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Composition of Isoflavone, Phytic Acid, and Saponins in Hypocotyls and Cotyledons of Six Traditional Korean Soybeans

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Abstract: The objective of present study was to examine the composition of functional components (isoflavones, phytic acid, and saponin) in hypocotyls and cotyledons of six traditional Korean soybeans, namely *Cheongtae*, *Seoritae*, *Jinjoori*, *Subaktae*, *Yutae*, and *Huktae*. Three 'family' of isoflavones, as four chemical structures were present in hypocotyls and cotyledons of six soybean cultivars: the aglycones genistein, daidzein, and glycitein; the glycosides genistin, daidzin, and glycitin; the acetylglycosides 6"-O-acetylgenistin, 6"-O-acetyldaidzin, and 6"-O-acetylglycitin; and the malonylglycosides 6"-O-malonylgenistin, 6"-O-malonyldaidzin, and 6"-O-malonylglycitin. Isoflavone contents of hypocotyls and cotyledons differed among the cultivars, and glucosides and malonylglucosides accounted for more than 90% of the total phytoestrogens, with the remaining 1~7% taken up by aglycones. Concentrations of isoflavones in cotyledons were approximately about 10~20% of respective hypocotyls. Contents of phytic acids in hypocotyls and cotyledons of the selected soybean cultivars were 1.21~1.70% and 2.59~3.01%, respectively. Hypocotyls of *Seoritae* showed the lowest content of phytic acid with 1.21%, while cotyledons of *Cheongtae* showed the highest content with 3.01%. The sapogenol concentrations ranged from 13.58 mg/100 g~20.82 mg/100 g for hypocotyls and 0.95 mg/100 g~2.55 mg/100 g for cotyledons showing that concentrations of saponin are 7~10 times higher in hypocotyls than in cotyledons of respective soybeans. For both hypocotyls and cotyledons the sapogenol A were present in higher concentrations than soyasapogenol B.

Keywords: traditional Korean soybeans, cultivars, isoflavone, phytic acid, saponin

Introduction

Soybean, a leguminous plant, is native to Far East, where it has been cultivated as a principal crop for at least 4,000 years. In Korea, there are many varieties of soybean in cultivation, producing beans of many sizes, shapes, and colors. Soybeans with black or green hulls are known to have health promoting effects, while soybeans with yellow hull are used mainly as the raw material for producing cooking oil, isolated soy protein or processed for making traditional foods such as soy paste, soy sauces, soymilk, and tofu. However, with the discovery of functional compounds present in soybean, much attentions are being focused on utilizing these substances.

Biologically active compounds of soybean include isoflavones, phytic acid, and saponins.[1-6] Isoflavones of soybean are mainly present as sugar conjugates. Isoflavones are known to have activities against cancer, cardiovascular diseases and osteoporosis in women.[7-10] Possible role in cancer protection of phytoestrogens have been intensively investigated and anticarcinogenic activity of genistein has been predominantly attributed to specific inhibition of protein tyrosine kinases.[8,11] Traditionally phytic acid was associated with reduced availability of zinc and calcium due to the formation of metal-phytate complex. However, mineral binding properties of phytic acid may prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract.[12] Discoveries are being made that phytic acid found in the fiber of legumes and grains are the major ingredient responsible for preventing colon cancer and other cancers.[13,14] Saponins are chemical structures consisting of triterpenoidal or steroidal aglycones with various carbohydrate moieties. Due to the presence of both hydrophilic and hydrophobic regions, saponins are excellent emulsifiers and foaming agents, and provide functional roles in foods. The ability of saponins to form emulsions in the intestine have lead to the investigation into their role for lowering serum cholesterol in humans.[15,16)

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Studies on compositions of functional components of traditional Korean soybeans are surprisingly lacking, even though the beans are consumed almost daily by Koreans, either fermented or with some processing.[17] In this study, we investigated composition of functional compounds of hypocotyls and cotyledons of six selected traditional Korean soybeans. Soybean hypocotyls were produced in the dehulling process of soybean as a byproduct. Soybean hypocotyls may be a good source of raw material for the production of isoflavones and other functional ingredients.[18,19]

Materials and Methods

Materials

Six traditional Korean soybean cultivars harvested in 2002, namely *Cheongtae, Seoritae, Jinjoori, Subaktae, Yutae,* and *Huktae* were purchased from Collective Farm Cooperation of North Cholla Province, Korea. Cotyledons and hypocotyls of the six soybean cultivars were hand separated and used for investigating compositions of the functional ingredients, namely isoflavones, phytic acids, and saponin. Isoflavone standards were kindly supplied by Fujico (Tokyo, Japan). Phytic acids were purchased from Sigma (St. Louis, MO, USA). Soybean saponin A and B were obtained from Wako Chemicals (Tokyo, Japan). Other reagents used were of reagent grade.

Isoflavone Detection

To identify the isoflavone contents of hand separated hypocotyls and cotyledons of the six soybean cultivars, the 12 samples were hammer milled and dried in a vacuum drier at 60°C for 12 hr. One tenth gram of each samples were dispersed in 0.5 m*l* of 70% aqueous ethanol containing 0.1% acetic acid and stirred for 24 hr at room temperature. The solutions were centrifuged at 10,000xg for 5 min and the supernatants were filtered with 0.45 μm membrane (PVDF syringe filter, Whatman, Germany) and analyzed with HPLC.[20] JASCO (Tokyo, Japan) HPLC system was used with ODS A303 (4.6×250 mm, YMC, Japan) column. Injection volume was 10 μ*l*. Detector used was UV detector at 254 nm, and the flow rate of the solvent was 1.0 m*l*/min. All the data were presented as means of three determinations. A linear HPLC gradient was composed of (A) 0.1% glacial acetic acid in H_2O and (B) 0.1% glacial acetic acid in acetonitrile. Following injection of the sample solutions, solvent B was increased from 15% to 35% over 50 min, and then held at 35% for 10 min.

Phytic Acid Detection: To determine phytic acid content of hypocotyls and cotyledons of the six soybean samples, spectrophotometric method was used.[21] Briefly, to powdered samples of 0.5 g, 30 m*l* of 2.4% HCl was added and stirred for 2 hr at room temperature. The mixture was centrifuged at 8,000 × g for 10 min and the supernatant of the acid treated sample was applied to 1.0 cm × 15 cm column packed with anion exchanger (AG1-X8, Bio-Rad Lab, Hercules, CA, USA) The resin had been previously activated with 0.7 M NaCl solution and washed with three bed volumes of deionized water. To elute phytate, 15 m*l* of 0.7 M NaCl solution was applied to the column. The eluate was made up 30 m*l* with deionized water. Three m*l* aliquots was subsequently mixed with 1 m*l* of Wade reagent (containing 0.03% ferric chloride and 0.3% sulfosalicylic acid) and the color developed was detected by reading absorbance at 500 nm.

Saponin Detection

To determine saponin contents of the soybean parts, 1 g of powdered hypocotyls or cotyledons were mixed with 100 m*l* of 80% methanol and stirred at 80°C for 4 hr to extract the crude saponins.

Table 1. Conditions for analysis of saponin by HPLC

HPLC system	Agilent 1100 series			
Column	Apex Silica column (4.6 × 150 mm, 3 μm, Jones Chromatography)			
Mobile phase	Ethanol/Petroleum ether			
Solvent condition	Times	A% (EtOH)	B% (pet.ether)	Flow rate (m*l*/min)
	0	0	100	1.2
	15	15	85	1.2
Column temp.	30°C			
Detector	ELSD (Alltech 2000, Neb. temp 40°C, N2 gas flow 1.5 m*l*/min)			
Injection volume	20 μ*l*			

Saponins separated were then powdered dried with rotary evaporator and added with 7% methanolic-HCl under refluxing condition for 24 hr, to form sapogenols (aglycones). Dried aglycones were solubilized with 50 m*l* petroleum ether, which were filtered and used for HPLC analysis. HPLC conditions are as in Table 1.

Results and Discussion

Isoflavone Contents

HPLC chromatogram of 12 isoflavone standards is as in Fig. 1. Three 'family' of isoflavones, as four chemical structures are known to present in soybeans: the aglycones genistein, daidzein, and glycitein; the glycosides genistin, daidzin, and glycitin; the acetylglycosides 6"-O-acetylgenistin, 6"-O-acetyldaidzin, and 6"-O-acetylglycitin; and the malonylglycosides 6"-O-malonylgenistin, 6"-O-malonyldaidzin, and 6"-O-malonylglycitin. Isoflavone concentrations of hypocotyls and cotyledons of six soybean cultivars are as shown in Table 2 and 3. Hypocotyls and cotyledons account for approximately 2% and 90% of soybean, on weight basis, respectively. Isoflavone concentrations of hypocotyls and cotyledons differed among the cultivars, though in all the beans studied glucosides and malonylglucosides accounted for more than

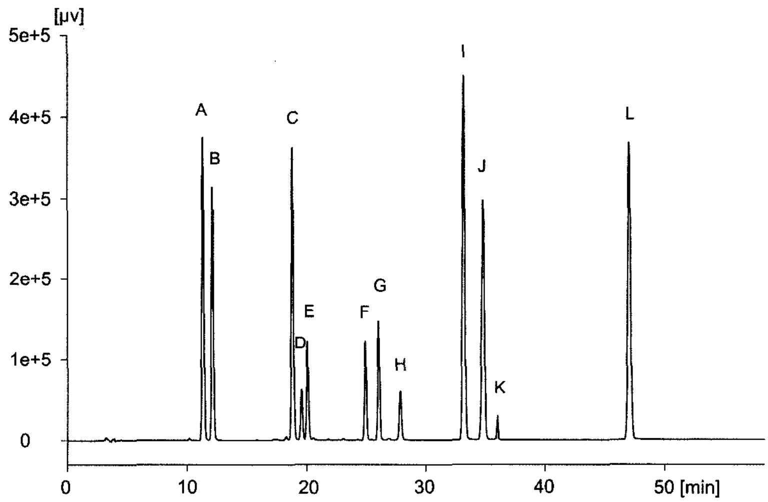

Fig. 1. HPLC chromatogram of isoflavone standards.
A Daidzin; B, Glycintin; C, Genistin; D, Malonyl-daidzin; E, Malonyl-glycitin; F, Acetyl-daidzin; G, Acetyl-glycitin; H, Malonyl-genistin; I, Daidzein; J, Glycitein; K, Acetyl-genistin; L, Genistein.

Table 2. Isoflavone content of hypocotyls in soybean samples (unit: mg/100 g)

Variety	Glucoside			Malonyl			Acetyl			Aglycon			Total
	Din	Glin	Gin	Din	Glin	Gin	Din	Glin	Gin	Dein	Glein	Gein	Isoflavones
Cheongtae	249.8	235.8	141.9	1724.6	516.3	702.8	21.8	31.5	1.6	53.6	41.3	26.6	3747.7
Seoritae	176.3	238.8	49.7	2190.6	675.4	586.4	21.5	22.1	nd	61.9	59.3	20.9	4103.0
Jinjoori	114.8	155.2	31.3	2137.5	562.5	555.9	17.9	23.7	1.9	39.3	38.3	15.0	3693.6
Subaktae	495.5	381.0	111.1	2615.7	646.3	596.9	24.3	25.7	1.4	102.4	54.1	27.8	5082.2
Yutae	128.4	146.3	70.9	1860.8	500.1	990.7	49.4	11.8	2.6	46.5	41.2	28.4	3877.0
Huktae	405.6	258.4	98.6	1973.5	402.6	473.8	67.1	31.2	tr	53.6	37.7	18.9	3821.8

All the data are means of three determinations.
Abbreviations: Din, daidzin; Glin, glycitin; Gin, genistin; Gein, daidzein; Glein, glycitein; Gein, genistein; tr, trace; nd, not detected (See Fig. 1).

Table 3. Isoflavone content of cotyledons in soybean samples (unit: mg/100 g)

Variety	Glucoside			Malonyl			Acetyl			Aglycon			Total
	Din	Glin	Gin	Din	Glin	Gin	Din	Glin	Gin	Dein	Glein	Gein	Isoflavones
Cheongtae	6.9	nd	28.5	18.3	3.5	150.8	8.1	nd	nd	tr	1.3	1.2	219.2
Seoritae	19.0	nd	47.0	179.5	8.3	387.0	24.5	nd	nd	3.2	2.5	7.0	678.0
Jinjoori	4.5	nd	14.1	56.3	4.1	177.6	10.3	nd	nd	1.0	2.1	2.3	272.3
Subaktae	31.5	nd	62.6	108.9	7.9	239.4	14.3	nd	nd	6.4	2.5	8.9	482.4
Yutae	10.5	nd	27.1	101.2	6.7	285.0	16.5	nd	nd	2.0	2.4	4.1	455.5
Huktae	27.4	nd	62.6	151.6	7.7	340.7	19.6	nd	nd	6.7	2.3	10.9	629.6

All the data are means of three determinations.
Abbreviations : Din, daidzin; Glin, glycitin; Gin, genistin; Gein, daidzin; Glein, glycitein; Gein, genistein; tr, trace; nd, no detected.

90% of the total phytoestrogens, with the remaining 1~7% taken up by aglycones. Among the hypocotyls of six cultivars tested, the soybean with green hull with green stripes, *Subaktae*, showed the highest concentrations of isoflavones with 5082 mg/100 g while that of *Cheongtae, Jinjoori, Yutae,* and *Huktae* were about 75% of *Subaktae,* in the range of 3700~3900 mg/100 g. Concentrations of isoflavones in cotyledons are approximately about 10~20% of respective hypocotyls as shown in Table 3. Cotyledons of *Cheongtae* showed lowest concentrations of isoflavones with 219.2 mg/100 g while *Seoritae* showed the highest with 678.0 mg/100 g. The glycosides and malonylglycosides (genistin, daidzin and glycitin) are the main components and small amounts of these three compounds exist as aglycones in the soybean. Degradation of malonyl glucosides at high temperature were reported by many researchers, but our results showed high percentages of malonyl forms for all the soybean samples. The differences were speculated to be due to relatively mild heat treatment employed for the isolating process in our research.[22,23]

Table 2, 3 show that large sized beans with black hull, such as *Seoritae* and *Huktae* contained higher concentration of isoflavones in both hypocotyls and cotyledons, while small sized seed with black hull such as *Jinjoori, Cheongtae,* and *Yutae* were low in isoflavone contents. Anlin *et al.* (1995) mentioned that soybean with green hull are low in isoflavone content while soybean with black hull tended to have high content of isoflavone.[24)] *Seoritae* has been traditionally known to promote health such as improving blood circulation and stamina among Koreans, which might be attributed to higher content of functional compounds such as isoflavone in the bean. The concentration of isoflavones in soybeans has been quantified but only on a limited scale using a few cultivars.[24,25)] It is generally agreed that concentrations of isoflavones are highly influenced by the environment. The date of planting was found to have a influence on levels of isoflavones, with early planted soybean having lower concentrations of isoflavones. Tsukamoto *et al.* (1995) discovered that high temperatures during seed development of soybeans significantly reduced concentrations of isoflavones but had no effect on levels of saponins.[26)]

Phytic Acid Content

According to our preliminary studies, recovery percentage of phytic acid using method developed by Harland and Oberleas (1977) was 98~100%.[27)] Contents of phytic acids in hypocotyls and cotyledons of the selected soybean samples were 1.21~1.70% and 2.59~3.01%, respectively, as shown in Table 4. Hypocotyls of *Seoritae* showed the lowest content

Table 4. Content of phytic acid in hypocotyls and cotyledons of soybean samples (%)

Variety	Hypocotyl	Cotyledon
Cheongtae	1.70	3.01
Seoritae	1.21	2.88
Jinjoori	1.40	2.95
Subaktae	1.50	2.62
Yutae	1.69	2.90
Huktae	1.65	2.59

All the data are means of three determinations.

of phytic acid with 1.21% while *Cheongtae* showed the highest content with 1.70%. As for cotyledons, *Cheongtae* showed the highest content of phytic acid with 3.01%. Latta and Eskin (1980) reported phytic acid content of their samples to be 1.8%[21] However, Chitra *et al.* (1995) reported phytic acid content of soybean to be 3.64%.[28] The discrepancies in phytic acid contents of soybeans by different researchers could be partly due to differences in the purification methods adopted by the researchers.

Finding concentration of phytic acid is important because the compound acts as a strong chelator of minerals such as calcium, magnesium, iron and zinc and therefore, can contribute to mineral deficiencies in certain people whose diets rely on foods containing legumes. Still, to certain people intake of phytic acid could be helpful because the mineral binding properties of phytic acid are known to prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract.[29] Encouraging consumption of traditional soybeans would be one of the means for improving public health.

Saponin Contents

Saponins are naturally occurring triterpenoids found in many food materials derived from plants. They are secondary plant metabolites containing a steroid or triterpenoid aglycone with a number of different carbohydrate moieties which are linked through either an ether or an ester linkage. Although there are numerous reports on the analysis of saponin glycosides in soybeans, many of these procedures are complex and occasionally inaccurate. In this report, saponin preparation method included alteration of the chemical structure of the naturally occurring saponins to sapogenols in order to minimize the errors resulting from numerous isomers present. Standard chromatogram of sapogenol A and sapogenol B is shown in Fig. 2. The sapogenol concentrations ranged from 13.58 mg/100 g~20.82 mg/100 g for hypocotyls and 0.95 mg/100 g~2.55 mg/100 g for cotyledons showing that concentrations of saponin are 7~10 times higher in hypocotyls than in cotyledons of respective soybeans (Table 5, 6). For both hypocotyls and cotyledons the sapogenol A were present in higher concentrations than soyasapogenol B.

Among the six varieties of soybean samples, *Huktae, Seoritae* and *Jinjoori* were higher in sapogenol concentrations than *Yutae, Subaktae*

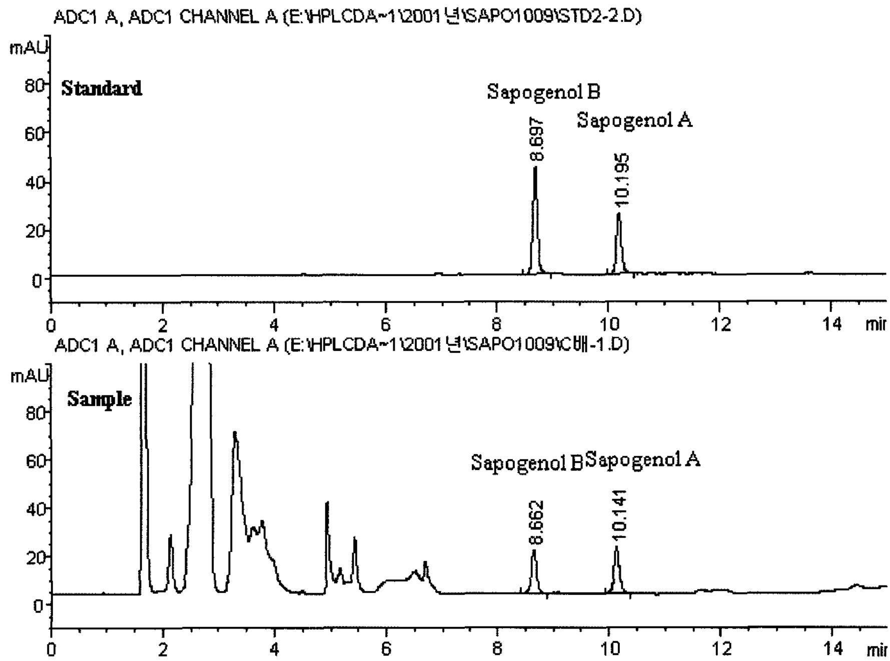

Fig. 2. Chromatogram of standard sapogenol A and sapogenol B using HPLC-ELSD.

Table 5. Content of saponin in hypocotyls of soybean samples (unit: mg/100 m*l*)

	Sapogenol A	Sapogenol B	Total
Cheongtae	7.76	5.82	13.58
Seoritae	9.10	5.48	14.58
Jinjoori	9.27	6.09	15.35
Subaktae	10.19	7.59	17.78
Yutae	11.73	7.23	18.96
Huktae	12.68	8.14	20.82

All the data are means of three determinations.

Table 6. Content of saponin in cotyledons of soybean samples (unit: mg/100 m*l*)

	Sapogenol A	Sapogenol B	Total
Cheongtae	0.61	0.50	1.11
Seoritae	1.01	0.67	1.68
Jinjoori	0.87	0.44	1.31
Subaktae	1.70	0.85	2.55
Yutae	0.63	0.32	0.95
Huktae	1.00	0.70	1.70

All the data are means of three determinations..

and *Cheongtae*. *Huktae*, *Seoritae* and *Jinjoori* all have black seedcoats. Both *Huktae* and *Seoritae* are large sized soybeans with 100 seed weight being 36.59 g and 43.09 g though both beans can be differentiated by seed shape or cotyledon color: *Seoritae* is oval shaped with greenish cotyledons while *Huktae* is round shaped with yellow cotyledons. *Jinjoori*, *Yutae*, *Subaktae* and *Cheongtae* are all small sized soybeans and can be easily differentiated by seedcoat color. *Jinjooi*, *Yutae*, *Subaktae* and *Cheongtae* are black, yellow, greenish with green stripes, and green, respectively. 100 seed weights of the these soybeans are 11.81 g, 10.20 g, 11.25 g, and 27.76 g, respectively. The relationship between seed color and content of functional compounds are still unclear at this time, however, and needs further researches.

Conclusions

Contents of functional ingredients present in hypocotyls and cotyledons of six soybean varieties are compared. The hypocotyl is a part of soybean that eventually develops into the stem during germination while the cotyledon becomes the embryonic first leaves of a seedling. Hypocotyl accounts for less than 2% of whole soybean and are normally removed during production of soymilk or soyprotein isolate processors, due to bitter taste. However, with the discovery of biologically active compounds present in high concentrations, soybean hypocotyl is attracting much attentions as functional foods among health-conscious consumers. Functional components with biological activities include isoflavones, phytic acids, and saponins. Three 'family' of isoflavones, as four chemical structures are present in soybean hypocotyl. Contents of isoflavones in cotyledons are approximately about 10~20% of respective hypocotyls. Contents of phytic acids in hypocotyls and cotyledons of the selected soybean samples were 1.21~1.70% and 2.59~3.01%, respectively. The sapogenol concentrations ranged from 13.58 mg/100 g~20.82 mg/100 g for hypocotyls and 0.95 mg/100 g~2.56 mg/100 g for cotyledons showing that concentrations of saponin are 7~10 times higher in hypocotyls than in cotyledons of respective soybeans. This results showed that soy hypocotyls, which are used mainly used as animal feeds, could be used as raw material for purifying highly value added products such as isoflavone and soponin.

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