets, being $0.5\sim2\mu$ located along the network in groups. Figures 10 and 11 are the microphotographs of tofu-gel which showed the maximum and minimum volume, respectively. By comparing Figs. 10 to 11, it is shown that tofu-gel having minimum volume has closer network of protein granules but has rather large vacancy in several places. In the tofu-gel having maximum volume, the network is extended entirely and vacancies are observed everywhere.

From the results obtained by using four varieties of soybean, it was indicated that phytic acid, naturally present in seeds or added to soybean milk, was attended with the slower coagulative reaction in tofu-making, which is preferable for tofu-makers, having wider allowance of calcium amount to secure good quality of tofu. But the inherent characteristics of soybean varieties which come out during tofu-making can not be clarified by phytic acid only, even when protein content in each variety is considered. It is to be resolved in our progressing work.

Acknowledgement. The authors wish to express their hearty thanks to Mr. Kei Arai, Department of Agriculture, University of Tokyo, for his kindful technical help in electron microscopic observation. The research was supported in part by a grant from U.S. Department of Agriculture under P. L. 480 to Japan Tofu Association.

FIG. 10. Microphotograph of Tofu Gel at Maximum Volume.

FIG. 11. Microphotograph of Tofu Gel at Minimum Volume.