Korean J. Crop Sci. 51(4): 340~347(2006)

Composition and Content of Soyasaponins and Their Interaction with Chemical Components in Different Seed-Size Soybeans

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ABSTRACT : Soyasaponins A_1, DDMP-conjugated group B soyasaponins αg and βg, non-DDMP counterpart soyasaponin I, II+III, and DDMP moiety were quantified in the large-, midium-, and small-seed soybean varieties. Protein contents were ranged from 38.1% to 41.8%, and oil contents were ranged from 15.5% to 18.9%, respectively. Oil contents in the large-seed varieties were significantly higher than those of medium- and small-seed varieties. Among detected soyasaponin peaks, βg was a major soyasaponin in DDMP-conjugated group B soyasaponins followed by soyasaponin I, DDMP moiety and A_1. Soyasaponin concentration among different seed size soybean varieties. The soyasaponin concentration of medium-seed (4014.5 μg/g) was slightly higher than those of large-seed (3755.0 μg/g) and small-seed varieties (3620.3 μg/g), however, the differences was statistically not significant. The composition rates of soyasaponins in the large-size seeds were 9.4% of soyasaponin A_1, 26.5% of DDMP-conjugated soyasaponins, 49.9% of non-DDMP counterpart soyasaponins, and 14.2% of DDMP moiety, respectively. Similar results were observed in the composition ratios of middle- and small-size seeds. Oil content and C:N ratio showed the significant positive correlations with total soyasaponin concentration, while the 100-seed weight, fiber, and ash contents showed the negative correlations with total soyasaponin but statistically not significant. It was noted that protein contents didn't have any relationship with group A, group B, DDMP moiety, and total soyasaponin. This fact suggested that protein contents are not affects the variation of soyasaponin concentration.

Keywords: soybean, seed size, soyasaponin, chemical components

Saponins are a family of steroid or triterpenoid glycosides with one or two polysaccharide chains found in a wide variety of plants (Kitagawa, *et al.*, 1988;. Okubo *et al*, 1992; Oakenfull *et al.*, 1990).

In general, saponins have detergent properties, forming oil-in-water emulsions and producing a foam when dissolved in water. For these characteristics, saponins are employed as foaming agents in foods and detergent agents in shampoos, facial cleansers, and various cosmetic products.

Soybeans have attracted the attention of scientists as a supply source for the biologically active secondary metabolites such as isoflavones and soyasaponins.

Soybean seed contain approximately 0.4~0.5% of soysaponins depending on the variety, cultivation year, location grown, and degree of maturity (Shiraiwa, *et al.*, 1991; Fenwick *et al.*, 1983; Fang *et al.*, 2004).

As shown in Fig. 1, soyasaponins can be classified into two groups, A and B, based on their aglycone structures (Berhow *et al.*, 2006). Group A acetylated saponins are mostly responsible for undesirable bitter and astringent taste (Kitagawa, *et al.*, 1988;. Okubo *et al*, 1992).

This is caused by the acetylation of the hydroxyl groups of terminal sugar chain attached at the C-22 position of soyasapogenol A (Burrows *et al.*, 1992). Therefore, breeders have been make an effort to improve a group A acetyl saponin-deficient mutants (Tsukamoto *et al.*, 1992).

Whereas, DDMP(2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyrran-4-one) and DDMP conjugated group B saponins have been suggested to possess multiple health promoting effects, such as lowering of cholesterol level by inhibiting its absorption (Oakenfull & Sidhu, 1990; Potter, 1995; Rao & Sung, 1995), being anticarcinogenic activity against various tumor cell lines (Rao & Sung, 1995; Okubo & Yoshiki, 1996; Berhow *et al.*, 2000), antihepatotoxic and anti-infectivity of HIV (Kinjo *et al.*, 1998; Miyao *et al.*, 1998; Okubo *et al.*, 1994).

Biosynthesis of soyasaponin occurs through the isoprenoid metabolism pathway (Ruzicka, 1953; Kurosawa *et al.*, 2002). In order to elucidate the physiological role of saponins in plants, it is necessary to understand their biosyn-

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thetic pathway and identify the regulatory mechanism of their metabolism.

It was reported that soyasaponins show a considerable variability in their concentration and composition according to seed parts. In case group A saponins were mostly detected in hypocotyls (Kim, 2003). Among group B, soyasaponins V and αg are detected exclusively in the hypocotyl, while soyasaponins II and βa are mainly detected in the cotyledon (Shiraiwa *et al.*, 1991; Tsukamoto *et al.*, 1995). Soyasaponin I and its DDMP-conjugate form βg are found in both parts (Rupasinghe *et al.*, 2003).

Kim (2003) reported that the total soyasaponin contents varied among different soybean varieties and concentrations in the cultivated soybeans were 2-fold lower than in the wild soybeans. But the contents of soyasaponin were not so influenced by environmental effects. The composition and concentration of soyasaponins were different among the soy products (soybean flour, soycurd, tempeh, soymilk, etc.) depending on the processing conditions.

Soybean seed size is traditionally classified into three types small, medium, and large seed by the 100-seed weight in Korea. In general, small-seed soybean is used for soybean sprouts but medium- and large-seed soybean are used for tofu, soymilk, soy sauce and Korean traditional fermented soy pastes such as chungkukjang and meju.

Despite the many studies on soyasaponins, little information is available for the variation of different seed size soybeans and their interaction with other chemical components mainly due to the difficulty of isolating and purifying of soyasaponins.

To effectively select soybean lines that produce high quality soy foods, it is necessary to understand the genetic parameters of the variation and to subsequently identify easily detectable traits that affect the quality of soybean.

Therefore, it is a very important to determine the properties of soyasaponins for supporting the proper usage of soybean varieties.

The present study focuses on the relationship among various types in soyasaponins of soybean and their interaction with major chemical components such as protein, oil, fiber, ash, and carbon to nitrogen (C:N) ratio to determine the association of soyasaponins with other seed traits of soybean to facilitate the development of cultivars with appropriate seed composition.

MATERIALS AND METHODS

Soybean cultivars

Twenty seven soybean varieties were grown and harvested at a field of National Institute of Crop Science, Suwon, Korea in 2005.

Soybeans were classified into three types small (<15 g), medium (15 g~24 g), and large (>24 g) base on Korean traditional soybean classification method by 100-seed weight, respectively.

Chemical components analysis

Soybean seeds were ground by using laboratory test mill (Brabender, Duisburg, Germany) about 100 mesh flour for the analysis of proteins, oil, fiber, ash, and C:N ratio. Protein content of soybeans was determined according to the Kjeldahl procedure using a Tecator Kjeltec Auto Analyzer, model 2400 (Foss Tecator, Huddinge, Sweden). Oil content was measured by Soxtherm Automatic System (Gerhardt, Hoffmannstre, Germany). The 5.0 g of homogenized sample was put into extraction thimble and add 140 mL of n-hexane. After boiling for 30 min at 180°C, extraction was performed for 80 min with 5 times of solvent reduction. Total oil contents were represented on a dry basis of soybean seeds.

Fiber content was determined by using a Fibertec^{TM} 2010 system (Foss Tecator, Huddinge, Sweden) according to van Soest *et al.*(1991) methods and sequential extractions including boiling, rinsing and filtration were performed under controlled conditions. The carbon to nitrogen ratio in soybeans measured by combustion assay (Elementar Vario MAX CN-analyzer, Hanan, Germany). The 100 mg of soybean flour was weighed into the tin-vessels and dropped into the combustion tube automatically, the helium carrier gas transfers the gaseous combustion. and detection was conducted by the thermoconductivity detector (TCD). The detector signals was integrated by using the calibration curves stored in the CN-analyzer system.

Saponin analysis

For quantitative saponin analysis, 0.25 g of defatted samples were placed in a vial and 3 mL of a dimethyl sulfoxide-methanol (1:1) solution was added. The vials were capped and wrapped with sealing tape and incubated in an oven for 72 h at 50°C. Then, the samples were sonicated for 15 min at 50°C and allowed to stand at room temperature for 2 h. An aliquot was removed from the vial and filtered through a 0.45 μm nylon 66 filter for HPLC analysis for saponins. Saponins were analyzed using an Agilent 2690 HPLC equipped with a photodiode array detection system Waters 2996 (Waters, Milford, MA, USA). The column used was an Inertsil ODS-3 reverse phase C-18, 5 μm, 250 mm 4.6 mm i.d., with a guard column (Varian, Torrance, CA).

The initial conditions were 30% acetonitrile and 0.025% trifluoroacetic acid (TFA) in water at a flow rate of 1 mL/

Table 1. Soyasaponin concentration (μg/g) in Korean soybean varieties.

Seed size	Varieties	100-seedwt (g)	Protein	Oil	Fiber	Ash	C:N ratio
			----------- g/100g DB ------------				
Large (>24 g)	Daemangkong	25.3	40.5	18.6	9.7	5.1	7.9
	Daolkong	28.5	41.3	17.6	8.5	5.3	7.3
	Daewonkong	26.5	41.1	18.9	7.1	4.5	8.3
	Hwangkeumkong	25.0	39.3	18.3	7.9	4.9	7.9
	Jangwonkong	31.4	38.1	18.7	8.2	5.1	7.7
	Jangyeobkong	33.6	38.6	17.3	8.5	5.3	7.3
	mean of large seeds	*28.4*	*39.8*	*18.2*	*8.3*	*5.0*	*7.7*
Medium (15 g~24 g)	Daepungkong	21.6	40.7	18.2	8.7	4.9	7.9
	Hojangkong	20.9	40.5	17.4	9.1	5.0	7.5
	Jinpumkong	22.6	41.1	16.5	7.4	4.4	8.4
	Mallikong	18.4	39.4	19.3	7.3	4.9	8.3
	Sinpaldalkong 2	19.5	39.6	18.9	6.8	5.0	7.6
	Taekwangkong	23.2	40.5	17.4	8.0	4.5	8.0
	mean of medium seeds	*21.0*	*40.3*	*18.0*	*7.9*	*4.8*	*8.0*
Small (<15 g)	Anpyeongkong	10.5	40.7	18.0	8.8	4.8	7.7
	Danbaegkong	12.9	40.3	15.9	6.5	4.9	6.5
	Kwangankong	11.5	39.2	15.5	8.5	5.1	6.5
	Pungsannamulkong	8.6	40.0	16.2	8.7	4.8	7.5
	Sobaeknamulkong	9.7	40.8	16.8	8.2	4.8	8.2
	Sowonkong	9.7	41.8	17.4	8.5	5.2	7.9
	mean of small seeds	*10.5*	*40.5*	*16.6*	*8.2*	*4.9*	*7.4*
LSD (p<0.05) among seed size		-	ns	1.10	ns	ns	ns

min. The effluent was monitored at 210 nm on the variable wavelength detector. After 20 μL injection, the column was developed to 50% acetonitrile and 0.025% TFA in a linear gradient over 45 min. Before the samples were run, an extinction coefficient for the saponins was determined from a linear standard curve based on mAbs units *vs* concentration injected. This curve was prepared from a dilution series of pure soyasaponin standard that had been purified in the USDA-ARS, Peoria, IL, USA (Berhow *et al.*, 2006).

Statistical analysis

There were three replicates for all measurements. The data obtained from the analysis were statistically analyzed using SAS release ver. 8.0 for Windows (Statistical Analysis Systems Institute Inc., Raleigh, NC, USA).

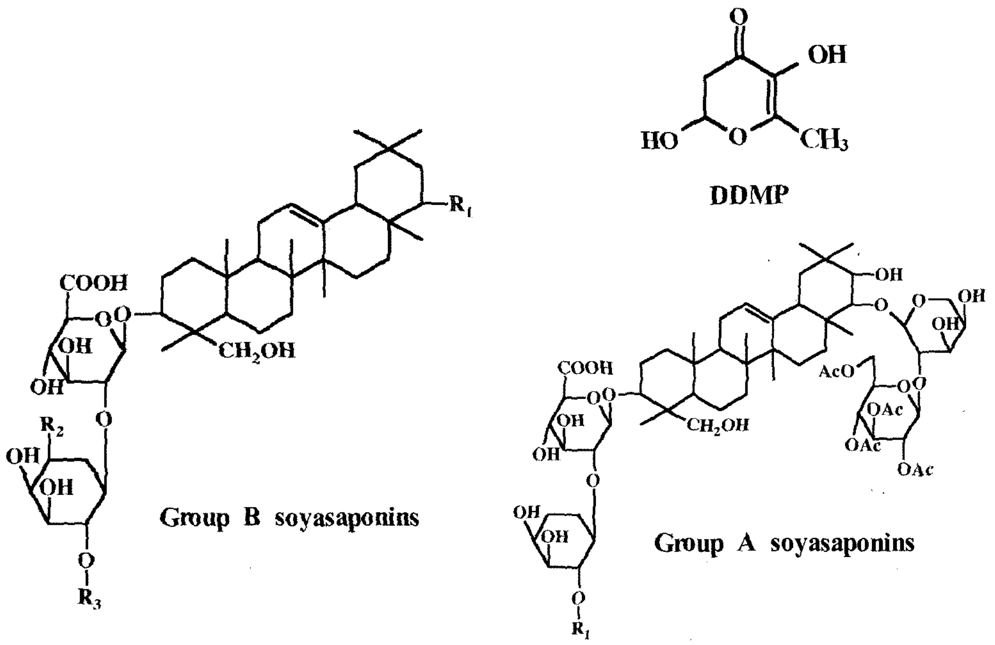
RESULTS AND DISCUSSION

Chemical components in soybeans

Protein, oil, fiber, ash and carbon to nitrogen (C:N) ratio in soybean seeds were represented in Table 1. Protein contents were ranged from 38.1% (Jangwonkong, large size seed) to 41.8% (Sowonkong, small-seed), and the mean value of protein contents in the small-seed varieties were slightly higher than those of large- and medium-seed varieties, however, the differences were statistically not significant. Oil contents were ranged from 15.5% (Kwangkong, small-seed) to 18.9% (Sinpaldalkong 2, medium-seed), and the mean value of oil contents in the large-seed varieties were significantly higher than those of medium- and small-seed varieties.

The importance of soybean worldwide is mainly due to its high protein and oil contents. Soybean breeders have looked for genotypes with high protein and oil contents along with high grain yield.

Many attempts have been made through breeding to elevate grain yield and quality improvement. However, the negative correlation existent between oil and protein contents as well as the negative correlation between protein and yield is a problem in obtaining cultivars with high yield and high protein contents. The large seeds are used in the production of tofu and meju, and the small seeds are used in the



Group A acetyl-saponins	MW	R_1		
Soyasaponin A1	1436	*O*-β-D-Glucose		
Group B saponins	**MW**	R_1	R_2	R_3
Soyasaponin I	942	OH	CH_2OH	*O*-β-D-Glucose
Soyasaponin II	912	OH	H	*O*-α-L-Rhamnose
Soyasaponin III	796	OH	CH_2OH	OH
Soyasaponin IV	766	OH	H	OH
Soyasaponin V	958	OH	CH_2OH	*O*-a-L-Rhamnose
Soyasaponin βg	1068	*O*-DDMP	CH_2OH	*O*-ß-D-Glucose
Soyasaponin αg	1084	*O*-DDMP	CH_2OH	*O*-a-L-Rhamnose

Fig. 1. Structures and nomenclature of the soybean saponins. The nomenclature of the group A and B soyasaponins used in this paper is those of Berhow *et al.* (2006).

production of sprouts and chungkukjang. Knowledge of the relationship between seed composition and seed size would be useful in developing these type of cultivars.

Soyasaponins

An extract of soybean sample was analysed by reversed-phase HPLC, using the gradient employed Berhow *et al.* (2006).

The extraction and analysis methods presented here are an accurate and reproducible one-step extraction procedure for the total soyasaponins in soybean samples by HPLC. This soyasaponin analysis has been expanded to include the A group soyasaponins, giving a more accurate chromatogram of the soyasaponins and analysis time was not excessive. HPLC chromatography reveled well resolved individual group A and B soyasaponins. Fig. 2 shows a chromatogram of these soyasaponins in Korean soybean variety Sinpaldalkong 2 that is one of the medium seed size varieties.

Soyasaponin A_1, and the DDMP-conjugated group B soyasaponins, αg and βg, and non-DDMP counterparts, soyasaponin I, II+III, IV, V and DDMP moiety, were quantified in the different seed size soybean varieties and presented in Table 2.

Among detected peaks, βg was a major soyasaponin in DDMP-conjugated group B soyasaponins followed by soyasaponin I, DDMP moiety and A_1. The soyasaponin concentration of medium-seed varieties (4014.5 μg/g) was slightly higher than those of large-seed (3755.0 μg/g) and small-seed varieties (3620.3 μg/g), however, the differences was statistically not significant.

As compared the soyasaponins individually among differ-

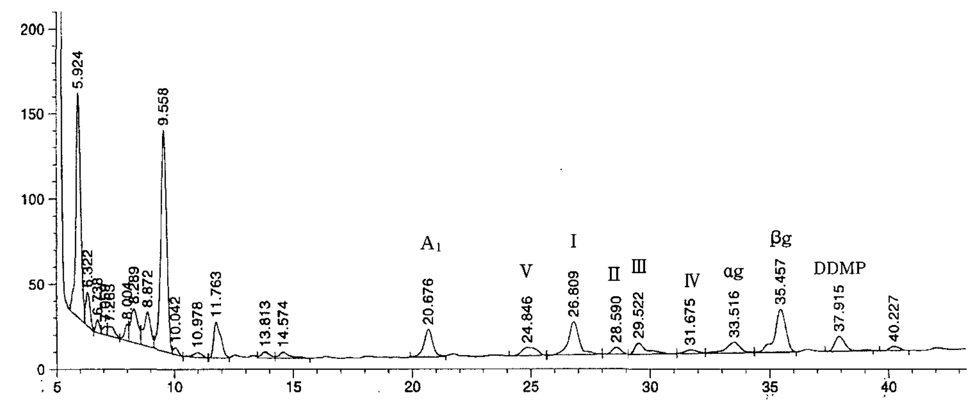


Fig. 2. HPLC chromatogram of soyasaponins in Korean soybean variety Sinpaldalkong 2.

Table 2. Soyasaponin concentration (μg/g) in Korean soybean varieties.

Seed size	Varieties	A_1	I	II+III	IV	V	αg	βg	DDMP	Total
Large (>24g)	Daemangkong	331.0	793.2	368.3	187.5	220.4	255.7	766.9	475.5	3398.6
	Daolkong	295.4	643.0	377.0	163.5	188.8	237.4	916.7	500.5	3322.3
	Daewonkong	467.2	1241.4	548.5	225.8	371.3	448.9	349.7	612.4	4265.1
	Hwangkeumkong	426.9	1318.3	620.7	277.2	461.5	504.7	398.9	882.3	4890.6
	Jangwonkonh	321.7	860.8	543.1	262.1	240.1	306.7	598.1	338.3	3470.9
	Jangyeobkong	269.4	708.6	290.7	145.6	188.8	271.1	920.1	387.9	3182.2
	mean of large seeds	*351.9*	*927.6*	*458.1*	*210.3*	*278.5*	*337.4*	*658.4*	*532.8*	*3755.0*
Medium (15g~24g)	Daepungkong	534.1	1106.5	532.1	336.7	258.4	395.3	1054.9	504.2	4722.1
	Hojangkong	219.8	703.3	373.6	175.8	200.5	260.7	720.3	404.9	3058.9
	Jinpumkong	449.6	748.9	377.3	145.6	280.4	346.7	906.0	496.8	3751.2
	Mallikong	256.4	987.3	398.3	204.8	196.5	393.3	877.5	474.2	3788.2
	Sinpaldalkong 2	685.3	959.5	485.8	201.1	415.6	413.5	1101.2	564.8	4826.8
	Taekwangkong	179.2	955.4	406.6	184.8	238.1	375.0	1130.0	470.5	3939.6
	mean of medium seeds	*387.4*	*910.2*	*429.0*	*208.1*	*264.9*	*364.1*	*965.0*	*485.9*	*4014.5*
Small (<15g)	Anpyeongkong	599.4	1062.6	268.4	219.8	241.1	348.3	900.8	279.4	3919.7
	Danbaegkong	322.7	677.0	245.8	135.9	276.1	329.7	1070.6	305.4	3363.0
	Kwangankong	390.3	756.6	172.5	172.5	248.1	267.1	713.0	192.8	2912.8
	Pungsannamulkong	568.8	921.4	305.0	177.8	373.3	338.7	685.3	318.0	3688.3
	Sobaeknamulkong	230.4	927.1	457.5	155.6	192.5	320.7	1165.8	523.5	3973.1
	Sowonkong	596.7	1001.7	266.7	215.1	326.3	355.3	829.2	273.7	3864.8
	mean of small seeds	*451.4*	*891.1*	*286.0*	*179.5*	*276.2*	*326.6*	*894.1*	*315.5*	*3620.3*
LSD ($p<0.05$) among seed size		ns	ns	102.7	ns	ns	ns	209.1	136.7	ns

ent seed size soybeans, statistically significant differences ($p<0.05$) were found in soyasaponin II+III, βg and DDMP moiety. The concentration of soyasaponin II+III in large- and medium-seed varieties was higher than that of small-seed varieties, while the concentration of βg and DDMP moiety were high in medium-seed varieties.

This results suggested that the major variation of soyasaponin concentration among different seed size varieties were mainly due to the differences in the concentration of soyasaponin II+III, βg and DDMP moiety.

The DDMP group B soyasaponins and the group A soyasaponins are accumulate as the final natural product in soybean seed. Variation of saponin composition occurs from the substitution of sugar moieties in the group A and B soyasaponins as well as the possible incomplete addition of all of the sugar moieties to the oligosaccharide chains (Berhow *et al.*, 2006).

In the large-seed varieties showed relatively a higher level of disaccharidic soyasaponins III (although soyasaponin II and III were poorly resolved each other) and IV. These soyasaponins are produced by the loss of the third sugar (α-L-rhamnose) of soyasaponins I and II, respectively (Fig. 1).

Hubert *et al.* (2005) reported that the concentration of trisaccharidic soyasaponins I and II was higher in the hypocotyl than in the cotyledon and these soyasaponins were degraded into soyasaponins III and IV under particular alkaline conditions or by enzymatic degradation.

This study results showed that the concentration of trisaccharidic soyasaponins I and II were relatively higher in the large-seed varieties than in the medium- and small-seed varieties. Consequently, it was considered that the concentration of trisaccharidic soyasaponins in the hypocotyl of large-seed varieties was higher than the concentration in the hypocotyl of medium- and small-seed varieties.

It is generally considered that most of soyasaponins B are DDMP-conjugated in the seed (Tsukamoto *et al.*, 1995; Hu *et al.*, 2002). The DDMP-conjugated soyasaponins were not altered by high temperature during seed development (Tsukamoto *et al.*, 1995). While the significant conversion of DDMP soyasaponin βg to soyasaponin I was observed in peas during 9 months of storage (Daveby *et al*, 1998).

Soyasaponin βg is a major saponin of soybean seed and has important biological activities, such as reactive oxygen-scavenging activity (Yoshiki & Okubo, 1995).

However the utilization of soyasaponin βg as a functional compound would be difficult due to soyasaponin βg hydrolyzed into soyasaponin I and DDMP moieties during the isolation process. Yoshiki *et al.* (2005) have proposed that group B soyasaponin is more favorable as the supply source of soybean functionality rather than DDMP-conjugated soyasaponins.

As showen in Fig. 3, the composition rates of soyasaponins were varied among different seed size varieties. The approximate composition rates of soyasaponins in the large-size seeds were 9.4% of soyasaponin A1, 26.5% of DDMP-conjugated soyasaponins, 49.9% of non-DDMP counterpart soyasaponins, and 14.2% of DDMP moiety, respectively. Similar results were observed in the composition ratios of middle- and small-size seeds. However, group A saponin was showed the lowest composition ratio in the large- and medium-size varieties, while the DDMP moiety showed the lowest composition ratio in small-size varieties. In contrast,

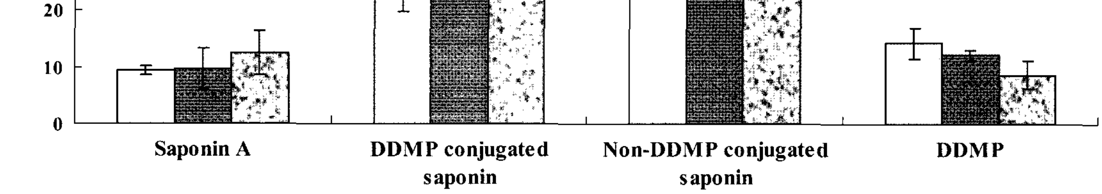


Fig. 3. Comparison of the soyasaponin composition rates among different seed size soybean varieties.

Table 3. Correlation coefficients among chemical components and soyasaponins

	A_1	I	II+III	IV	V	αg	βg	DDMP	Total
100-seed wt	-0.371	-0.061	0.534*	0.199	-0.119	-0.027	-0.293	0.478*	-0.008
Protein	0.204	0.058	-0.144	-0.123	-0.014	0.012	0.228	0.001	0.123
Oil	0.077	0.502*	0.687**	0.580**	0.124	0.418*	-0.243	0.533*	0.500*
Fiber	-0.106	-0.157	-0.203	0.126	-0.388	-0.540*	-0.126	-0.248	-0.353
Ash	-0.062	-0.379	-0.247	-0.007	-0.252	-0.518*	-0.020	-0.258	-0.372
C: N ratio	0.034	0.531*	0.599**	0.329	0.093	0.477*	-0.077	0.534*	0.528*

*, **means the significant differences at P <0.05 and P<0.01, respectively.

the mean value of soyasaponin A1 was considerably high in the small-seed varieties, although the differences were statistically not significant and the highest concentration was found in medium-seed varieties Sinpaldalkong 2.

Considering the results, the soyasaponin concentration and composition are varied among different seed-size soybeans and this may depend on the variety of soybeans.

Soayasaponin A_1 and A_4 which were nominated as Ab and Aa, respectively, by Shiraiwa et al. (1991) are the major constituents of group A soyasaponins. Most of soybean varieties contain either soyasaponin A_1 or A_4 (Shiraiwa et al., 1991).

Soyasaponin A_4 contains a xylose residue at the C-22 position of soyasapogenol A, whereas soyasaponin A_1 contains a glucose at this position (Fig. 1).

In general, group A soyasaponins have a stronger undesirable taste than group B and E saponins. This is caused by the acetylation of the hydroxyl groups of terminal sugar chain attached at the C-22 position of soyasapogenol A (Burrows *et al.*, 1992). In spite of health benefits, soybean is still predominantly processed by Western people only to extract oil because of its peculiar taste and flavour.

Therefore, a group A acetyl saponin-deficient mutant would be expected to improve the taste of soybean foods. Although much efforts had been expended to identify such mutant from cultivated soybeans, this has not yet been successful. Therefore, a evaluation of soyasaponin concentration and its composition in soybean varieties not only will be valuable for the soybean breeding and but also will be clarified the health-promoting effects of soy products and decrease undesirable taste in soybeans.

Relationship between soyasaponins and chemical components

Statistical analysis of correlation among protein, oil, fiber, ash, and C:N ratio with soyasaponins in the soybean seeds was conducted to determine whether these components affected the relative contents of soyasaponins (Table 3).

Among the tested chemical components, oil content and C:N ratio showed the significant positive correlations with total soyasaponin concentration, while the 100-seed weight, fiber, and ash contents showed the negative correlations with total soyasaponin but statistically not significant.

Oil content and C:N ratio of soybean seed have a strong relationship with DDMP moiety, αg, and soyasaponin I, II+III. However, it was noted that protein contents didn't have any relationship with group A, group B, DDMP moiety, and total soyasaponin. This fact suggested that protein contents, although they are most abundant chemical components in soybean seeds, are not affects the variation of soyasaponin concentration.

From the results, it was suggested that the accurate characterization of the soyasaponin biosynthesis in soybean seed is necessary for the understanding the interactions concerned and the improvement of soybean quality and soybean utilization.

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