한작지(Korean J. Crop Sci.), 55(2): 165~176(2010)

Comparison of Soyasapogenol A, B Concentrations in Soybean Seeds and Sprouts

Eun-Young Kang*, Seung-Hyun Kim**, Sun-Lim Kim***, Su-Hyun Seo*, Eun-Hye Kim*, Hong-Keun Song*, Joung-Kuk Ahn*, and Ill-Min Chung*†

** Department of Applied Life Science, College of Life and Environmental Science, Konkuk University, Seoul 143-701, Korea*
***Agricultural and Environmental Sciences, University of California, Davis, CA 95616, USA*
**** Rural Development Adminstration Resarch Plicy Bureau, Suwon 441-707, Korea*

ABSTRACT Soybean seeds contain many biologically active secondary metabolites, such as proteins, saponins, isoflavones, phytic acids, trypsin inhibitors and phytosterols. Among them, saponins in soybeans have attracted considerable interest because of their health benefits. Soyasaponin A and B are the most abundant types of saponins found in soybeans along with soyasapogenol (aglycone), which is a precursor of soyasaponin. The main purpose of this experiment was to determine the concentration of soyasapogenol in soybean seeds and sprouts as a function of seed size, usage, seed coat color and seed cotyledon color. The 79 Korean soybean varieties were cultivated at Yesan of Chungnam in 2006 for the analysis of soyasapogenol using HPLC with Evaporative Light Scattering Detection (ELSD). The total average concentration of soyasapogenol was 1313. 52 μg g^{-1} in soybean seeds and 1377.72 μg g^{-1} in soybean sprouts. Soybean sprouts were about 5% higher than soybean seeds in average total soyasapogenol concentration. In the process of sprouting, the average soyasapogenol A content decreased by approximately 1.6%, but soyasapogenol B and total soyasapogenol increased by 8.31% and 4.88%, based on the content of soybean seeds. When classified according to the size of seeds, the total soyasapogenol concentration of soybean seeds were not significantly different (p<0.05) On average, small soybean seeds were increased by as much as 103.14 μg g^{-1} in sprouting process. As a function of the use of the seeds, The total soyasapogenol in soybean seeds were significantly different (p<0.05). While, the soybean sprouts were not significant different (p<0.05). Altogether, sprout soybean seeds show the greatest change in content during the germination process. When seeds with different coat colors were compared, the total soyasapogenol concentration of soybean with yellow seed coats (1357.30 μg g^{-1}) was slightly higher than that of soybean with black (1260.30 μg g^{-1}) or brown (1263.62 μg g^{-1}) seed coats. For the color of the cotyledon, the total soyasapogenol concentration was significantly increased in green cotyledon during the germination and seedling process. The results of this study suggest the functional characteristics of soybeans through quantitative analysis of soyasapogenol. In addition, the concentration of soyasapogenol exhibited a change during the germination process, which was evaluated by the nutritional value of the soybean sprouts.

Keywords : Soybean, soyasapogenol A and B, seeds, sprouts

†Corresponding author: +82-2-450-3730
(E-mail) imcim@konkuk.ac.kr < Received June 8, 2010 >

Soybean that has been cultivated for long time as a principle food crop in Asia is one of the critical international food resources, containing approximately 40% protein and 20% fat (Kim & Kim 2001; Kim *et al*., 2006). In Korea, soybean has been utilized as a protein source to supplement the inadequate nutrition of rice. Soybean is used in various ways, such as cooked with rice, paste, curd, sprout, etc.

Soybeans have secondary metabolites, such as amino acids, phenolic compounds, isoflavones, saponins, phytic acids and peptides, with various functions (Kim *et al*., 2006). These substances are generated to protect the soybeans from external attack (Oh *et al*., 2003; Oh *et al*., 2007). Previously known as antinutritional factors, these substances have a physiological function, and much research is actively under way on their content in soybeans and their health benefits (Chi *et al*., 2005).

Soyasaponin (saponins in soybean) constitutes 0.5% of the total soybean dry weight. The variety, cultivation year, location grown and degree of maturity affect the total soyasaponin content (Rupasinghe *et al*., 2003; Shiraiwa *et al*., 1991). Soyasaponins have attracted attention because of their various physiological activities; for example, antiviral activity against HIV, prevention of hypercholesterolemia, inhibition of cell damage, antihepatitis, anticancer and antioxidative effects (Shiraiwa *et al*., 1991; Berhow *et al*., 2000; Oh *et al*., 2003; Rupasinghe *et al*., 2003; Berhow *et al*., 2006). Ellington *et al*. (2005) reported that group B soyasaponin inhibits proliferation and spread of human colorectal cancer (HCT-15). According to Zhang *et al*. (2008), soyasapogenol A and B are more effective than soybean saponin extract in suppressing Hep-G2 (liver cancer cell) proliferation. Kuzuhara *et al*. (2000) reported that soyasapogenol A regulates the number of inflammatory cells in the liver and prevents apoptosis of hepatocytes.

Soyasaponin is a triterpenoid glycoside including soyasapogenol (aglycone) connected with a polysaccharide (Rupasinghe *et al*., 2003, Kim *et al*., 2006). The soyasaponins are classified into two categories depending on their soyasapogenol (aglycone) structure. Group A soyasaponin consists of soyasaponin A1 and A2, and group B soyasaponin includes soyasaponin I, II and III (Kudou *et al*., 1993; Gurfinkel & Rao, 2002). Soyasapogenol is the precursor of group A and B soyasaponin and the aglycone of soyasaponins. Structurally, for soyasapogenol A and B, the group at the C-21 position is different (Rupasinghe *et al*., 2003). Group B soyasaponin is present in all edible seeds, while group A soyasaponin only exists in the hypocotyls of soybean seeds (Shiraiwa *et al*., 1991).

Soybean seed can be used as a soybean sprout after the germination process. Soybean sprout contains high-quality protein and vitamin C; therefore, it can be called an ideal food. Seed nutrition changes in the germination process by increasing the content of saponin, the highest content being shown after 5-6 days (Oh *et al*., 2003). During germination, the soyasapogenol A concentration of the seeds was unchanged, while the soyasapogenol B content was increased (Rupasinghe *et al*., 2003). Shiraiwa *et al*. (1991) reported that the saponin content in soybean seed cotyledons and hypocotyls depended on the variety, rather than the cultivation year. Soyasaponin concentration in hypocotyls was higher than in cotyledons, and the seed coat showed extremely low saponin content (Shimoyamada *et al*., 1990; Shimoyanada *et al*., 1991). The concentration of saponin was increased by chitosan treatment of the soybean sprout (Oh *et al*., 2007).

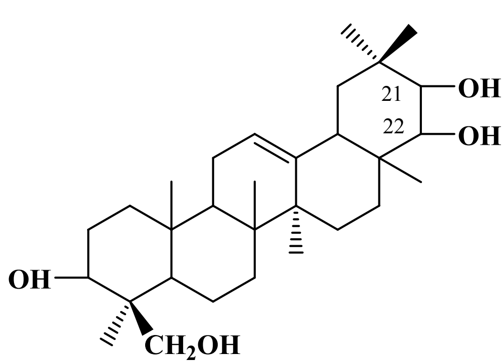
Research on the secondary metabolites in soybeans is actively under way, but there is a relative lack of research on soyasapogenol. In this experiment, we examined the variation of soyasapogenol content according to different seed size, usage, seed coat color and cotyledon color, as well as the process of seed germination.

MATERIALS AND METHODS

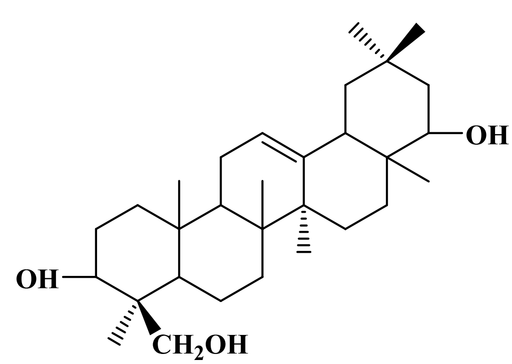
Preparation of samples

The 79 Korean soybean varieties were cultivated at Yesan of Chungnam in 2006, according to standard cultivation methods. Soybean seeds were harvested with triplicate replications in each cultivar and stored at 4°C for analysis of soyasapogenol A and B (Fig. 1). The varieties were classified according to the seed coat color, cotyledon color, 100-seed weight and usage.

In this study, soybeans were classified into three types, large (above 25 g, 22 varieties), medium (15–25 g, 27



Soyasapogenol A



Soyasapogenol B

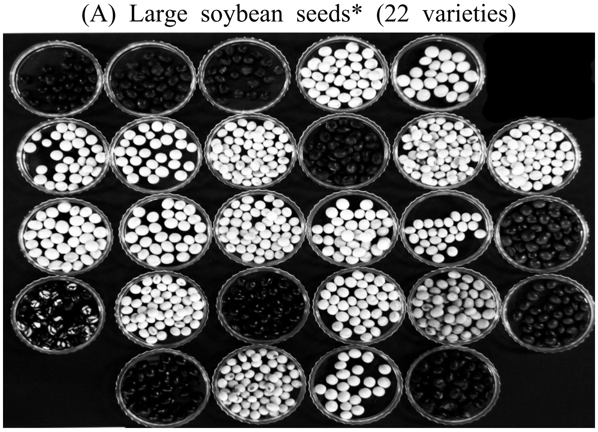
Fig. 1.

varieties) and small (below 15 g, 30 varieties) by 100-seed weight (Fig. 2).

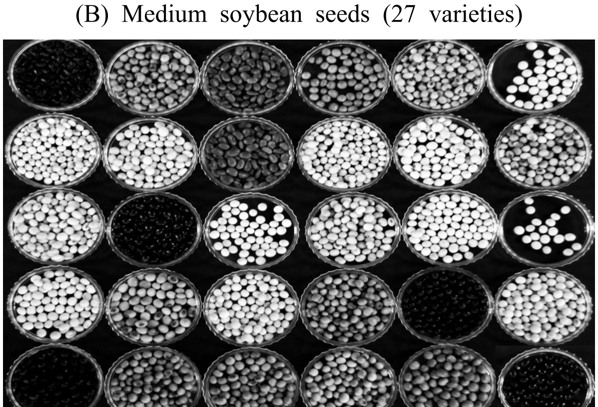
Soybean sprouts were prepared by the following procedure. 4 g of soybean seed was washed and submerged in tap water at 20 ± 2°C for 12 h. Seeds were spread uniformly in a conical tube with holes and given sufficient water more than six times a day and cultivated for five days in a dark chamber.

After this, the soybeans and soybean sprouts were freeze-dried at -47°C and then crushed and stored in a desiccator for analysis. When the soybean sprouts had grown by absorbing water, their weight was increased by a factor of four compared with the weight of fresh seeds. However, freeze-drying to remove all the water decreased the weight by 22% compared with the weight of fresh seeds. Similarly, Shimoyamada *et al*. (1991) reported that during seed germination, seeds fresh weight was increased about eight times, while the seeds dry weight was unchanged.

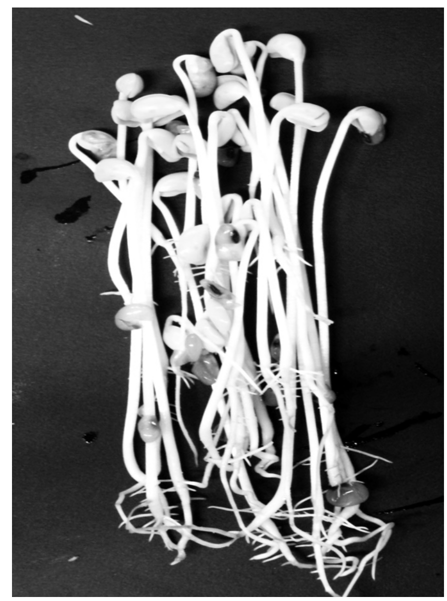
(A) Large soybean seeds* (22 varieties)



(B) Medium soybean seeds (27 varieties)



(C) Small soybean seeds (30 varieties)



(D) Parannamulkong sprout

(E) Pureunkong sprout

Fig. 2.

Analysis of soyasapogenol A and B

Sample preparation

The content of soyasapogenol A, B was analyzed by the method of Rupasinghe *et al*. (2003). 0.2 g of finely ground soybean seed and sprout powder was extracted with 30 mL of 80% EtOH and horizontally stirred in a water bath/shaker at 50°C for 2 h. The suspension was centrifuged at 3000 rpm, 4°C for 5 min. 15 mL of the supernatant liquid was transferred to a 100 mL round flask and evaporated to dryness under reduced pressure using a rotary vacuum evaporator, below 40°C, and the remaining residue was reconstituted with 8 mL of 1 N HCl in methanol. The resuspension was horizontally stirred in a water bath/shaker at 75°C for 2.5 h to release the soyasapogenol from soyasaponins as a result of acid hydrolysis. The solution was placed in a cartridge (Vac 6CC 500 mg C-18 Sep-Pak cartridge) and washed with water, and the soyasapogenol was eluted with methanol. The aliquot samples were filtered through a 0.2 μm nylon syringe filter (TITAN, USA) and analyzed by Evaporative Light Scattering Detection (ELSD). All of the solvents were used as HPLC grade (J. T. Baker, USA) without further purification.

Analysis of soyasapogenol A, B

The instrumentation for ELSD analysis was applied by the method of Rupasinghe *et al*. (2003). The ELSD system consisted of an Alltech 2000 ES ELSD, a TSP (Thermo Separation Products, USA) AS1000 auto injector and a Young-Lin Acme 9000 pump (Korea). Nitrogen was used as the ELSD nebulizer gas (2 mL/min), and the tube temperature was set to 70°C. The separation of soyasapogenol A and B was performed using an ODS C18 column (25 0×4.6 mm I.D.). The mobile phase was: acetonitrile: water: 1-propanol: 0.1% acetic acid = 80: 13.9: 6: 0.1, isocratic. The analysis time was 15 min, and the flow rate was 0.9 mL/min. The standard compounds of soyasapogenol A and B were purchased from Tauto Biotech (China) and were used to establish the calibration curve. The soyasapogenol A, B standards were prepared in MeOH at several concentrations-soyasapogenol A: 12.5, 25, 50, 75, 100 and 150 μ g/mL; soyasapogenol B: 37.5, 75, 150, 225, 300 and 450 μg/mL-and a high linearity was obtained. The interpretation of the soyasapogenol A, B was supported by its retention time (soyasapogenol A: 5.7 min, soyasapogenol B: 9.5 min).

Statistical analysis

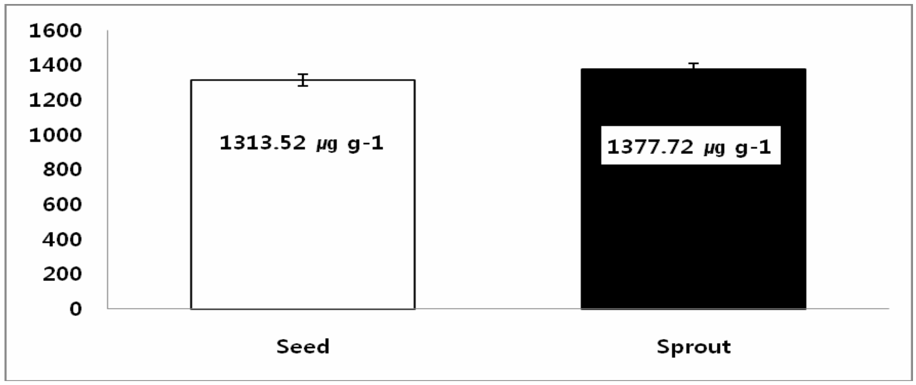
The statistical analysis was undertaken using the general linear model procedure (GLM) of the statistical analysis program (SAS, 2000). All experiments were performed in duplicate. The least significant difference (LSD) test was based on the 0.05 probability level.

RESULTS ANS DISCUSSION

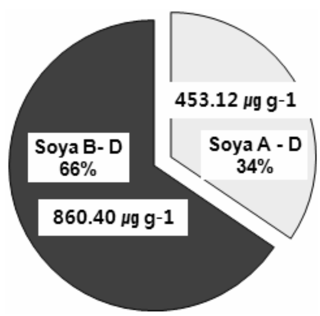
The total average concentration of soyasapogenol was 1313.52 μg g^{-1} in soybean seeds and 1377.72 μg g^{-1} in soybean sprouts. Soybean sprouts were almost 5% higher than soybean seeds in average total soyasapogenol concentration. However, the total average concentrations of soyasapogenol in soybean seeds and sprouts was not significantly different (p<0.05). In soybean seeds, soyasapogenol A content was 453.12 μg g^{-1} and soyasapogenol B content was 860.40 μg g^{-1}. This accounts for 34% and 66%, respectively. In contrast, soyasapogenol A content was 445.85 μg g^{-1} and soyasapogenol B content was 931.88 μg g^{-1} for soybean sprouts, accounting for 32% and 68%, respectively (Fig. 3). Rupasinghe *et al*. (2003) reported that the content of soyasapogenol B was 2.5-4.5 fold higher than the content of soyasapogenol A. In this experiment, 0.81-2.71 times were found in soybean seeds and 1.75-3.65 times in soybean sprouts.

In soybean seeds, the total soyasapogenol concentration of Jineunjeori-kong (2094.90 μg g^{-1}) was the highest, and that of Geomjeong-kong3 (1149.88 μg g^{-1}) was the lowest. In soybean sprouts, the total soyasapogenol concentration of Dachae-kong (2718.09 μg g^{-1}) showed the highest content, in contrast to Geomjeongsaeol-kong (1157.32 μg g^{-1}), which showed the lowest content.

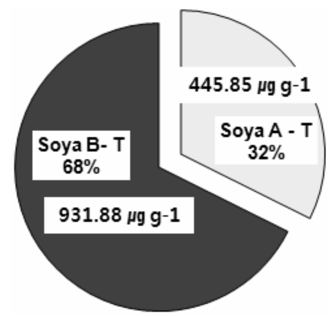
In the process of sprouting, the average content of soyasapogenol A was decreased by approximately 1.6%, but soyasapogenol B and total soyasapogenol were increased by 8.31% and 4.88%, respectively, based on the content of soybean seeds (Fig. 4). Although they showed differences between varieties, the total soyasapogenol and soyasapogenol B contents increased during germination. Also, Rupasinghe *et al*. (2003) reported that during seed germination, the



(A)



(B)



(C)

Fig. 3.

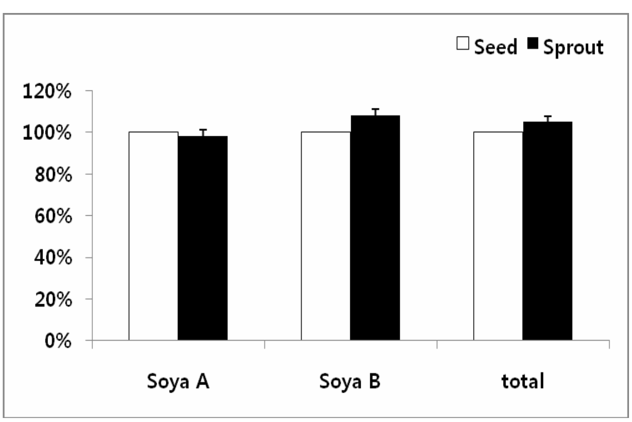


Fig. 4.

concentration of soyasapogenol A was not changed, but soyasapogenol B was increased. Shimoyamada *et al*. (1990) reported that soyasapogenol B was synthesized in the germination process. The composition and content of soyasapogenol were influenced by the seed variety. These results were similar to those of Shiraiwa *et al*. (1991) and Kim (2003), who reported that the contents of soyasapogenol A, B depend on the varieties rather than the environments. The total amount of soyasapogenol and soyasapogenol B exhibited similar patterns. Comparing soyasapogenol A and soyasapogenol B, the amount of soyasapogenol B constituted over 60% of the total soyasapogenol.

The result of this experiment may be used for breeding food that has high soyasaponin content.

All of these experiments were performed using ELSD, and a typical chromatogram of soyasapogenol standard and Danbaek-kong is shown in Fig. 5.

Concentration of soyasapogenol according to the seed size

In this study, soybean varieties were classified into three types: large (above 25 g, 22 varieties), medium (15-25 g, 27 varieties) and small (below 15 g, 30 varieties) by 100-seed weight.

The total soyasapogenol concentration of soybean seeds ranged from 1149.88 to 1655.54 μg g^{-1} in large soybean seeds, from 1171.30 to 1694.45 μg g^{-1} in medium soybean seeds and from 1189.10 to 2094.9 μg g^{-1} in small soybean seeds. In soybean sprouts, the total soyasapogenol concen-

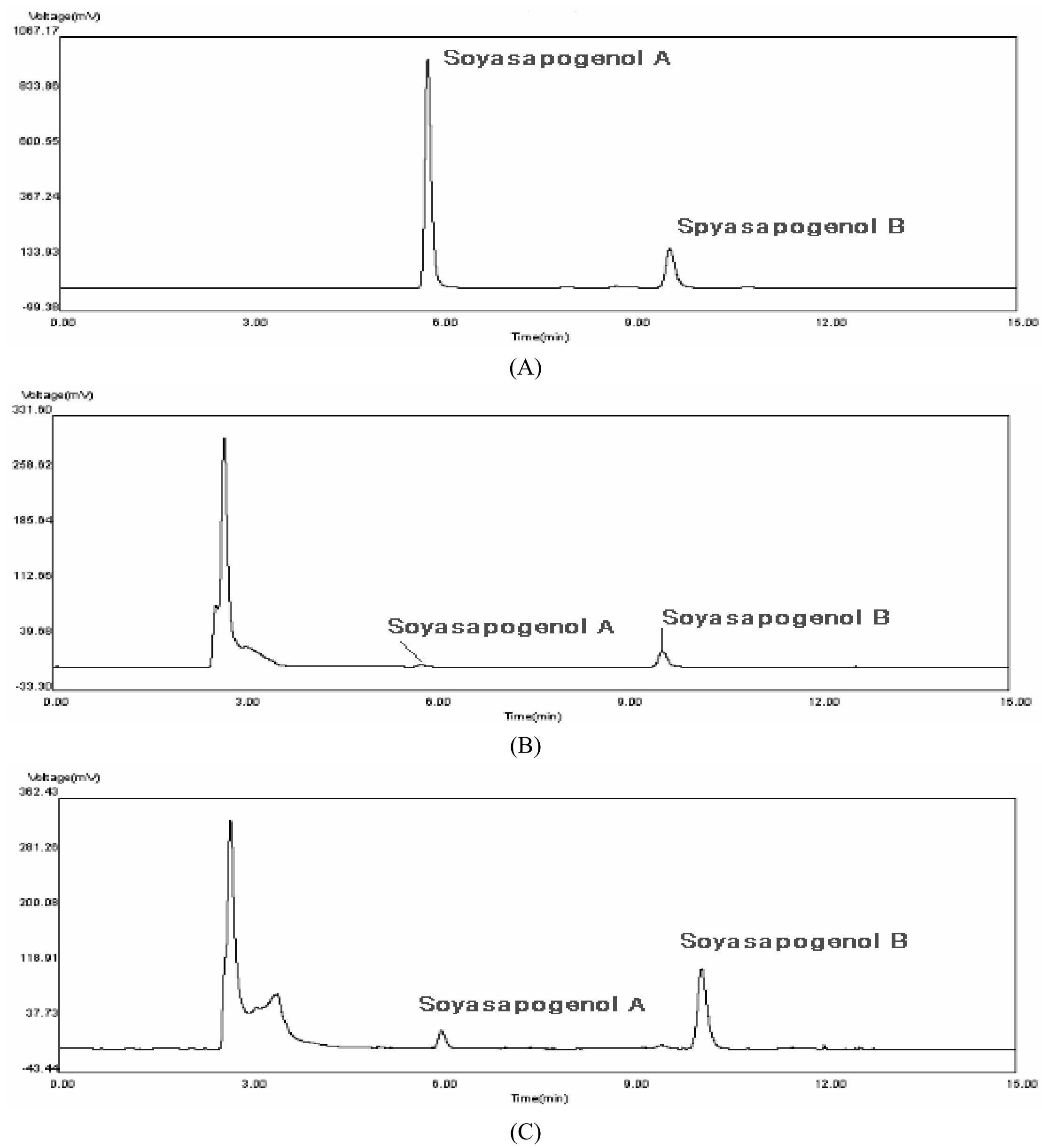


Fig. 5.

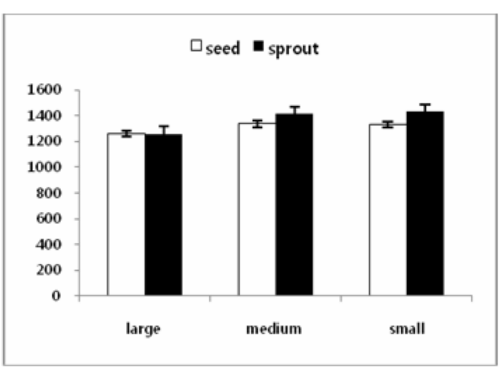
tration ranged from 1157.32 to 1747.63 $\mu g\ g^{-1}$ in large soybean sprouts, from 1196.56 to 2585.91 $\mu g\ g^{-1}$ in medium soybean sprouts and from 1176.51 to 2718.09 $\mu g\ g^{-1}$ in small soybean sprouts (Table, 1). The total soyasapogenol in soybean seeds as a function of their seed size was not significantly different (p<0.05). However, soybean sprouts of a different size showed a significant difference (p<0.05). On average, the total content in large soybean seeds was decreased by 3.60 $\mu g\ g^{-1}$ and that in medium soybean seeds was increased by 76.20 $\mu g\ g^{-1}$ while that in small soybean seeds was increased by as much as 103.14 $\mu g\ g^{-1}$ in the sprouting process (Fig. 6A).

Among each substance, the soyasapogenol A concentration of soybean seeds ranged from 419.30 to 543.82 $\mu g\ g^{-1}$ in large soybean seeds, from 423.99 to 602.26 $\mu g\ g^{-1}$ in medium soybean seeds and from 425.65 to 887.72 $\mu g\ g^{-1}$ in small soybean seeds. In soybean sprouts, the soyasapogenol A concentration ranged from 420.57 to 509.24 $\mu g\ g^{-1}$ in large soybean sprouts, from 421.69 to 582.66 $\mu g\ g^{-1}$ in medium soybean sprouts and from 424.27 to 802.88 $\mu g\ g^{-1}$ in small

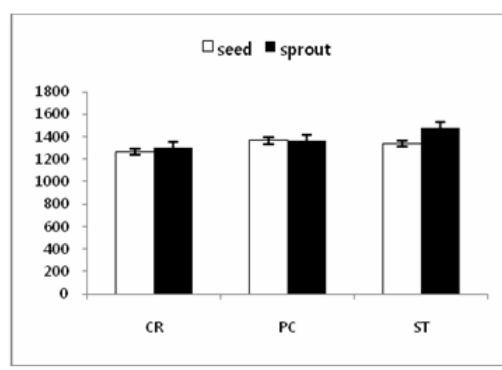
Table 1. Variationof soyasapogenol concentration according to seed size.

Seed size		Soya A - D*	Soya B - D	Soya T - D	Soya A - T	Soya B - T	Soya T - T
		g/g					
Large seed (22 varieties)	Maximum	543.82	1121.09	1655.54	509.24	1238.39	1747.63
	Minimum	419.30	729.61	1149.88	420.57	736.76	1157.32
	Mean	444.14	816.26	1260.41	430.47	826.34	1256.81
	CV (%)	0.50	0.55	0.43	0.18	1.13	0.79
	LSD (0.05)	4.64	9.31	11.35	1.57	19.41	20.51
Medium seed (27 varieties)	Maximum	602.26	1212.25	1694.45	582.66	2029.80	2585.91
	Minimum	423.99	746.41	1171.30	421.69	774.87	1196.56
	Mean	446.74	891.33	1338.07	447.65	966.62	1414.27
	CV (%)	0.72	0.53	0.46	0.56	0.68	0.58
	LSD (0.05)	6.59	9.72	12.69	5.17	13.48	16.82
Small seed (30 varieties)	Maximum	887.72	1529.74	2094.90	802.88	1990.10	2718.09
	Minimum	425.65	721.00	1189.10	424.27	751.13	1176.51
	Mean	465.43	864.93	1330.36	455.50	978.01	1433.51
	CV (%)	0.69	0.84	0.60	0.56	1.11	0.92
	LSD (0.05)	5.78	14.89	16.26	5.17	22.15	26.87

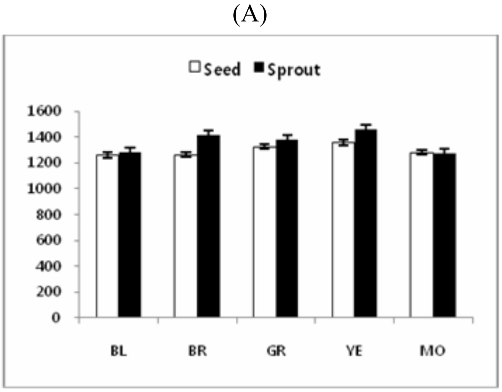
* Soya A-D : soyasapogenol A in soybean seeds, Soya A-T : soyasapogenol A in soybean sprouts, Soya B-D : soyasapogenol B in soybean seeds, Soya B-T : soyasapogenol B in soybean sprouts, Soya T - D : total soyasapogenol in soybean seeds, Soya T - T : total soyasapogenol in soybean sprouts



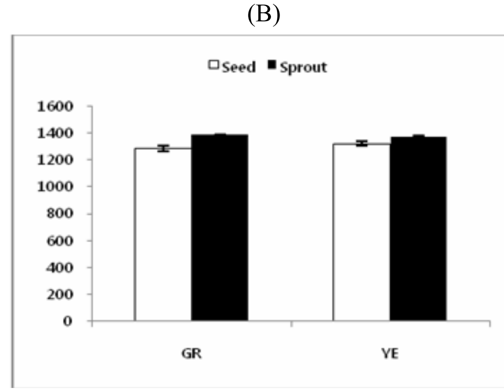
(A)



(B)



(C)



(D)

Fig. 6.

soybean sprouts. The soyasapogenol A concentrations in soybean seeds and sprouts as a function of their seed size were not significantly different (p<0.05). On average, the content of soyasapogenol A in large soybean seeds was decreased by 13.68 $\mu g\ g^{-1}$ and that in small soybean seeds was decreased by 9.93 $\mu g\ g^{-1}$ while that in medium soybean seeds was increased by 0.91 $\mu g\ g^{-1}$ in the sprouting process.

The soyasapogenol B concentration of soybean seeds ranged from 729.61 to 1121.09 $\mu g\ g^{-1}$ in large soybean seeds, from 746.41 to 1212.25 $\mu g\ g^{-1}$ in medium soybean seeds and from 721.00 to 1529.74 $\mu g\ g^{-1}$ in small soybean seeds. In soybean sprouts, the soyasapogenol B concentration ranged from 736.76 to 1238.39 $\mu g\ g^{-1}$ in large soybean sprouts, from 774.87 to 2029.80 $\mu g\ g^{-1}$ in medium soybean sprouts and from 751.13 to 1990.10 $\mu g\ g^{-1}$ in small soybean sprouts. The soyasapogenol B concentrations in soybean seeds and sprouts as a function of their seed size were significantly different (p<0.05). On average, the soyasapogenol B content in large soybean seeds was increased by 10.08 $\mu g\ g^{-1}$, in medium soybean seeds by 75.28 $\mu g\ g^{-1}$ and in small soybean seeds by 113.08 $\mu g\ g^{-1}$ in the sprouting process. Rupasinghe *et al*. (2003) reported that the total soyasapogenol content depended significantly on the size of the soybean seeds. Furthermore, Lee *et al*. (2008) reported that the contents depended on the difference between growing regions, but the contents of phenolic compounds and anthocyanins in small soybean seeds were higher than those of large seeds.

This study indicated that the total soyasapogenol content increased during the sprouting process. Furthermore, the change in saponin content for the germination process depends on the seed size. In addition, the concentration of total soyasapogenol depends on the seed size and sprouting.

Concentration of soyasapogenol according to the seed usage

Soybean seeds were divided into three types, depending on the usage: cooked with rice (33 varieties), paste and curd (18 varieties) and sprout (28 varieties). The total soyasapogenol concentration of soybean seeds ranged from 1149.88 to 1608.72 $\mu g\ g^{-1}$ in soybean seed cooked with rice, from 1185.78 to 1694.45 $\mu g\ g^{-1}$ in paste and curd soybean seed and from 1189.10 to 2094.90 $\mu g\ g^{-1}$ in sprout soybean seed. In soybean sprouts, the total soyasapogenol concentration ranged from 1157.32 to 1927.12 $\mu g\ g^{-1}$ in soybean sprouts cooked with rice, from 1196.56 to 2585.91 $\mu g\ g^{-1}$ in paste and curd soybean sprouts and from 1176.51 to 2718.09 $\mu g\ g^{-1}$ in sprout soybean sprouts (Table, 2). The

Table 2. Differences of soyasapogenol concentration in soybean seeds used for various purpose.

Seed usage		Soya A - D	Soya B - D	Soya T - D	Soya A - T	Soya B - T	Soya T - T
		g/g					
Cooked with rice (33 varieties)	Maximum	887.72	981.08	1608.72	525.48	1405.31	1927.12
	Minimum	419.30	721.00	1149.88	420.57	736.76	1157.32
	Mean	454.66	811.91	1266.58	435.28	865.00	1300.28
	CV (%)	0.56	0.60	0.40	0.27	0.93	0.68
	LSD (0.05)	5.15	9.85	10.46	2.42	16.45	17.88
Paste and curd (18 varieties)	Maximum	534.46	1212.25	1694.45	556.11	2029.80	2585.91
	Minimum	423.99	759.72	1185.78	421.69	774.87	1196.56
	Mean	449.22	915.31	1364.53	439.56	925.42	1364.98
	CV (%)	0.89	0.46	0.55	0.63	0.64	0.58
	LSD (0.05)	8.36	9.26	15.80	5.84	12.36	16.72
Sprout (28 varieties)	Maximum	638.33	1529.74	2094.90	802.88	1990.10	2718.09
	Minimum	429.02	757.67	1189.10	424.27	751.13	1176.51
	Mean	453.80	882.25	1336.05	462.33	1014.86	1477.19
	CV (%)	0.48	0.84	0.58	0.56	1.15	0.95
	LSD (0.05)	4.44	15.11	15.99	5.35	24.00	28.63

total soyasapogenol in soybean seeds depended significantly on the seed usage (p<0.05). However, there was no significant difference for different usage of soybean sprouts (p< 0.05). The average content of total soyasapogenol in the soybean seeds cooked with rice increased by 33.70 μg g^{-1}, in paste and curd soybean seeds by 0.45 μg g^{-1} and in sprout soybean seeds by 141.14 μg g^{-1} in the sprouting process (Fig. 6B).

Among each substance, the soyasapogenol A concentration of soybean seeds ranged from 419.30 to 887.72 μg g^{-1} in soybean seeds cooked with rice, from 423.99 to 534.46 μg g^{-1} in paste and curd soybean seeds and from 429.02 to 638.33 μg g^{-1} in sprout soybean seeds. In soybean sprouts, the soyasapogenol A concentration ranged from 420.57 to 525.48 μg g^{-1} in soybean sprouts cooked with rice, from 421.69 to 556.11 μg g^{-1} in paste and curd soybean sprouts and from 424.27 to 802.88 μg g^{-1} in sprout soybean sprouts. The soyasapogenol A in soybean seeds and sprouts did not depend significantly on their seed usage (p<0.05). The average content of total soyasapogenol in the soybean seeds cooked with rice decreased by 19.38 μg g^{-1} and that in paste and curd soybean seeds decreased by 9.66 μg g^{-1} while that in sprout soybean seeds increased by 8.53 μg g^{-1} in the sprouting process.

The soyasapogenol B concentration of soybean seeds ranged from 721.00 to 981.08 μg g^{-1} in soybean seeds cooked with rice, from 759.72 to 1212.25 μg g^{-1} in paste and curd soybean seeds and from 757.67 to 1529.74 μg g^{-1} in sprout soybean seeds. In soybean sprouts, the soyasapogenol B concentration ranged from 736.76 to 1405.31 μg g^{-1} in soybean sprouts cooked with rice, from 774.87 to 2029.80 μg g^{-1} in paste and curd soybean sprouts and from 751.13 to 1990.10 μg g^{-1} in sprout soybean sprouts. The soyasapogenol B in soybean seeds depended significantly on their usage (p<0.05); the concentration when used for paste and curd or for sprout was higher than when cooked with rice. However, in soybean sprouts, there was no significant difference as a function of different usage (p<0.05). The average soyasapogenol B content in the soybean seeds cooked with rice increased by 53.08 μg g^{-1}, in paste and curd soybean seeds by 10.11 μg g^{-1} and in sprout soybean seeds by 132.61 μg g^{-1} in the sprouting process.

As a result, the concentration of total soyasapogenol increased during the sprouting process, and it depended on the usage of the soybean seeds. Usually, among 76 varieties, soybeans cooked with rice and paste and curd soybeans were the large and medium soybean seeds, and small soybean seeds were used as sprout seeds. This result is similar to the result of dependence on the seed size.

Concentration of soyasapogenol according to the seed coat color

The seed coat color could be classified into five types: black (21 varieties), brown (5 varieties), green (17 varieties), yellow (31 varieties) and mottled (5 varieties). The total soyasapogenol concentration of soybean with yellow seed coat (1357.30 μg g^{-1}) was slightly higher than that of soybean with black (1260.30 μg g^{-1}) or brown (1263.62 μg g^{-1}) seed coats. However, the differences were not statistically significant (p<0.05) (Table 3). In soybean sprouts, the total soyasapogenol concentration was 1280.36 μg g^{-1} in black seed coat sprouts, 1415.52 μg g^{-1} in brown seed coat sprouts, 1376.66 μg g^{-1} in green seed coat sprouts, 1455.13 μg g^{-1} in yellow seed coat sprouts and 1272.55 μg g^{-1} in mottled seed coat sprouts. The content differences were not significant (p<0.05). During germination, the content of total soyasapogenol decreased in mottled (7.51 μg g^{-1}) seed coat soybeans. However, soybeans with brown (151.91 μg g^{-1}), yellow (97.83 μg g^{-1}), green (52.73 μg g^{-1}) and black (20.06 μg g^{-1}) seed coats increased in content (Fig. 6C).

Black (462.48 μg g^{-1}), mottled (455.84 μg g^{-1}), green (454.43 μg g^{-1}), yellow (449.07 μg g^{-1}) and brown (431.71 μg g^{-1}) seed coats showed a high soyasapogenol A content in soybean seeds. However, the content differences were not significant (p<0.05) (Table, 3). Yellow (459.15 μg g^{-1}), brown (448.80 μg g^{-1}), green (440.09 μg g^{-1}), black (433.49 μg g^{-1}) and mottled (431.91 μg g^{-1}) seed coats showed a high soyasapogenol A content in soybean sprouts. During germination, the soyasapogenol A content was decreased in soybeans with black (29.00 μg g^{-1}), mottled (23.93 μg g^{-1}) and green (14.34 μg g^{-1}) seed coats but was increased in soybeans with brown (17.09 μg g^{-1}) and yellow (10.08 μg g^{-1}) seed coats.

The soyasapogenol B contents in soybean seeds as a function of their seed coat color were: 797.82 μg g^{-1} in

Table 3. Comparison of soyasapogenol concentration between soybean seeds and sprouts as a function of seed coat color.

Seed coat color		Soya A - D	Soya A - D	Soya T - D	Soya A - T	Soya A - T	Soya T - T
		g/g					
Black (21 varieties)	Maximum	887.72	893.63	1608.72	525.48	1338.16	1863.64
	Minimum	419.30	721.00	1149.88	420.57	736.76	1157.32
	Mean	462.48	797.82	1260.30	433.49	846.87	1280.36
	CV (%)	0.69	0.63	0.44	0.31	0.93	367.00
	LSD (0.05)	6.68	10.38	11.56	2.77	16.35	17.80
Brown (5 varieties)	Maximum	436.61	965.64	1402.25	521.81	1405.31	1927.12
	Minimum	425.65	783.25	1214.56	425.02	771.10	1201.52
	Mean	431.71	831.90	1263.62	448.80	966.72	1415.52
	CV (%)	0.19	0.24	0.16	0.21	1.28	0.93
	LSD (0.05)	2.12	2.13	5.27	2.48	31.72	33.67
Green (17 varieties)	Maximum	638.33	1529.74	2094.90	564.47	1990.10	2554.57
	Minimum	421.36	736.63	1157.99	422.58	776.04	1200.88
	Mean	454.43	869.50	1323.93	440.09	936.58	1376.66
	CV (%)	0.85	0.86	0.67	0.30	0.69	0.55
	LSD (0.05)	8.18	15.80	18.69	2.76	13.62	16.09
Yellow (31 varieties)	Maximum	534.46	1212.25	1694.45	802.88	2029.80	2718.09
	Minimum	423.99	759.16	1185.78	421.69	751.13	1176.51
	Mean	449.07	908.23	1357.30	459.15	995.98	1455.13
	CV (%)	0.48	0.66	0.52	0.68	1.10	0.92
	LSD (0.05)	4.39	12.14	14.51	6.37	22.28	27.35
Mottled (5 varieties)	Maximum	543.82	887.15	1341.58	440.06	992.63	1432.69
	Minimum	426.63	772.32	1198.95	424.27	794.68	1226.78
	Mean	455.84	824.23	1280.06	431.91	840.64	1272.55
	CV (%)	0.29	0.38	0.16	0.19	0.66	0.50
	LSD (0.05)	3.36	8.13	5.33	2.13	14.31	16.27

black, 831.90 μg g^{-1} in brown, 869.50 μg g^{-1} in green, 908.23 μg g^{-1} in yellow and 824.23 μg g^{-1} in mottled. The soyasapogenol B concentration in yellow seed coat was higher than that in the others, and the differences were statistically significant ($p<0.05$). On the other hand, in soybean sprouts, the soyasapogenol B in soybean seed as a function of their seed coat color was not statistically significant ($p<0.05$). The average soyasapogenol B concentration for black seed coat was 797.82 μg g^{-1}, brown seed coat was 831.90 μg g^{-1}, green seed coat was 869.50 μg g^{-1}, yellow seed coat was 908.23 μg g^{-1} and mottled seed coat was 824.23 μg g^{-1}. Yellow seed coat soybean sprouts had the highest concentration of soyasapogenol B; on the other hand, mottled seed coat soybean sprouts had the lowest. During germination, the content of soyasapogenol B increased in soybeans of all seed coats: brown (134.82 μg g^{-1}), yellow (87.75 μg g^{-1}), green (67.07 μg g^{-1}), black (49.05 μg g^{-1}) and mottled (16.41 μg g^{-1}).

Soybean seed coats did not seem to be related to saponin content, although the content of phenolic compounds and anthocyanins is known to be related to the seed coat color (Kim *et al*., 2006). This result is similar to that of Shimoyamada *et al*. (1990), who reported that there was no significant correlation between seed coat color and content of soyasapogenol in soybean seeds and sprouts.

Table 4. Comparison of soyasapogenol concentration between green cotyledon and yellow cotyledon varieties.

Seed cotyledon color		Soya A - D	Soya B - D	Soya T - D	Soya A - T	Soya B - T	Soya T - T
		g/g					
Green (15 varieties)	Maximum	638.33	981.08	1438.30	564.47	1990.10	2554.57
	Minimum	426.77	772.09	1199.71	421.85	775.79	1197.63
	Mean	459.54	823.61	1283.15	444.16	941.86	1386.02
	CV (%)	0.28	0.54	0.37	0.34	0.76	0.61
	LSD (0.05)	2.77	9.44	10.19	3.26	15.34	18.04
Yellow (64 varieties)	Maximum	887.72	1529.74	2094.90	802.88	2029.80	2718.09
	Minimum	419.30	721.00	1149.88	420.57	736.76	1157.32
	Mean	451.61	869.02	1320.63	446.24	929.54	1375.78
	CV (%)	0.68	0.70	0.54	0.52	1.03	0.82
	LSD (0.05)	6.13	12.11	14.24	4.62	19.09	22.45

Concentration of soyasapogenol according to the seed cotyledon color

For the cotyledon color, the green (15 varieties) and yellow (64 varieties) were divided into two kinds. The total soyasapogenol concentration of soybeans with yellow cotyledon (1320.63 $\mu g\ g^{-1}$) was slightly higher than that of soybeans with green cotyledon (1283.15 $\mu g\ g^{-1}$). However, the differences were not statistically significant ($p<0.05$). In soybean sprouts, the total soyasapogenol concentration was 1386.02 $\mu g\ g^{-1}$ in green cotyledon sprouts and 1375.78 $\mu g\ g^{-1}$ in yellow cotyledon sprouts. However, the differences were not statistically significant ($p<0.05$) (Table, 4). During germination, the content of total soyasapogenol increased in green (102.87 $\mu g\ g^{-1}$) and yellow (55.14 $\mu g\ g^{-1}$), the increase in the green being significant (Fig. 6D).

The soyasapogenol A concentration of soybeans with green cotyledon (459.54 $\mu g\ g^{-1}$) was higher than that of soybeans with yellow cotyledon (451.61 $\mu g\ g^{-1}$). However, the differences were not statistically significant ($p<0.05$). In soybean sprouts, the dependence of soyasapogenol A in soybean seeds on their seed cotyledon color was not statistically significant ($p<0.05$). The soyasapogenol A concentration was 444.16 $\mu g\ g^{-1}$ in green cotyledon sprouts and 446.24 $\mu g\ g^{-1}$ in yellow cotyledon sprouts. During germination, soyasapogenol A content decreased more in green (15.37 $\mu g\ g^{-1}$) than in yellow (5.37 $\mu g\ g^{-1}$).

The soyasapogenol B concentration of soybeans with yellow cotyledon (869.02 $\mu g\ g^{-1}$) was higher than that of soybeans with green cotyledon (823.61 $\mu g\ g^{-1}$). However, the differences were not statistically significant ($p<0.05$). In soybean sprouts, the soyasapogenol B in soybean seeds as a function of their seed cotyledon color was not statistically significant ($p<0.05$). The soyasapogenol B concentration was 941.86 $\mu g\ g^{-1}$ in green cotyledon sprouts and 929.54 $\mu g\ g^{-1}$ in yellow cotyledon sprouts. During germination, soyasapogenol B content increased more in green (118.25 $\mu g\ g^{-1}$) than in yellow (60.52 $\mu g\ g^{-1}$).

In conclusion, total soyasapogenol accumulation was related to seed coat color, especially green seed coat, and the content was influenced by the germination process.

ACKNOWLEDGEMENTS

"This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-313-2007-2-F00004)."

REFERENCES

Berhow, M. A., S. B. Kong, K. E. Vermillion, and S. M. Duval. 2006. Complete quantification of group A and group B soyasaponins in soybeans. J. Agric. Food Chem. 54: 2035-2044.

Berhow, M. A., E. D. Wagner, S. F. Vaughn, and M. J. Plewa. 2000. Characterization and antimutagenic activity of soybean saponins. Mut. Res. 448: 11-22.

Chi, H. Y., J. S. Roh, J. T. Kim, S. J. Lee, M. J. Kim, S. J. Hahn, and I. M. Chung. 2005. Light quality on nutritional composition and isoflavones content in soybean sprouts. Korean J. Crop. Sci. 50: 415-418.

Ellington, A. A., M. Berhow, and K. W. Singletary, 2005. Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. Carcinogenesis. 26: 159-167.

Gurfinkel, D. M. and A. V. Rao. 2002. Determination of saponins in legumes by direct densitometry. J. Agric. Food Chem. 50: 426-430.

Kim, J. A., W. S. Jung, S. C. Chun, C. Y. Yu, K. H. Ma, J. G. Gwag, and I. M. Chung. 2006. A correlation between the level of phenolic compounds and the antioxidant capacity in cooked-with-rice and vegetable soybean (*Glycine max* L.) varieties. Eur. Food Res. Technol. 224: 259-270

Kim, M. J. and K. S. Kim. 2001. Change in amino acid composition of soybean during germination. Bull. Nat. Sci. 6: 97-102.

Kim, S. L., M. A. Berhow, J. T. Kim, H. Y. Chi, S. J. Lee, and I. M. Chung. 2006. Evaluation of soyasaponin, isoflavone, protein, lipid, and free sugar. J. Agric. Food Chem. 54: 10003-10010.

Kim, Y. H. 2003. Biological activities of soyasaponins and their genetic and environmental variations in soybean. Korean J. Crop. Sci. 48: 49-57.

Kim, Y. J., Y. J. Oh, S. K. Cho, J. G. Kim, M. R. Park, and S. J. Yun. 2006. Variations of isoflavone contents in seeds and sprouts of sprout soybean cultivars. Korean J. Crop. Sci. 51: 160-165.

Kudou, S., M. Tonomura, C, Tsukamoto, T, Uchida, T. Sakabe, N. Tamura, and K. Okubo. 1993. Isolation and structural elucidation of DDMP-conjugated soyasaponins as genuine saponins from soybean seeds. Biosci. Biotechnol. Biochem. 57: 546-550.

Kuzuhara, H., S. Nishiyama, N. Minowa, K. Sasaki, and S. Omoto. 2000. Protective effects of soyasapogenol A on liver injury mediated by immune response in a concanavalin A-induced hepatitis model. Eur. J. Pharmacol. 391: 175-181.

Lee, S. J., J. J. Kim, H. I. Moon, J. K. Ahn, J. K., S. C. Chun, W. S. Jung, O. K. Lee, and I. M. Chung. 2008. Analysis of isoflavones and phenolic compounds in the Korean soybean (*Glycine max* L. (Merrill)) with different seed weights. J. Agric. Food Chem. 56: 2751-2758.

Oh, B. Y., B. H. Park, and G. S. Ham. 2003. Changes of saponin during the cultivation of soybean sprout. Korean J. Food Sci. Technol. 35: 1039-1044.

Oh, B. Y., B. H. Park, and K. S. Ham. 2007. Effects of chitosan treatment on changes of soyasaponin contents in soybean sprouts. Korean Soc. Food Sci. Nutr. 36: 584-588.

Rupasinghe, H. P. V., C. J. C. Jackson, V. Poysa, C. D. Berardo, J. D. Bewley, and J. Jenkinson. 2003. Soyasapogenol A and B distribution in soybean (*Glycine max* L. Merr.) in relation to seed physiology, genetic variability, and growing location. J. Agric. Food Chem. 51: 5888-5894.

Shimoyamada, M., K. Harada, and K. Okubo. 1991. Saponin composition in developing soybean seed (*Glycine max* (L.) Merrill, cv. Mikuriyaao). Agric. Biol. Chem.55: 1403-1405.

Shimoyamada, M., S. Kudo, K. Okubo, F. Yamauchi, and K. Harada. 1990. Distributions of saponin constituents in some varieties of soybean plant. Agric. Biol. Chem.54: 77-81.

Shimoyamada, M., and K. Okubo. 1991. Variation in saponin contents in germinating soybean seeds and effect of light irradiation. Agric. Biol. Chem., 55: 577-579.

Shiraiwa, M., K. Harada, and K Okubo. 1991. Composition and content of saponins in soybean seed according to variety, cultivation year and maturity. Agric. Biol. Chem. 55: 323-331.

Shiraiwa, M., S. Kudo, M. Shimoyamada, K. Harada, and K. Okubo. 1991. Composition and structure of group A saponin in soybean seed. Agric. Biol. Chem. 55: 315-322.

Zhang, W. and D. G. Popovich. 2008. Effect of soyasapogenol A and soyasapogenol B concentrated extracts on Hep-G2 cell proliferation and apoptosis. J. Agric. Food Chem. 56: 2603-2608.