

Germination Effect of Soybean on Its Contents of Isoflavones and Oligosaccharides

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Abstract Three Korean soybean varieties - Shinpaldal-2, Seomoktae and Seoritae - were investigated for changes in their physical properties and the amount of functional components (i.e. isoflavones and oligosaccharides), during germination. Soybeans were germinated at 20°C for 96 hr in complete darkness. The dry weights of cotyledone, hypocotyl, seed coat, and hilum of Seoritae were heavier than those of other varieties. The dry weights of the three bean varieties decreased steadily in spite of root growth. The largest amount of isoflavone content was observed from Shinpaldal-2 (1.824 mg/g), followed by Seoritae (1.216 mg/g) and Seomoktae (1.125 mg/g). Total isoflavone content increased by 13% during initial germination, and then decreased thereafter. Aglycone types such as daidzein and genistein dominated the increase in isoflavone contents. The increase in genistein content of Shinpaldal-2 was 17.5 fold compared with ungerminated soybean, while the amount of daidzein was 6.7 times as much as ungerminated Shinpaldal-2 over an 18-hr germination period. Oligosaccharide contents such as raffinose (Raf) and stachyose (Sta) rapidly decreased during germination, while the sucrose (Suc) content remained constant until 36-48 hr of germination. From these results, it was clearly shown that the germination process significantly changed the contents of functional nutrients in soybeans. Therefore, the optimization of germination process should be considered to improve the biological functionality of soybeans in food processing.

Keywords: soybean, germination, isoflavone, aglycone, oligosaccharides

Introduction

Isoflavone, a functional compound in soybeans, has been intensively studied for its preventative effects on chronic diseases such as cancer of the breast, prostate and colorectal, and cardiovascular disease, osteoporosis, and diabetes (1-4). Soybeans have shown to be a major source of functional isoflavones such as genistein, genistin, daidzein, and daidzin. It has been demonstrated that genistein and genistin promote health by reducing the incidence of specific cancer and atherosclerosis (5, 6). Daidzein and daidzin are known to have protective effects against osteoporosis (7) and soy isoflavones relieve menopausal symptoms (8). The oligosaccharides, another functional soybean component, are important nutrients in food, and reports indicate that there are effective bifidus factors in the colon. Additionally, the biologically functional soy oligosaccharides have been identified as stachyose (Gal-Gal-Glc-Fru) and raffinose (Gal-Glc-Fru) (9-12).

Germinated soybean sprouts are served as a staple vegetable in many Asian countries and are used in soups, salads and side dishes (13). Germination may cause changes in the nutrients, including functional substances through aerobic respiration and biochemical metabolism. Previously, it was reported that non-protein nitrogen increased (14) during germination, while protein and lipids decreased (15). Germination also affected the level of oligosaccharides by significantly reducing it by 60-100% (16-18). However, there is no report on the effects of

germination changing isoflavone content.

The objectives of this study were to investigate the effects of initial germination on the contents of isoflavone and oligosaccharides in soybean. A secondary concern is to select the optimal germination time in terms of isoflavone increases for further use as a functional food source.

Materials and Methods

Materials Three soybean varieties used in this experiment were purchased from Nonghyup (Seoul, Republic of Korea), which were harvested in 2002. The two black bean varieties were Seomoktae and Seoritae, and the third one was a Shinpaldal-2 yellow bean. Isoflavone standards (daidzein, daidzin, genistein, genistin, glycitein and glycitin), and oligosaccharides standards (sucrose, raffinose and stachyose) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other reagent grade chemicals and the HPLC grade solvents (acetonitrile, ethanol and acetic acid), were obtained from J. T. Baker (J. T. Baker. Co., USA).

Germination The soybeans were placed in a plastic container (30 cm×15 cm) and germinated in an incubator at 20°C in complete darkness for four days, with water sprinkling five times a day. The germinated soybeans were dehydrated in a drying oven at 60°C for 24 hr. All beans were ground using a waring blender to pass a 30-mesh sieve and were then stored at -20°C until use.

Measurement of weight, size and root length of soybean Randomly sampled 100 soybeans were weighed and measured for g whole soybean/100 seeds, g cotyledone/100 seeds, g hypocotyl/100 seeds, and g seed

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coat/100 seeds. The length and width of 20 soybeans were measured using a vernier caliper (Mitutoyo, Japan; 5/100 mm), whereby the mean values of all three measurements were calculated. The root length and dry weight of germinated soybeans were measured as well. The dry weights of the germinated soybeans were measured after preliminary drying at 60°C, followed by drying at 105°C for 2 hr. Using a Color Difference Calculating Meter (CR-200, Minolta Inc., Japan), the color of the whole, cotyledone and seed coat of germinated soybeans were measured.

Extraction and analysis of isoflavone One gram of ground samples was extracted with 20 mL of 80% ethanol at 50°C for 1 hr, with using an ultrasonicator. Prior to HPLC analysis, the extract was centrifuged at 12,000 × *g* for 15 min, then the supernatant was filtered through a syringe filter (0.22 μm, Waters Co., Milford, Massachusetts, USA). Analysis of isoflavone was performed with the HPLC (Waters Co., Milford, Massachusetts, USA) and equipped with a Waters 486 Absorbance UV Detector set at 254 nm. The column used was an XTerra™ RP₁₈ column (5 μm, 4.6 × 250 mm, Waters Co., USA). The HPLC eluent consisted of 0.1% (v/v) acetic acid in water (solvent A), and 0.1% (v/v) acetic acid in acetonitrile (solvent B). The solvent gradient was as follows: the proportion of solvent B increased from 15% to 23% for 0-40 min, increased to 27% within the next 30 min, and then increased to 35% within the next 15 min. It finally reached 40% within the last 5 min at a flow rate of 1 mL/min. Standard curves were obtained by injecting the standards of daidzein, daidzin, genistein, genistin, glycitein and glycitin based on the corresponding peak area on the HPLC chromatograms. The isoflavone sample was determined using standard curves. The average amount of each isoflavone was calculated using triplicate analysis (19).

Extraction and analysis of oligosaccharide One gram of soybean powder was extracted with 10 mL of 10% ethanol for 1 hr using an ultrasonicator. The extract was centrifuged at 12,000×*g* for 20 min. Supernatant, in the amount of 0.5 mL, was added to 0.5 mL of 10% lead acetate and 0.5 mL of water. Then it was centrifuged at 12,000 × *g* for 5 min to remove the proteins. The supernatant was added to 10% oxalic acid to precipitate surplus lead acetate, which was followed by filtration through a syringe filter (0.22 μm, Waters Co., USA), prior to the HPLC analysis (20). Oligosaccharides were analyzed using the HPLC (Waters Co., USA), using a Waters 2414 Refractive Index Detector and a Carbohydrate analysis column (3.9 × 300 mm). The eluent of 65% acetonitrile was used in the isocratic mode at the flow rate of 1.3 mL/min for 20 min. Standard curves were prepared for sucrose (Suc), raffinose (Raf), and stachyose (Sta). The peak area of individual components on the chromatogram was calculated, and the quantitative amount of each oligosaccharide was expressed in relative terms to the peak areas obtained with standard solution. Triplicate samples were analyzed and average values were calculated.

Statistical analysis Statistical analysis was performed with one-way ANOVA using SPSS software for Windows

12.0. A significant difference at the 5% probability level was estimated using Duncan's Multiple Range Test.

Results and Discussion

Changes in Grain Weights and Root Lengths during Germination The physical properties of the three soybean varieties, such as grain weights, size and color, were determined and presented in Tables 1 and 2. The initial dry weights (ungerminated 100 grains) of Seoritae, Shinpaldal-2 and Seomoktae were 37.5 g, 23.36 g and 10.94 g, respectively. The weights of cotyledone, hypocotyl, seed coat, and hilum of Seoritae were heavier than those of other varieties. The weight ratios of hypocotyls- and seed coat-to-whole soybean were 1.6% and 6.9% for Seoritae, 2.6% and 5.8% for Shinpaldal-2, and 2.3% and 8.3% for Seomoktae, respectively. These data demonstrated that the difference in weight ratio of seed coats was due to the size of soybean. The seed coat of Shinpaldal-2 was light yellow, while Seoritae and Seomoktae were black. Shinpaldal-2 displayed the highest levels of L (lightness), a (redness), and b (yellowness) values, while Seomoktae and Seoritae were low in these values.

The change in soybean root length during germination is shown in Table 3. It took 24 hr to observe the root

Table 1. Physical characteristics of three soybean varieties

Physical characteristics	Soybean varieties		
	Seoritae	Seomoktae	Shinpaldal-2
Weight ^{b)} (g)			
Seed	37.50	10.94	23.36
Cotyledon	33.66	9.27	21.25
Hypocotyl	0.60	0.25	0.60
Seed coat	2.60	0.91	1.36
Hilum	0.22	0.12	0.15
Size (mm)			
Whole length	9.65±0.85	5.84±0.57	8.27±0.54
Long width	5.83±0.43	4.24±0.56	6.5±0.34
Short width	7.97±0.43	5.23±0.49	7.27±0.30
Hilum length	2.93±0.41	2.00±0.33	2.79±0.21

^{b)}All of the weights are based on 100 seeds

Table 2. Color values of whole seeds, cotyledon and seed coats of three soybean varieties

Physical characteristics	Soybean varieties		
	Seoritae	Seomoktae	Shinpaldal-2
Whole seed			
L	74.39±0.59	73.80±0.47	86.41±0.55
a	-4.83±0.19	-4.36±0.43	-2.27±0.14
b	13.76±0.27	12.61±0.91	19.54±0.48
Seed coat			
L	44.1±0.33	38.41±0.89	67.96±0.17
a	0.08±0.02	-0.31±0.13	-0.25±0.17
b	1.74±0.26	1.61±0.35	17.24±0.53
Cotyledon			
L	82.65±0.20	82.56±0.31	87.21±0.23
a	-6.28±0.04	-5.47±0.27	-2.23±0.02
b	16.61±0.10	15.30±0.95	18.71±0.16

budding off from the embryo in Shinpaldal-2 and Seomoktae, and 48 hr for Seoritae. The small-sized Seomoktae was well germinated and evenly grown, while the medium-sized Shinpaldal-2 and Seoritae were unevenly grown including cracking of tissue and some discoloration after a 48-hr germination period.

During the soybean germination at 20°C, dry weights steadily decreased in spite of root growth. The decrease in dry weight is most likely due to protein, lipids and oligosaccharides (reserve carbon source) consumption for germination without supplying of new nutrients. The dry weights decreased by 14.29% of Seoritae, 10.29% of Seomoktae and 11.82% of Shinpaldal-2 over a 96-hr germination period. The decrease in dry weight may be related to a rapid decrease in oligosaccharides during the initial stage (16). Additionally, a slow decrease in lipids and nitrogen compounds occurs at a later germination stage (14).

Changes of isoflavone contents The initial isoflavone content (0.16%, db) of Shinpaldal-2 was significantly larger than those of Seomoktae (0.10%) and Seoritae (0.11%) as shown in Tables 5-7. It was observed from all the three soybean varieties that total isoflavones content increased during a short period (6-24 hr) within germination, and then gradually decreased thereafter. The germination

time displaying maximal isoflavone content varied according to the type of soybean (13.2% of Shinpaldal-2 was observed at 12 hr, 12.5% of Seomoktae at 6 hr and 12.1% of Seoritae at 24 hr).

The changes in the amount of individual types of isoflavone, such as daidzin, daidzein, genistein, genistin, glycitin and glycitein, were monitored during a 96-hr germination period. Among the analyzed isoflavones, the contents of aglycone-type genistein and daidzein changed most drastically. In case of Shinpaldal-2, the increase in genistein content was 17.5 times over an 18-hr germination period, while the corresponding glycoside, genistin, decreased by only 7%. Thus, this metabolic conversion does not explain the entire picture of genistein production. It was possible that another biochemical pathway in the plant system produced this specific compound. Another form of isoflavone, daidzein, displayed 6.7 times as much as ungerminated Shinpaldal-2 over a 12-hr germination period. Such a drastic change was not observed from glycitein, but still more than two-fold increase of glycitein occurred. The level of glycitin was constant over an 18-hr germination period. Other soybean varieties also showed similar changes in individual isoflavone content.

This data indicated that isoflavone was not only produced from biosynthetic pathways, but also metabolized from glycosides to aglycone during germination by β -glucosidase in soybean (21, 22). Meanwhile, certain types of malonyl and acetyl esterases could be induced and expressed during germination (23). Most likely, these esterases converted isoflavone isomers, such as acetyl and malonyl isoflavones into their relevant glycoside forms, which eventually metabolized to aglycone forms. Further investigation is needed to identify the exact biochemical pathways of aglycone type isoflavone production.

Changes of oligosaccharide contents The changes in the amount of major soybean oligosaccharides such as Suc, Raf, and Sta during germination are shown in Table 8. The total oligosaccharide contents rapidly decreased as the germination process proceeded. Among the three varieties of soybean, Seomoktae contained the greatest oligosaccharide content (13.6%) followed by Shinpaldal-2 (12.1%) and Seoritae (8.7%). The Raf and Sta contents drastically decreased compared with the Suc content. After 24 hr of germination, Sta reduced its content by 34-64%, Raf by 30-45% and Suc by 4-18%. The level of Suc in soybean was relatively constant compared with those of Raf and Sta during 36-48 hr of germination. They were then subject to decrease noticeably (16). For instance, the amounts of Raf and Sta in Shinpaldal-2 decreased by 42 and 53%, respectively, while Suc displayed only 15% reduction during a 36-hr germination period. It was expected that the constant level of Suc resulted from an active metabolic balance between the Raf and Sta production and the hydrolysis for germination energy generation.

This result confirmed that Raf and Sta were energy sources for seed germination as described in previous studies (24). The decreased amount of Raf and Suc is likely due to the sequential hydrolysis of Sta to Raf, and then Suc by α -galactosidases (24, 25).

After the initial soybean germination for 12-24 hr, the

Table 3. Changes in root length of soybean sprouts during germination at 20°C
(unit : mm)

Germination (hr)	Soybean varieties		
	Seoritae	Seomoktae	Shinpaldal-2
0	0 ^c	0 ^c	0 ^c
6	0 ^c	0 ^c	0 ^c
12	0 ^c	0 ^c	0 ^c
24	0 ^c	2.85±0.33 ^e	3.25±1.54 ^c
30	0 ^c	4.01±1.56 ^{de}	4.05±2.35 ^c
36	0 ^c	10.25±1.75 ^{cd}	7.75±1.71 ^c
48	2.25±0.408 ^{bc}	15.25±2.22 ^c	9.05±4.21 ^{bc}
72	14.82±6.55 ^b	28.75±6.27 ^b	19.65±7.21 ^b
96	40.37±21.00 ^a	52.48±9.64 ^a	42.35±17.06 ^a

a,b,c,d,e: Values with different letters within the same column are significantly different at 5% level by Duncan's multiple range test.

Table 4. Changes in the dry weights of soybeans during germination at 20°C¹⁾

Germination (hr)	Soybean varieties		
	Seoritae	Seomoktae	Shinpaldal-2
0	100	100	100
6	98.47	98.24	99.56
12	97.59	96.14	99.01
18	97.40	95.87	98.48
24	97.03	95.57	97.35
30	94.49	95.01	96.67
36	93.17	94.81	95.48
48	91.72	94.16	94.93
72	87.00	93.04	94.27
96	85.80	89.71	88.18

¹⁾Initial dry weights of soybeans were converted to 100 for the comparison of weight loss during germination.

Table 5. Changes in isoflavone content of Seoritae during germination at 20°C

Type of isoflavone	Germination time (hr)										(mg/g)
	0	6	12	18	24	30	36	48	72	96	
Daidzin	0.219±0.009 ^a	0.180±0.011 ^a	0.211±0.090 ^a	0.235±0.020 ^a	0.221±0.009 ^a	0.192±0.010 ^a	0.201±0.008 ^a	0.202±0.011 ^a	0.187±0.010 ^a	0.194±0.010 ^a	
Genistin	0.753±0.050 ^a	0.598±0.010 ^{de}	0.625±0.030 ^{bcd}	0.640±0.010 ^{bc}	0.724±0.010 ^a	0.626±0.010 ^{bcd}	0.649±0.010 ^{bcd}	0.607±0.010 ^{cde}	0.607±0.010 ^{cde}	0.582±0.010 ^e	
Daidzein	0.029±0.002 ^a	0.015±0.001 ^d	0.014±0.001 ^d	0.020±0.001 ^c	0.014±0.002 ^d	0.021±0.002 ^c	0.021±0.001 ^c	0.019±0.001 ^c	0.024±0.002 ^b	0.025±0.002 ^b	
Genistein	0.022±0.001 ^c	0.283±0.001 ^{ab}	0.237±0.020 ^{ab}	0.210±0.020 ^{ab}	0.213±0.010 ^{ab}	0.214±0.090 ^{ab}	0.294±0.010 ^a	0.187±0.090 ^b	0.301±0.090 ^a	0.231±0.010 ^{ab}	
Subtotal	1.023±0.110 ^a	1.076±0.100 ^a	1.087±0.100 ^a	1.105±0.010 ^a	1.172±0.100 ^a	1.053±0.090 ^a	1.165±0.050 ^a	1.015±0.100 ^a	1.119±0.010 ^a	1.032±0.100 ^a	
Glycitin	0.031±0.002 ^{abc}	0.032±0.001 ^{ab}	0.028±0.000 ^{de}	0.030±0.001 ^{bcd}	0.028±0.000 ^{de}	0.029±0.001 ^{cd}	0.033±0.002 ^a	0.029±0.001 ^{cd}	0.029±0.001 ^{cd}	0.026±0.001 ^c	
Glycitein	0.005±0.000 ^g	0.027±0.000 ^a	0.028±0.000 ^a	0.027±0.001 ^a	0.016±0.001 ^d	0.010±0.001 ^f	0.022±0.001 ^b	0.013±0.002 ^e	0.014±0.001 ^c	0.019±0.001 ^c	
Total	1.085±0.010 ^a	1.135±0.100 ^a	1.143±0.130 ^a	1.162±0.100 ^a	1.216±0.100 ^a	1.092±0.120 ^a	1.220±0.150 ^a	1.057±0.100 ^a	1.162±0.100 ^a	1.077±0.120 ^a	

^{a,b,c,d,e,f,g}: Values with different letters within the same row are significantly different at 5% level by Duncan's multiple range test.

Table 6. Changes in isoflavone content of Seomoktae during germination at 20°C

Type of isoflavone	Germination time (hr)										(mg/g)
	0	6	12	18	24	30	36	48	72	96	
Daidzin	0.228±0.018 ^a	0.175±0.021 ^{bc}	0.167±0.009 ^{cd}	0.171±0.009 ^c	0.195±0.019 ^b	0.127±0.009 ^c	0.145±0.009 ^{de}	0.159±0.011 ^{cd}	0.151±0.010 ^{cde}	0.151±0.010 ^{cde}	
Genistin	0.624±0.011 ^a	0.573±0.040 ^{abc}	0.590±0.031 ^a	0.578±0.020 ^{ab}	0.571±0.028 ^{abc}	0.502±0.044 ^d	0.500±0.050 ^d	0.568±0.032 ^{abc}	0.525±0.021 ^{bcd}	0.514±0.031 ^{cd}	
Daidzein	0.088±0.009 ^c	0.210±0.010 ^a	0.154±0.010 ^b	0.164±0.009 ^b	0.098±0.008 ^c	0.046±0.001 ^e	0.062±0.001 ^d	0.049±0.001 ^e	0.073±0.001 ^d	0.095±0.009 ^c	
Genistein	0.023±0.002 ^{de}	0.122±0.011 ^a	0.106±0.020 ^b	0.097±0.005 ^b	0.049±0.001 ^c	0.031±0.002 ^d	0.044±0.001 ^c	0.026±0.002 ^{de}	0.016±0.001 ^e	0.020±0.001 ^{de}	
Subtotal	0.963±0.091 ^{ab}	1.079±0.098 ^a	1.017±0.097 ^a	1.010±0.010 ^a	0.914±0.190 ^{abc}	0.707±0.120 ^d	0.751±0.100 ^{cd}	0.802±0.100 ^{bcd}	0.765±0.094 ^{bcd}	0.780±0.087 ^{bcd}	
Glycitin	0.031±0.000 ^{cd}	0.033±0.001 ^{bc}	0.030±0.002 ^d	0.035±0.000 ^b	0.038±0.001 ^a	0.027±0.001 ^{ef}	0.030±0.001 ^d	0.030±0.002 ^d	0.029±0.002 ^{de}	0.025±0.001 ^f	
Glycitein	0.006±0.000 ^c	0.012±0.002 ^a	0.012±0.002 ^a	0.010±0.001 ^{ab}	0.008±0.001 ^{bc}	0.006±0.002 ^c	0.008±0.001 ^{bc}	0.007±0.001 ^c	0.006±0.001 ^c	0.006±0.002 ^c	
Total	1.000±0.087 ^{abc}	1.125±0.190 ^a	1.059±0.120 ^{ab}	1.055±0.110 ^b	0.960±0.200 ^{abcd}	0.740±0.050 ^e	0.789±0.100 ^{cd}	0.839±0.100 ^{cd}	0.800±0.100 ^{cd}	0.811±0.100 ^{cd}	

^{a,b,c,d,e,f}: Values with different letters within the same row are significantly different at 5% level by Duncan's multiple range test.

Table 7. Changes in isoflavone content of Shinpaldal-2 during germination at 20°C

Type of isoflavone	Germination time (hr)										(mg/g)
	0	6	12	18	24	30	36	48	72	96	
Daidzin	0.364±0.020 ^a	0.339±0.010 ^{abc}	0.350±0.010 ^{abc}	0.332±0.010 ^{abc}	0.326±0.040 ^{abc}	0.315±0.010 ^{bc}	0.321±0.040 ^{abc}	0.299±0.030 ^c	0.331±0.020 ^{abc}	0.321±0.010 ^{abc}	
Genistin	1.093±0.010 ^a	0.984±0.070 ^{ab}	1.025±0.100 ^{ab}	1.016±0.120 ^{ab}	1.017±0.100 ^{ab}	0.826±0.080	0.950±0.010 ^{ab}	0.994±0.100 ^{ab}	0.985±0.100 ^{ab}	1.019±0.100 ^{ab}	
Daidzein	0.006±0.000 ^a	0.032±0.000 ^a	0.043±0.001 ^a	0.040±0.000 ^a	0.033±0.002 ^a	0.027±0.000 ^a	0.027±0.000 ^a	0.031±0.001 ^a	0.031±0.001 ^a	0.047±0.002 ^a	
Genistein	0.015±0.001 ^g	0.224±0.010 ^b	0.252±0.010 ^a	0.263±0.020 ^a	0.145±0.010 ^c	0.109±0.010 ^d	0.106±0.010 ^d	0.082±0.001 ^e	0.059±0.001 ^f	0.069±0.002 ^{ef}	
Subtotal	1.478±0.100 ^{abc}	1.579±0.200 ^{ab}	1.670±0.200 ^a	1.651±0.100 ^{ab}	1.521±0.140 ^{abc}	1.277±0.110 ^e	1.404±0.100 ^{bc}	1.406±0.100 ^{bc}	1.406±0.100 ^{bc}	1.456±0.100 ^{abc}	
Glycitin	0.109±0.010 ^a	0.113±0.010 ^a	0.113±0.020 ^a	0.110±0.000 ^a	0.105±0.000 ^a	0.102±0.010 ^a	0.109±0.010 ^a	0.105±0.020 ^a	0.119±0.010 ^a	0.108±0.020 ^a	
Glycitein	0.025±0.001 ^c	0.040±0.002 ^b	0.041±0.001 ^b	0.055±0.001 ^a	0.024±0.000 ^c	0.019±0.001 ^d	0.018±0.001 ^{de}	0.017±0.001 ^e	0.009±0.000 ^g	0.015±0.001 ^f	
Total	1.612±0.100 ^{ab}	1.732±0.300 ^{ab}	1.824±0.300 ^a	1.816±0.100 ^a	1.650±0.010 ^{ab}	1.398±0.300 ^b	1.531±0.100 ^{ab}	1.528±0.100 ^{ab}	1.534±0.100 ^{ab}	1.579±0.100 ^{ab}	

^{a,b,c,d,e,f,g}: Values with different letters within the same row are significantly different at 5% level by Duncan's multiple range test.

Table 8. Changes in oligosaccharide content of three soybean varieties during germination at 20°C (mg/g)

	Germination time (hr)									
	0	6	12	18	24	30	36	48	72	96
Shinpaldal-2										
Sucrose	71.3±0.09 ^a	70.1±1.24 ^a	61.5±1.56 ^c	60.6±0.89 ^{cd}	61.6±1.25 ^c	64.0±1.76 ^b	60.3±1.45 ^{cd}	58.7±1.29 ^d	52.3±1.37 ^e	46.3±1.64 ^f
Raffinose	5.2±0.12 ^{ab}	4.9±0.08 ^b	5.9±0.04 ^a	3.8±0.08 ^c	3.6±1.01 ^{cd}	3.2±0.08 ^{cde}	3.0±0.46 ^{cde}	2.9±0.78 ^{cde}	2.7±0.59 ^{de}	2.4±0.42 ^e
Stachyose	44.5±0.04 ^a	40.3±0.01 ^b	31.8±0.12 ^c	28.1±0.45 ^d	27.0±0.85 ^e	23.7±0.06 ^f	20.9±0.76 ^f	18.7±0.39 ^h	15.4±0.28 ⁱ	8.8±0.12 ^j
Total	121.0±0.98 ^a	115.3±1.58 ^b	99.2±1.36 ^c	92.5±0.98 ^d	92.2±1.45 ^d	91.0±1.07 ^d	84.2±1.32 ^e	80.3±1.19 ^f	70.4±1.27 ^g	57.5±1.71 ^h
Seomoktae										
Sucrose	64.0±0.01 ^a	53.3±2.83 ^c	52.2±0.07 ^c	52.1±0.02 ^c	52.4±3.54 ^e	53.4±4.53 ^c	57.6±2.19 ^b	51.3±0.85 ^c	44.1±0.92 ^d	33.0±0.49 ^e
Raffinose	10.3±0.02 ^a	5.1±0.01 ^{cd}	7.6±2.74 ^b	7.0±0.05 ^{bc}	6.2±1.48 ^{bc}	5.2±2.05 ^{cd}	3.9±0.57 ^{de}	2.2±0.85 ^{ef}	0.6±0.07 ^f	0.4±0.07 ^f
Stachyose	62.1±0.01 ^a	42.2±4.56 ^{cd}	47.6±3.18 ^b	43.4±1.81 ^c	41.2±2.66 ^{cd}	38.6±2.67 ^d	30.9±1.70 ^e	12.2±0.71 ^f	4.7±0.14 ^g	0.4±0.07 ^h
Total	136.4±0.01 ^a	100.5±6.75 ^c	107.4±7.84 ^b	102.5±3.42 ^{bc}	99.8±3.01 ^c	97.1±2.19 ^{cd}	92.5±1.13 ^d	65.7±1.06 ^e	49.4±1.34 ^f	33.8±0.85 ^g
Seoritae										
Sucrose	50.5±1.10 ^c	55.8±1.13 ^a	55.0±0.09 ^a	52.7±2.47 ^b	48.7±1.17 ^c	49.2±0.85 ^c	46.6±0.52 ^d	44.0±1.42 ^e	34.7±1.33 ^g	38.8±0.09 ^f
Raffinose	14.4±0.07 ^a	11.1±0.10 ^b	11.3±0.02 ^b	6.4±1.75 ^c	7.9±1.25 ^c	1.5±0.47 ^d	ND	ND	ND	ND
Stachyose	22.5±0.02 ^s	19.3±0.08 ^b	22.7±2.18 ^a	8.4±1.20 ^c	8.1±0.09 ^{cd}	6.0±0.65 ^e	6.7±0.42 ^{de}	5.6±0.68 ^e	3.5±0.29 ^f	2.4±0.05 ^f
Total	87.5±1.27 ^s	86.2±1.89 ^s	89.0±3.12 ^s	67.5±3.04 ^b	64.6±2.12 ^b	56.7±1.75 ^c	53.3±0.75 ^d	49.6±1.12 ^e	38.2±1.03 ^f	41.2±0.08 ^f

a,b,c,d,e,f,g,h,i,j: Values with different letters within the same row are significantly different at 5% level by Duncan's multiple range test.

total isoflavone content increased by 13% and oligosaccharides remained at 40-70%. In this study, we observed relatively short germination time significantly increased aglycone-type isoflavone content. Therefore, this initial germination process can be used for food processing to improve functionality and some physical properties of soybean products.

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References

- Messina MJ, Persky V, Setchell KDR, Barnes S. Soy intake and cancer risk; A review of the in vitro and in vivo data. *Nutr. Cancer* 21: 113-131 (1994)
- Barnes S. Evolution of the health benefits of soy isoflavones. *Proc. Soc. Exp. Biol. Med.* 217: 386-392 (1998)
- Tham DM, Gardner CD, Haskell WL. Potential health benefits of dietary phytoestrogens: A review of the clinical, epidemiological, and mechanistic evidence. *J. Clin. Endocr. Metab.* 83: 2223-2235 (1998)
- Bingham SA, Atkinson C, Liggins J, Bluck L, Coward, A. Phytoestrogens: Where are we now? *Br. J. Nutr.* 79: 393-406 (1998)
- Lee HP, Gourley L, Duffey SW, Esteve J, Lee J, Day NE. Dietary effects on breast-cancers in Singapore. *Lancet* 337: 1197-2000 (1991)
- Witztum JL. The oxidation hypotheses of atherosclerosis. *Lancet* 344: 793 (1994)
- Arjmandi BH, Smith BJ. Soy isoflavones' osteoprotective role in postmenopausal women: mechanism of action. *J. Nutr. Biochem.* 13: 130-137 (2002)
- Aldercreutz H, Hamalainen E, Gorbachh S Goldin B. Dietary phyto-estrogens and the menopause in Japan. *Lancet* 339: 1233 (1992)
- Hidaka H, Eida T, Takizawa T, Tokunaga T, Tashiro Y. Effect of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora* 5: 37-50 (1986)
- Okazaki M, Fujikawa S, Mtsumoto N. Effect of xylooligosaccharides on the growth of bifidobacteria. *Bifidobacteria Microflora* 9: 77-86 (1990)
- Kim JR, Yook C, Kwon HK, Hong SY, Park CK, Park KH. Physical and physiological properties of isomaltoligosaccharides and fructooligosaccharides. *Korea J. Food Technol.* 27: 170-175 (1995)
- Kato Y, Ikeda N, Iwanami T, Ozaki A, Ohmura K. Change of soybean oligosaccharides in the digestive tract. *J. Jpn. Soc. Nutr. Food Sci.* 44: 29-35 (1991)
- Kim YH, Kim SD, Hong EH. Characteristics of soy sprouts cultivated with soybeans for sprout. *RDA J. Agri. Sci.* 36: 107-112 (1994)
- Yang CB, Kim ZU. Changes in nitrogen compounds in soybean sprout. *J. Korean Agric. Chem. Soc.* 23: 7-13 (1980)
- Lee SH, Chang DH. Studies on the effects of plant growth regulator on growth and nutrient compositions in soybean sprout. *J. Korean Agric. Chem. Soc.* 25: 75-82 (1982)
- Kim WJ, Smit CJB, Nakayama TOM. The removal of oligosaccharides from soybeans. *Lebensm. Wiss. U. Technol.* 6: 21-24 (1973)
- Sathe SK, Dehpande SS, Reddy NR, Goll DE, Salunkhe DK. Effects of germination on proteins, raffinose oligosaccharides and antinutritional factors in the Great Northern Beans. *J. Food Sci.* 48: 1796-1800 (1983)
- Kadlec P, Skullinova M, Kaasova J, Bubnik Z, Pour V, Dostalova J, Valentova H, Hosnedl V. Changes in composition of pea during germination, microwave treatment and drying. *Food Sci. Biotechnol.* 12: 213-218 (2003)
- Choi YB, Rhee JS, Lee YB, Nam SY, Kim KS. Extraction of isoflavones from soybean hypocotyls using aqueous ethanol. *Food Sci. Biotechnol.* 13: 700-706 (2004)
- Mok CK, Ku KH, Park DJ, Kim N, Sohn HS. Ultrafiltration of soybean cooking water for the production of soy-oligosaccharides. *Korean J. Food Sci. Technol.* 27: 181-184 (1995)
- Ha EYW, Morr CV, Seo A. Isoflavone aglucones and volatile organic compound in soybeans; effect of soaking treatments. *J. Food Sci.* 57: 414-417 (1992)
- Hendrich S. Bioavailability of isoflavones. *J. Chromatogr. B.* 777: 203-210 (2002)
- Hsieh MC, Graham TL. Partial purification and characterization of a soybean β -glucosidase with high specific activity towards isoflavone conjugates. *Phytochemistry* 58: 995-1005 (2001)
- Kim SL, Moon JK, Yun HT, Park KY, Lee YH, Ryu YH, Ku JH, Kim SD. Varietal variation of oligosaccharides during germination in soybean. *Korean J. Breed.* 34: 244-250 (2002)
- Cruz R, Batistela JC, Wosiacki G. Microbial α -galactosidase for soymilk processing. *J. Food Sci.* 46: 1196-1200 (1981)