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Effect of Germination on Phytic Acid and Soluble Minerals in Soymilk

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발아에 의한 콩우유의 Phytic Acid와 가용성 무기물의 함량변화

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Abstract

Soybeans were soaked in water for 3 hrs and germinated at 25°C for 5 days followed by hot water extraction and boilding for 30 min. The prepared soymilk was analyzed for chemical composition, phytic acid and soluble minerals. Germination resulted in a slow decrease in total solids while protein recovery increased to a maximum of 92.5% after 2 days. Carbohydrate content was reduced to 65% by the 5th day of germination. The intial phytic acid content in soymilk of 1.26 *g* recovered from 100 *g* dry soybean was decreased by 41.3% while inorganic phosphorus increased more than four times from 31.3 *mg* to 135.0 *mg*. Total soluble minerals was also steadily increased.

Introduction

Soybeans, high in protein content with a well balanced amino acid composition, have been steadily increasing their use as a low cost protein source. Utilization of soybean protein, however, is limited for human consumption due to the undesirable flavor and antinutritional factors. Among the antinutritional factors, phytic acid in dry beans interacts with several minerals[1] and reduces considerably the biological availability of dietary copper, manganese, iron and zinc,[2,3] Phytic acid was also reported to interact with protein, thus decreasing the availability of protein when subjected to proteolytic digestion.[4]

Phytic acid is widespread in nature, particularly in cereal grains and in legumes, as a complex salt of calcium and magnesium.[5] The content of phytic acid in soybeans was reported between 1.0-1.47% in dry weight basis which represented 51.4-57.1% of total phosphorus in the soybean.[6] De Boland *et al.*[7] reported that the phytate in soybean flakes and corn germ were very soluble in water (appr. 90%).

Recently many attempts were made to reduce or eliminate phytate content in foods. These methods studied fermentation in whole wheat meal,[8,9] ultrafiltration of aqueous extract of soybean,[10] combination of ion exchange treatment[11] and enzymatic hydrolysis of phytate during germination[12,13] and incubation of presoaked beans.[1]

In our recent study on better soymilk preparation,[14] germination of soybeans prior to grinding with boiling water was found to be effective in reduction of oligosaccharides and improvement of sensory quality. The present work was carried out to improve the nutritional quality of soymilk. Changes in phytic acid, soluble minerals and chemical composition of soymilk prepared from germinated soybeans were determined.

Materials and Methods

Germination and Soymilk Preparation

Kwangyo variety certified soybeans grown in Korea were used in this study. Soybeans were soaked in water at room temperature for 3 hours and germinated in dark moist chamber at 23°C over a period of 5 days. At the end of each day of germination, 20 beans were measured for average of root length and average amount of water absorbed. For preparation of soymilk, the germinated soybeans were dehulled by hand followed by grinding in boiling water in the ratio of 1:1.5 (beans:water) using Waring blendor. The slurry was filtered through Whatman No. 4 filter paper. The residue was ground five more times in boiling water in the ratio of 1:1 (residue:water) and then filtered. The filterates of above were combined and boiled for 30 min. The prepared soymilk were stored in refrigerator until analysis.

Analytical Methods

Proximate analysis was carried out by standard procedures. For nitrogen analysis, the AOAC micro-Kjeldahl procedure[15] and a conversion factor of 6.25 were used. Phenol sulfuric acid method of Dubois *et al.*[16] and Roese-Gottlieb method (AOAC 16, 052)[15] were followed for determination of sugars and lipids, respectively. For moisture contents, cotyledon root, hull and soymilk were dried at 105°C to constant weight.

For determination of phytic acid and phytate phosphorus, the method of Wheeler and Ferrel[17] with some modification was used. A 50 *ml* aliquot of soymilk prepared from germinated soybeans was mixed with 1.5 *g* trichloroacetic acid (TCA) and stirred for 30 min using a magnetic stirrer, then centrifuged at 18,000 X g for 30 min.

To 10 *ml* of supernatant in centrifuge tubes were added 0.2 *ml* of saturated $FeCl_3 \cdot 6H_2O$ in 3% TCA solution and 1-2 drops of 3% Na_2SO_4 solution, and then mixed well. The mixture in the tube was then heated in boilding water for one hr. After cooling, the tube was centrifuged at 18,000 X g for 30 min. The supernatant was carefully decanted and discarded, the precipitate was washed two more times with 10 *ml* of 3% TCA, followed by heating, cooling and centrifugation. The rest of the procedures were followed as described in the Wheeler and Ferrel's method.[17] Phytic acid and phytate phosphorus were calculated by using a conversion factor of 2.98 to Fe value and the atomic ratio of 4:6 Fe/P, respectively.

Total phosphorus contents were determined by the phosphomolybdate colorimetric method of Allen[18] and inorganic phosphorus by the method of Pons and Guthrie.[19]

The mineral contents of Mg, Ca, Zn, Fe, Cu, Mn in soymilk were determined using a Varian AA 575 atomic absorption spectrophotomer. Samples were ashed and the ash was dissolved with conc-HNO_3 and diluted to appropriate volume with 5% HCl.

The soluble minerals were also analyzed by atomic absorption spectrophotometry. Soymilks were centrifuged at 12,000 X g for 20 min and the supernatant was ashed by dry ashing method. The ash was dissolved and analyzed for soluble minerals as described for total minerals.

Results and Discussion

Germination has been known to cause net loss of stored compounds to provide substrate and energy for various processes of growth. Table 1 shows increase in moisture absorbed during soaking and germination, growth of root length and changes in dry weights. During the five days of germination, water absorbed increased to 3.65 *g* water/*g* dry bean and the total dry weight was reduced by approximately 9%.

Changes in chemical composition of soymilk

Table 1. Effect of germination on the water absorbed, root length and dry weights

		Germination time (days)				
	Soaked[a]	1	2	3	4	5
Water absorbed[b]	0.86	1.34	1.81	2.80	2.80	3.65
Root length (*cm*)	0.0	1.92	5.28	11.30	13.10	16.70
Dry weight[c]						
Cotyledon	0.84	0.80	0.78	0.74	0.70	0.64
Root	0.00	0.02	0.04	0.08	0.11	0.15
Hull	0.07	0.06	0.06	0.05	0.04	0.04
Total	0.91	0.88	0.88	0.87	0.85	0.83

[a] Soaked in water at room temperature for 3 hrs.
[b] *g* of water absorbed/*g* dry soybean.
[c] Dry weight per *g* of dried soybean after gemination.

Table 2. Effect of germination on chemical composition of soymilk prepared from 100g of dry soybeans

Germination time (Days)	Total solid (g)	Protein (g)	Carbohydrate (g)	Lipid (g)	Ash (g)
0 [a]	72.2	28.3	24.6	14.6	4.7
1	71.0	29.4	22.2	13.6	4.8
2	68.4	29.8	20.4	13.4	4.8
3	65.9	28.7	19.4	13.2	4.7
4	63.3	27.6	18.3	12.7	4.7
5	57.2	25.8	15.8	11.0	4.6

[a] Soaked in water at room temperature for 3 hrs.

prepared from soaked and germinated soybens (100 *g* of dry soybean basis) are shown in Table 2. The main observation is that the amount of protein recovered from dry soybeans was increased during the initial stages of germination, while total solids were steadily decreased. This result may indicate the breakdown of insoluble protein by proteolytic enzymes to soluble form before utilization for growth metabolism. This result is agreeable with the report of Suberbie *et al.*[20] who found the increase of protein dispersibility index of fullfat soy flour when prepared from germinated soybean. A considerable reduction of carbohydrates was probably due to major decrease in oligosaccharides which were reported to be rapidly reduced during germination.[21] The ash recovered was very close to the amount prior to germination while the lipids showed a significant decrease in the later stage of germination.

Effect of germination on phytic acid and inorganic phosphorus in soymilk is shown in Table 3. The level of phytic acid decreased steadily throughout germination and reached 59% on the 5th day. At the same time inorganic phosphorus was liberated as a result of breakdown of phytic acid. The content of inorganic phosphorus in soymilk increased rapidly from 31.3 *mg* to 135.0 *mg* (100 *g* dry soybean basis) which accounts for 22.5% of total phosphorus. The organic phosphorus other than phytic acid was also gradually increased. The increased was probably due to the intermediates of inositol phosphates such as inositol pentaphosphate, inositol tetraphosphate, inositol triphosphate, inositol diphosphate and inositol monophosphate which were reported to be formed by enzymatic hydrolysis of phytic acid during germination.[22] The data of changes in phosphorus fractions of soymilk in the present study agree with the results of Reddy *et al.*[23] who investigated phytate phosphorus in black gram seeds during germination.

The contents of total and soluble minerals of Mg, Ca, Fe, Zn, Cu and Mn in soymilk (Tables 4 and 5) were changed but not uniformly. In general, however, the ratio of soluble minerals to total minerals were slowly and steadily increased in soymilk by germination. The highest content of mineral, in soymilk besides phosphorus, was Mg followed by Ca, Fe, Zn, Mn and Cu.

The results presented in this paper indicate that germination of soybeans before preparation of soymilk would improve the nutritional quality of soymilk by reduction of phytate content resulting in a potential increase in availability of minerals for humans. An apparent increase in protein recovered during the initial stages of germination would require further work in order to find the optimal conditions for germination which would improve the protein yield and nutritional quality of soymilk.

Table 3. Effect of germination on the contents of phosphorus compounds in soymilk

Time (Days)	Phytic acid [a] (*mg*)	Phosphourus (*mg*) [b]			Phytate P as total P (%)	Inorganic P as total P (%)	Organic P other than phytate P (%)
		Total	Phytate	Inorganic			
0 [c]	1264.3	642.5	347.1	31.3	54.0	4.9	41.1
1	1190.8	624.1	326.9	69.3	52.4	11.1	36.5
2	1155.6	609.1	317.2	72.8	52.1	12.0	36.1
3	942.7	588.4	258.8	98.3	52.0	16.7	42.6
4	861.6	592.0	236.5	103.3	44.0	17.5	42.6
5	742.4	599.0	203.8	135.0	34.0	22.5	43.4

[a] *mg* of total phytic acid in soymilk prepared from 100*g* dry soybean germinated.
[b] *mg* of phosphorus in soymilk prepared from 100*g* dry soybean germinated.
[c] Soaked in water at room temperature for 3 hrs.

Table 4. Effect of germination on the contents of total and soluble Mg, Ca and Fe in soymilk[a]

Time (days)	Mg			Ca			Fe		
	Total (*mg*)	Soluble (*mg*)	Soluble as total (%)	Total (*mg*)	Soluble (*mg*)	Soluble as total (%)	Total (*mg*)	Soluble (*mg*)	Soluble as total (%)
0[b]	124.0	79.8	64.4	25.6	16.6	64.8	16.1	6.6	41.0
1	118.4	77.6	65.5	24.8	15.5	62.5	25.1	12.0	47.8
2	116.1	80.2	69.1	24.3	15.5	63.8	25.3	12.3	48.6
3	110.8	72.4	65.3	24.4	14.7	60.2	18.0	13.0	72.2
4	107.7	74.7	69.4	24.1	14.4	59.8	17.8	8.6	48.3
5	100.1	85.6	85.6	22.5	12.4	55.1	29.5	6.1	20.7

[a] Soymilk prepared was based on the use of 100*g* of dry soybeans.
[b] Soaked at room temperature for 3 hrs.

Table 5. Effect of germination on the contents of total and soluble Zn, Cu and Mn in soymilk[a]

Time (days)	Zn			Cu			Mn			Soluble minerals as total minerals[c] (%)
	Total (*mg*)	Soluble (*mg*)	Soluble as total (%)	Total (*mg*)	Soluble (*mg*)	Soluble as total (%)	Total (*mg*)	Soluble (*mg*)	Soluble as total (%)	
0[b]	4.9	2.3	46.9	1.7	1.1	64.7	2.5	0.9	36.0	61.4
1	4.9	4.4	89.8	1.7	1.2	70.6	2.7	0.9	33.3	62.8
2	4.4	3.9	88.6	1.8	1.1	61.1	2.7	0.8	29.6	65.2
3	4.9	3.2	65.3	2.0	1.2	60.0	2.8	1.0	35.7	64.8
4	5.0	4.4	88.0	1.9	1.2	63.2	2.8	1.1	39.3	65.5
5	4.4	3.8	86.4	2.1	1.1	52.4	3.1	1.1	35.5	68.1

[a] Soymilk prepared was based on the use of 100*g* of dry soybeans.
[b] Soaked at room temperature for 3 hrs.
[c] Sum of total or soluble Mg, Ca, Fe, Zn, Cu and Mn.

요 약

콩우유 중의 무기성분과 결합하여 이들의 이용성을 저하시키는 phytic acid의 함량을 감소시키고저 콩을 마쇄하기 전에 25℃에서 5일동안 콩을 발아시켜 콩우유를 제조하였으며 제조된 콩우유는 phytic acid와 수용성 무기성분, 그리고 일반성분과 수율의 변화를 조사하였다. 100*g*의 콩을 기준으로 할 때 제조된 콩우유의 고형분은 발아가 진행되면서 서서히 감소한 반면 회수된 단백질의 양은 발아 2일까지 증가했다가 감소하는 경향을 보였고 탄수화물과 지방질은 지속적인 감소를 보였다. 한편 발아시키지 않은 100*g*의 콩으로 제조된 콩우유는 1.26*g*의 phytic acid가 함유되었었으나 발아 5일후에는 41.3%가 감소된 0.74*g*이 회수되었으며 이 결과 inorganic P는 31.3*mg*에서 135.0*mg*으로 증가하였다. 가용성 무기성분인 Mg, Ca, Zn등의 변화에는 뚜렷한 경향은 없었으나 전체 무기성분에 대한 수용성 무기물의 비율은 점차 증가하였다.

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