Effect of Phytic Acid on the Texture of Tofu and the Precipitation Reaction in Tofu Making

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ABSTRACT

Soybean curd or tofu is widely used as daily foods by people in Indonesia. One of the delicate steps in the preparation of tofu is the addition of a salt to precipitate soy protein. The common salt of calcium sulfate and the peeled soybeans were used to produce the non phytic acid soybean milk. Furthermore the adjustment of phytic acid content in soybean milk was done by addition of phytic acid. The result showed that phytic acid is the most important factor in the formation of precipitated protein aggregate in tofu at above isoelectric point. Weight and moisture content were affected by both phytic acid and amount of calcium sulfate as a coagulant. The results may conclude that either natural phytic acid present in soybean seeds or phytic acid added to soybean milk, have a significant effect on the texture of tofu. The higher the phytic acid content in soybean milk is the stronger the interaction among soybean protein, calcium and phytic acid. This is important in producing tofu of desirable hardness.

INTRODUCTION

Soybean milk, tempeh and soybean curd are widely used as daily foods by people in the Orient. Soybean curd is one of the important nonfermented soybean products which was widely used in a variety of dishes by Oriental people for many centuries, especially in Indonesia. This is a highly digestible and nutritive product (Schroder et al, 1993; Liener, 1972) and it also serves as an inexpensive protein source for people.

The consumption of soybean for food in Indonesia has been estimated at 9.5 kg/capita/year or 1.325 million tonnes in 1988. The nonfermented bean curd, tofu, make up 40% of this total. Tofu is the gel-like precipitate obtained by adding calcium sulfate to heated soybean milk (Smith and Circle, 1972). Certain mineral salts and acids are employed to precipitate soybean protein from soybean milk. If the precipitated mass is allowed to drain and is subsequently washed, a white curd is produced which is called by the Chinese name "*Tofu*".

Miller et al (1952) cit. Tseng et al (1977) indicated a low retention of thiamin (18%) when soybean is made into soybean curd and preserved in water by the usual commercial process. They also indicated that the water drained from the soybean curd contains more vitamins than the tofu itself. Storage of the blocks of tofu causes further reduction in the amount of water and water soluble nutrients present. The traditional way of soybean curd preparation involves first preparation of soy milk, addition of calcium salt, and then molding the resulting curd into cakes. It has been recognized that the selection and addition of salt at the proper level is the most important step in the preparation of soybean curd. Insufficient amount of salt may result in incomplete precipitation of soy protein and make the subsequent filtration difficult, whereas excess amount of salt makes the texture of soybean curd hard and unpalatable (Lu *et al*, 1980).

Schroder et al. (1973) and Wu and Salunkhe (1977) used calcium sulfate as the precipitation agent. Wang (1967) made use of a mixture consisting of calcium sulfate and magnesium sulfate to precipitate soybean protein. It appears that calcium sulfate is the most commonly employed salt in soybean curd preparation. The use of calcium sulfate, however causes some problems. This salt has a low aqueous solubitility, and accodingly the addition of this salt needs skill, to a constant quality product.

Glucono-delta-lactone (GDL) has been tested both by Javanese and Chinese food researchers in the production of soybean curd. They found that using GDL to adjust the pH value to approximately 5.4 yields a firm smooth, compact mass of curd with better water holding capacity than curd precipitated wit calcium sulfate. Phytate, the hexaphosphate salt of myoinositol (Erdman, 1979) is the principal storage

form of P in the soybean (Okubo et al, 1976). Phytate possesses six phosphoric acid residues per mole cule with 8 negative charges between pH 6.3 and 9.7 and 6 negative charges between pH 1.8 and 6.3 (Crean and Haisman, 1963; de Rham and Jost, 1979). As a result of this polyvalent nature, it is strongly associated with soy proteins above their isoelectric point (pH > 4,5), especially in the presence of Ca^{++} and Mg^{++} ions (Hartman, 1979). Phytate is also associated with soy pro teins at pH values below their isoelectric point by electrostatic interaction involving their cationic lysyl, histidyl, arginyl and terminal amino groups (Okubo et al, 1976).

At low pH, the protein can associate with phytate to form protein-phytate complexes and at pH values above their isoelec tric point can form protein-mineral-phytate (Setyono, 1988). The phytic acid and protein content of soybean milk influence the texture of tofu. The phytic acid gives remarkable effects on solubility characteristics or affinity of calcium ion to soybean meal protein. Moreover, it is considered that the additional of phytic acid to soybean milk during tofu-making may control the physical properties of tofu-gel prepared. The purpose of the present investigation was to study the texture of soybean curd as influenced by phytic acid. The other objective of the investigation was to clarify the interaction between soybean protein, calcium and phytic acid in soybean milk and also in the precipitate by calcium sulfate.

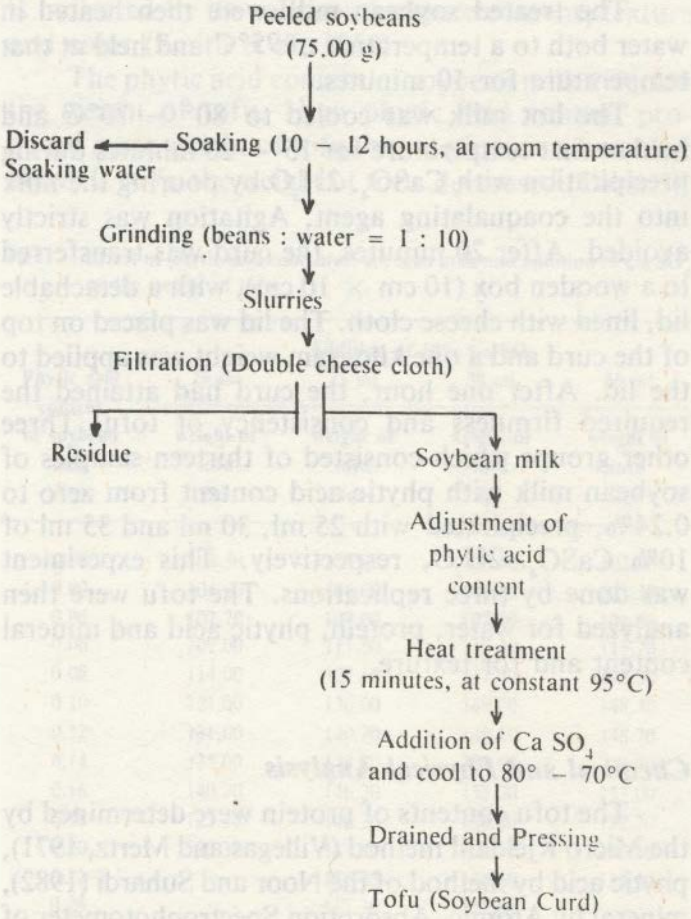

Diagram 1. **Flow diagram for the production of soybean milk and tofu (soybean curd)**

MATERIALS AND METHODS

The production of soybean curd consists principally of two processes, (i) the production of soybean milk and (ii) the pro duction as soybean curd from soybean milk. Diagram 1 indicates procedures used and conditions applied.

Soybean Milk Production

The Orba variety of soybeans was peeled by the Satake Owner Manual TM-05 type abrassive roller to eliminate the phytic acid content of soybean. Seventy five gram of the peeled soybean was soaked overnight in 500 ml of water at room temperature. The soybeans were then drained and rinsed in tap water. The rised soybeans were then blended with 750 ml destilled water in a Waring Blendor for 2 to 3 minutes. The resulting soybean slury was filtered through two layers of cheese cloth to yield a coarse residue and a soybean milk. The soybean milk was analyzed for protein content by micro kjeldahl method (villegas and Mertz, 1971), phytic acid by Wheeler and ferrel method (Wheeler and Ferel, 1971) and minerals (Zn, Mg, Fe and Ca) by atomic absorp tion spectrophotometer method (AOAC, 1970).

Phytic acid content of soybean milk was adjusted to 13 levels from zero to 0.24% (w/v), by adding phytic acid. For the precipitation of the soybean protein used 20 ml, 25 ml, 30 ml and 35 ml of 10% (w/v) $Ca\ SO_4 . 2H_2o$ were used respectively. The experiment was conducted by three replica tions and needed 156 (13 × 4 × 3) samples of soybean milk.

Soybean Curd Production

The treated soybean milk were then heated in water both to a temperature of 95°C and held at that temperature for 10 minutes.

The hot milk was cooled to 80° – 70°C and held at that temperature for 15 – 20 minutes during precipitation with $CaSO_4$. $2H_2O$ by pouring the milk into the coaqualating agent. Agitation was strictly avoided. After 20 minutes, the curd was transferred to a wooden box (10 cm × 10 cm), with a detachable lid, lined with cheese cloth. The lid was placed on top of the curd and a one kilogram weight was applied to the lid. After one hour, the curd had attained the required firmness and consistency of tofu. Three other groups which consisted of thirteen samples of soybean milk with phytic acid content from zero to 0.24%, precipitated with 25 ml, 30 ml and 35 ml of 10% $CaSO_4$. $2H_2O$, respectively. This experiment was done by three replications. The tofu were then analyzed for water, protein, phytic acid and mineral content and for texture.

Chemical and Physical Analysis

The tofu contents of protein were determined by the Micro Kjeldahl method (Villegas and Mertz, 1971), phytic acid by method of the Noor and Suhardi (1982), mineral by Atomic- Absorption Spectrophotometer of the AOAC (1970), and texture by penetrometer.

The unweighted penetrometer was adjusted to zero with the tip on the surface of the tofu exactly. Applying a constant load, the needle penetrated the tofu to a depth depend on the sofness or hardness of the tofu.

RESULT AND DISCUSSION

Protein-mineral-phytic Acid Interaction in Soybean Milk

Soybean meal contain 500 – 600 mg of phosphorus (d. b.) per 100 g, 79 – 80% of which account for the phosphorus of phytic acid. Phytic acid is myoinositol 1, 2, 3, 4, 5, 6 – hexakis (dihydrogen phosphate). Phytic acid is a strong chelating agent that can bind mono and divalent metal ions and protein to form the complex phytate (Oberleas, 1973). It has been reported that phytic acid influences the solubility characteristic of the meal protein by the combination with basic groups of the protein molecule below its isoelectric point (Fontain et al, 1946; Setyono, 1988). In the course of the investigation, it was found that fairly large quantity of phosphorus precipitates with protein at about pH 5.7 by calcium salt, and practically, 70 – 80% of the water extractable phosphorus transfered into the curd.

In soybean milk, solubility of protein, mineral and phytic acid are affected by pH. In low pH (below its isoelectric point), protein formed insoluble protein-phytate complexes with phytic acid.

$$\text{Protein}^{+} + \text{Phytate} \longrightarrow \text{Protein-Phytate}$$

In high pH (above its isoelectric point), addition of calcium salt, protein formed insoluble Protein – Calcium-Phytate Complexes.

$$\text{Protein} + {}^{+}\text{Calcium}^{+} - \text{Phytate} \longrightarrow \text{Protein-Calcium-Phytate}$$

Calsium - Phytic Acid Molar Ratio

Addition of Ca SO_4 to the soybean milk caused increases of the molar ratio of calcium (Ca) to Phytic Acid (PA) in soybean milk, from 8.27 to 161.22 (Table 1). Addition of $CaSO_4$ also caused increases in the

Table 1. **Effect of phytic acid content of soybean milk[1] and addition of Ca SO_4 on molar ratio of calcium/phytic acid (Ca/PA).**

Phytic acid of soybean milk (%)	Total of phytic acid (m mol)	Molar Ratio Ca/PA			
		B4	B1	B2	B3
0.00	0.00	–	–	–	–
0.02	0.18	100.00	120.50	140.89	161.22
0:04	0.36	50.06	60.25	70.44	80.61
0.06	0.55	32.76	39.44	46.11	52.76
0.08	0.73	24.68	29.71	34.74	39.75
0.10	0.91	19.80	23.84	27.84	31.89
0.12	1.09	16.53	19.90	23.27	26.62
0.14	1.27	14.19	17.08	19.97	22.85
0.16	1.45	12.43	14.96	17.49	20.01
0.18	1.64	10.99	13.23	15.46	17.70
0.20	1.82	9.90	11.92	13.93	15.95
0.22	2.00	9.01	10.85	12.68	14.51
0.24	2.18	8.27	9.95	11.63	13.31

Explanation.

1) : Soybean milk from extraction of 75.00 g peeled soybean with 750 ml destilled water

B_1 : Ca total = 134.26 mg + 588.00 mg = 723.26 mg = 18.02 m mol.
B_2 : Ca total = 134.26 mg + 735.00 mg = 869.26 mg = 21.69 m mol.
B_3 : Ca total = 134.26 mg + 822.00 mg = 1,016.26 mg = 25.36 m mol.
B_4 : Ca total = 134.26 mg + 1,029.00 mg = 1,163.26 mg = 29.02 m mol.

molar ratio of mineral - phytic acid. Molar ratio of M/PA in soybean milk samples varied from 11.82 to 204.22 (Table 2).

The molar ratio of Ca/PA affected the solubility of Ca and phytic acid (Grynspan and Cheryan, 1983). Increasing molar ratio of Ca/PA in the soybean protein extract caused decreasing on Ca and phytic acid solubility. Grynspan and Cheryan (1983) indicated that molar ratio of Ca/PA = 4 and Ca/PA = 12.67 caused precipitation of the phytic acid 90% and 100%, respectively.

If the molar ratio of Ca/PA was very high, calcium did not all bind with phytic acid, and if the molar ratio of Ca/PA was very low, the calcium was not enough to precipitate all of the phytic acid.

Table 2. **Effect of phytic acid content of soybean milk[1] and addition of Ca SO_4 on molar ratio of mineral/phytic acid (M/PA)**

Phytic acid content of soybean milk (%)	Total of phytic acid (m mol)	Molar ratio of Mineral/Phytic acid[2]			
		B1	B2	B3	B4
0.00	0.00	–	–	–	–
0.02	0.18	143.11	163.50	183.72	204.22
0.04	0.36	71.56	81.75	91.86	102.11
0.06	0.55	46.84	53.51	60.13	66.84
0.08	0.73	35.29	40.32	45.30	50.36
0.10	0.91	28.31	32.34	36.34	40.40
0.12	1.09	23.63	23.63	27.00	33.72
0.14	1.27	20.28	23.17	26.04	28.94
0.16	1.45	17.77	20.30	22.81	25.35
0.18	1.64	15.71	17.95	20.16	22.41
0.20	1.82	14.15	16.17	18.17	20.20
0.22	2.00	12.88	14.72	16.54	18.38
0.24	2.18	11.82	13.50	15.17	16.86

Explanation.

1) : Soybean milk from extraction of 75.00 g peeled soybean with 750 ml destilled water.

2) : the minerals are Zn, Mg, Fe and Ca.

B_1 : total mineral. Zn + Mg + Fe + Ca = (0.09 + 7.49 + 0.16 + 18.02) = 2.76 m mol.

B_2 : Total minerals Zn + Mg + Fe + Ca = (0.09 + 7.49 + 0.16 + 21.69) = 29.43 m mol.

B_3 : Total minerals Zn + Mg + Fe + Ca = (0.09 + 7.49 + 0.16 + 25.36) = 33.07 m mol.

B_4 : Total minerals Zn + Mg + Fe + Ca = (0.09 + 7.49 + 0.16 + 29.02) = 36.76 m mol.

Effect of Phytic Acid and Calcium Content on Weight and Moisture Content of Tofu

Lu *et al* (1980) showed that the pH, is by far the most important factor in the precipitation of soy protein. Soybean curd actually is a soy protein isolate which is precipitated at pH 6.0. The quality of soybean curd is indicated by its volume, moisture content, texture and color (Smith et al, 1960).

The phytic acid content of soybean milk affected the weight of tofu. High phytic acid content produced more weight of tofu, but at phytic acid content above 0.16%, the weight of tofu decreased (Table 3).

Table 3. **Effect of phytic acid content of soybean milk and addition of Ca SO_4 on the weight of tofu**

Phytic acid content of soybean milk (%)	Addition of 10% Ca SO_4 20 ml	25 ml	30 ml	35 ml
	weight of tofu (g)	weight of tofu (g)	weight of tofu (g)	weight of tofu (g)
0.00	103.30	103.00	103.80	102.00
0.02	104.00	106.00	106.95	103.30
0.04	107.70	109.00	107.00	108.00
0.06	109.00	111.50	117.30	115.70
0.08	114.00	127.50	147.20	146.30
0.10	121.00	136.00	148.00	148.30
0.12	131.00	140.20	148.20	148.70
0.14	135.00	148.20	151.00	149.70
0.16	140.70	146.20	158.00	153.00
0.18	123.30	148.20	156.30	152.70
0.20	120.30	149.80	169.70	145.80
0.22	–	131.70	164.50	133.30
0.24	–	115.30	133.80	–

Addition 20 ml, 25 ml, 30 ml and 35 ml of 10% Ca SO_4 to the samples of soybean milk containing various amounts of phytic acid produced 103.30 g to 140.70 g, 103.00 g to 149.80g, 103.80 g to 169.70 g and 102.00 g to 153.00 g of tofu, respectively (Table 3). The phytic acid content of soybean milk also affected the moisture content of tofu. Higher the phytic acid contents in soybean milk resulted in higher the moisture contents in tofu up to phytic acid contents of 0.16% or 0.18%. At still higher to it, the moisture contents of tofu decreased (Table 4).

The weight and moisture content of tofu were not affected significantly by the amount of added Ca SO_4 to the soybean milk, (Tables 3 and 4). Lu et al, (1980) indicated that the effect of calcium ions on soy protein precipitation is not clearly understoond. But Setyono (1988) has shown that addition of calcium salt to the soybean milk or soybean axtract would form a protein-calcium-phytic acid aggregate.

Table 4. **Effect of phytic acid content of soybean milk and addition of Ca SO_4 on the moisture content of tofu**

Phytic acid content of soybean milk (%)	Addition of 10% Ca SO_4 20 ml	25 ml	30 ml	35 ml
	Moisture content of tofu (%)	Moisture content of tofu (%)	Moisture content of tofu (%)	Moisture content of tofu (%)
0.00	73.08	73.11	73.23	72.75
0.02	73.22	74.57	74.51	73.88
0.04	74.52	75.16	74.80	75.03
0.06	74.98	75.43	76.60	76.27
0.08	76.16	78.56	81.56	81.52
0.10	77.63	80.06	81.70	81.69
0.12	79.85	80.49	81.70	81.55
0.14	80.07	81.72	81.97	82.25
0.16	80.78	81.42	82.93	81.25
0.18	77.66	81.18	82.39	81.96
0.20	76.57	79.98	81.92	79.63
0.22	–	76.93	82.67	76.82
0.24	–	73.54	77.01	–

Previous studies indicate that heating causes a significant decrease in the beany flavor and inactivatives the enzymic reaction of native soybeans. Heating of soybean milk at a temperature above 75°C causes gelation of soy protein, which is related not only to the degree of hydration of the protein molecules, but also to an unfolding of the protein structure as it changes from globular to fibrous form. Disulfide bonds are an important factor to heat gelation. Intermoleculer crosslinks formed by sulfhydryl disulfide interchange may help stabilize the protein network, thereby favoring other interaction required for gelation.

Hashizume et al (1975) states 7-S protein (glycoprotein) of soy protein is sensitive to heating at higher ionic strengths, forming aggregates. Where as 11-S protein (pure globulin of soybean) is sensitive to heat at lower ionic strengths, dissociating to subunits, which partly aggregate. Also 7-S protein cannot form a firm gel by Glucono-Delta- Lactone after heating, while that 11-S protein can form a firm gel under reasonable heating.

Saio et al (1988) indicated that a single molecule of cold insoluble fraction protein (CIF) combines with a larger number of calcium ions by many binding sites and that the co-existences of phytic acid with cold insoluble fraction protein (CIF) gives the changes in the binding behavior of CIF with calcium. Bound calcium increases as added phytic acid increases as far as the range of phytic acid in the experiment.

Effect of Phytic Acid Content on the Texture of Tofu

Texture qualities of tofu were determined by a penetrometer in a millimeter penetrometer scale unit. The higher the penetrometer reading, the smoother and softer the texture of the tofu. Lue et al (1980) indicated that, in general, soybean curd prepared from calcium salts had a higher yield than the non calcium compounds. The important factors for tofu-making are the protein concentration of soybean milk, phytic acid content of a soybean milk and total addition of calcium salts as coagulant.

If the soybean milk does not contain phytic acid, the texture of tofu is very soft and brittle and the amount of the calcium salt addition does not affect the texture of tofu significantly (Table 5). Even though the addition of the calcium sulfate is increasing, but texture of tofu from non phytic acid soybean milk is constant soft, about 9.7 mm to 9.6 mm (Table 5). If the phytic acid content of soybean milk is increased, the texture of the tofu gets hardering up to a phytic acid content in soybean milk of 0.16%. It was also observed that at phytic acid contents in soybean milk more than 0.24%, addition of Ca S04 did not form protein aggregates. This is due to the affinity of

Table 5. **Effect of phytic acid content of soybean milk and total addition of Ca SO_4 on the texture of tofu (mm scale of penetrometer)**

Phytic acid content of soybean milk (%)	Addition of 10% Ca SO_4 20 ml	25 ml	30 ml	35 ml
	Texture (mm)	Texture (mm)	Texture (mm)	Texture (mm)
0.00	9.7	9.7	9.6	9.6
0.02	8.2	8.0	7.8	7.6
0.04	5.9	5.7	5.5	4.6
0.06	3.9	3.5	3.1	2.7
0.08	3.5	3.1	2.9	2.5
0.10	3.2	2.9	2.7	2.3
0.12	2.7	2.6	2.4	2.0
0.14	2.9	2.7	2.2	2.0
0.16	5.8	3.9	3.5	2.6
0.18	6.2	4.2	3.6	2.9
0.20	7.7	5.2	4.8	4.5
0.22	–	6.0	5.7	7.9
0.24	–	6.7	6.6	–

phytic acid to the cation (Setyono, 1988). The texture of commercial tofu if about 4.0 mm to 4.1 mm scale of penetrometer (Setyono, 1988).

CONCLUSION

Precipitation of soy protein by calcium sulfate in tofu making represents the interaction between soy protein and phytic acid by calcium ion intermediary. Phytic acid-calcium molar ratio is the most important factor in formation precipitated protein aggregate in tofu making.

In the production of tofu, the phytic acid content of the soybean milk and the amount of added calcium ions affect the weight and moisture content of the tofu, as well as the texture of tofu. Ideal phytic acid content of the soybean milk for tofu making is about 0.06 percent to 0.16 percent depending on the amount of addition of calcium sulfate.

The results indicated that phytic acid present in soybean seeds or added to soybean milk gave significant effect on the quality of tofu.

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