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Evaluation of Soyasaponin, Isoflavone, Protein, Lipid, and Free Sugar Accumulation in Developing Soybean Seeds

SUN-LIM KIM,[†,‡] MARK ALAN BERHOW,[‡,§] JUNG-TAE KIM,[||] HEE-YOUN CHI,[†] SUN-JOO LEE,[||] AND ILL-MIN CHUNG*[,||]

National Institute of Crop Science, RDA., Suwon 441-857, Korea; National Center of Agricultural Utilization Research, USDA-ARS, 1815 N, Peoria, Illinois 61604; and Department of Applied Life Science, College of Life and Environment Science, Konkuk University, KwangJinKu HwaYangDong, Seoul 143-701, Korea

A combination of analytical techniques was used to examine and quantify seed compositional components such as protein, lipid, free sugars, isoflavones, and soyasaponins during soybean development and maturation in two Korean soybean cultivars. Protein accumulation was rapid during reproductive stages, while lipid content was only relatively moderately increased. The major carbohydrate saccarides sucrose and stachyose constantly increased during the reproductive stage. Previously published results suggest that the free sugar and lipid content reached their maximal concentrations at a relatively early stage of seed development and remain constant in comparison to other chemical components. The malonylglucosides were the predominant isoflavone form followed by the glucosides, acetyl glucosides, and aglycone forms. As soybean seed matures, total soyasaponin concentration was constantly decreased until the R8 stage. Soyasaponin βg was the major soyasaponin in DDMP-conjugated group B soyasaponins, followed by the non-DDMP counterpart soyasaponin I and soyasaponin A_1. The ratio of total isoflavone to total soyasaponin in the developing soybean increased from 0.06 to 1.31. Protein, lipid, and free sugar contents in the developing soybean seeds showed significant positive correlations with conjugated isoflavones and total isoflavone concentration, while the lipid contents showed a negative correlation with the isoflavone aglycone. Protein, lipid, and free sugar contents showed a negative correlation with total group A and B soyasaponins and total soyasaponins; however, only the soyasaponin A content was significantly negatively correlated with free sugar content. Total soyasaponin content was negatively correlated with isoflavone content ($r = -0.828$ at $p < 0.01$).

KEYWORDS: Soybean; protein; lipid; free sugar; isoflavone; soyasaponin; reproductive stage

INTRODUCTION

Soybeans (*Glycine max* L. Merr.) are recognized worldwide as an excellent source of high-quality protein and lipids and for their benefit to human health. Recently, special emphasis has been given on genetically manipulating the chemical composition of soybean for improving various functional ingredients as well as for developing new soybean varieties. Soybean seed which are used to make processed products are also a good source of biologically active secondary metabolites such as saponins and isoflavones.

Isoflavones are a group of naturally occurring heterocyclic phenols which have been shown to be phytoestrogens that have weak estrogen-like or antiestrogenic activity (*1*, *2*). The principal isoflavones of soybean seed—daidzein, genistein, and glycitein—are synthesized via the phenylpropanoid pathway and stored as glucoside conjugates in the cell vacuoles (*3*).

Isoflavones are biosynthetically modified with certain functional groups, with the malonylated isoflavone glycosides being the major isoflavone constituents found in mature soybean seed. The malonyl forms are thermally unstable and easily converted into their corresponding isoflavone acetyl-glycoside and glucoside forms (*3*, *4*). The total concentration of isoflavones in mature soybean seeds fluctuates depending on both the cultivar grown and the environmental conditions which occur during the seed filling stage (*4*−*9*).

The reproductive stage of soybean begins at the time of first bloom and ends at maturity. It is divided into four phases: flowering (R1 and R2), pod development (R3 and R4), seed growth (R5 and R6), and seed/plant maturation (R7 and R8). At the R5 stage soybean seeds are beginning growth and their size is approximately 3 mm long and at the R6 stage soybean pod contains green seeds that fill the pod cavity. At the R7

* To whom correspondence should be addressed. Tel: +82-2-450-3730. Fax: +82-2-446-7856. E-mail: imcim@konkuk.ac.kr.
[†] National Institute of Crop Science.
[‡] The authors contributed equally to this work.
[§] National Center of Agricultural Utilization Research.
[||] Konkuk University.

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Table 1. Changes of Seed Dry Weight, Length, Width, and Chemical Components during Reproductive Stages of the Korean Soybean Cultivars 'Sojinkong' and 'Daepungkong'

		seed characteristics			chemical components		
cultivars	RS[a]	dry weight (mg/seed)	length (mm)	width (mm)	protein (%)	lipid (%)	free sugar[b] (%)
Sojinkong	R5	38.1 ± 3.1[c]	5.88 ± 0.5	4.11 ± 0.3	34.8 ± 1.1	16.5 ± 0.6	13.8 ± 2.4
	R6	81.2 ± 4.6	6.36 ± 0.5	4.34 ± 0.5	35.5 ± 1.2	17.9 ± 0.7	15.3 ± 2.1
	R7	112.3 ± 8.1	7.56 ± 0.8	5.38 ± 0.6	38.7 ± 1.1	18.2 ± 1.2	14.6 ± 1.8
	R8	119.2 ± 7.1	7.16 ± 0.6	6.79 ± 0.7	39.2 ± 1.3	18.1 ± 1.1	14.5 ± 1.5
Daepungkong	R5	63.0 ± 4.2	6.69 ± 0.5	5.42 ± 0.4	37.7 ± 1.2	16.0 ± 0.5	13.0 ± 2.2
	R6	108.1 ± 7.9	8.15 ± 0.7	6.15 ± 0.6	38.8 ± 1.1	17.6 ± 1.1	15.2 ± 1.1
	R7	211.3 ± 7.6	8.98 ± 0.9	6.84 ± 0.8	40.2 ± 2.1	18.0 ± 1.2	15.7 ± 2.0
	R8	221.7 ± 9.1	8.52 ± 0.7	7.27 ± 0.6	41.1 ± 1.9	17.8 ± 1.2	15.5 ± 1.8

[a] RS: reproductive stage. [b] Free sugar represents the sum value of fructose, glucose, maltose, sucrose, raffinose, stachyose, trisaccharide, hexasaccharide, and heptasaccharide. [c] Mean ± SD, $n = 3$.

stage soybean seeds are beginning maturity and physiologically mature. At the final R8 stage soybean seeds are in the state of full maturity and approximately 95% of the pods have reached mature pod color (*10*).

Significant environmental effects on the final isoflavone concentrations in soybean have been reported. Analysis of isoflavone concentrations in soybean cultivars grown in different locations have shown significant variations in two or more years for both total and individual isoflavone contents (*11*, *12*). Tsukamoto et al. (*9*) reported that the isoflavone content was significantly lower in seeds that developed at high temperatures during seed filling than in seeds that developed at lower temperatures.

Isoflavones have been reported to reduce the risk of breast cancer and heart disease and total cholesterol levels and demonstrated to have some antioxidant activity (*2*, *13*, *14*). Several investigators have suggested that soy food consumption may contribute to lower rates of chronic diseases such as hormone-dependent cancers, cardiovascular diseases, and osteoporosis (*13*−*15*).

Soyasaponins are triterpenoid glycosides with one or two polysaccharide chains that are mainly present in legumes (*16*−*18*). The presence of saponins in soybean has attracted considerable interest because of both their potential health benefits and their adverse sensory characteristics. The soyasaponins can be divided into two groups based on their aglycone structure—the group A and group B soyasaponins. The group A acetylated saponins are mostly responsible for undesirable bitter and astringent taste (*16*, *17*). The DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one-conjugated group B saponins may possess multiple health promoting effects, such as lowering cholesterol levels by inhibiting its absorption (*18*, *19*). They also show anticarcinogenic activity against various tumor cell lines (*20*−*22*) and have antihepatotoxic properties and antiinfectivity to HIV (*23*−*25*). Soybean seeds on average contain about 0.5% of soyasaponins, but this value can vary widely depending on the variety, cultivation year, location grown, and degree of maturity (*26*, *27*).

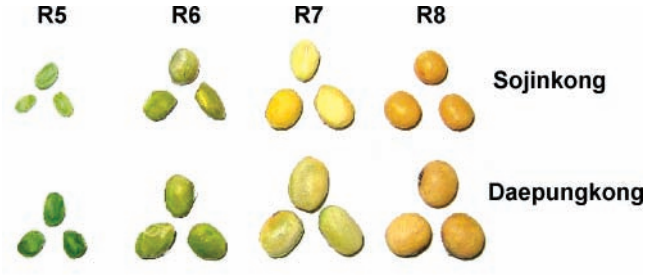


Figure 1. Profile of soybean seed development during the reproductive stages. Seeds of R5 and R6 stages were freeze-dried immediately after sampling.

Relatively little information is available on isoflavone and soyasaponin concentrations and their interaction in developing soybean seeds. This study focuses on the changes of isoflavone and soyasaponin concentration during seed development and their relationship to the other major chemical components such as protein, lipids, and free sugars.

MATERIALS AND METHODS

Soybean Cultivars. Two Korean soybean cultivars, 'Sojinkong' (small seed size, mainly used for soybean sprouts) and 'Daepungkong' (medium seed size, mainly used for tofu, soymilk, and fermented soybean paste), were grown and harvested in the field of the National Institute of Crop Science, Suwon, Korea, in 2005. The chemical characteristics of the experimental soil were pH 6.7, organic matter 1.5%, and available P_2O_5 321 ppm. Exchangeable K, Ca, and Mg cations were 0.48, 2.5, and 1.1 me/100 g, respectively. For analysis of nutrients in soybean seeds during the reproductive stages, soybean seeds were sampled from the R5 to R8 stages. Collected seeds were freeze-dried immediately after sampling and stored at 4 °C until analysis.

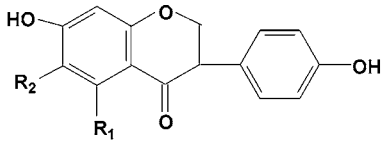
Protein, Lipid, and Free Sugar Analysis. Soybean seeds were ground to flour using an about 100-mesh laboratory test mill (Brabender, Duisburg, Germany) to analyze protein, lipids, amino acids, and fatty acids. The protein contents of seed samples were determined according to the Kjeldahl procedure using a Tecator Kjeltec Auto Analyzer, model 2400 (Foss Tecator, Huddinge, Sweden). Lipid contents were measured by a Soxtherm Automatic System (Gerhardt, Hoffmannstr, Germany). The extraction beaker was filled with a few boiling stones and then dried at 105 °C. Homogenized samples (5.0 g) were put into an extraction thimble, to which 140 mL of n-hexane was added. After boiling for 30 min at 180 °C, the extraction was performed 5 times with solvent reduction after each step for a total time of 80 min. After extraction, the beakers were dried at 105 °C for 1 h, cooled to room temperature in a desiccator, and then weighed. Total lipid contents were represented on a dry matter basis of soybean seeds.

The fatty acid was analyzed as follows; 0.5 g of freeze-dried soybean seed was heated with a reagent containing methanol:heptane:benzene: 2,2-dimethoxypropane:H_2SO_4 (37:36:20:5:2, v/v). The simultaneous digestion and lipid transmethylation took place in a single phase at 80 °C. After they were cooled at room temperature, the upper phases containing the fatty acid methyl esters (FAMEs) were prepared for the capillary GC analysis. The GC analysis was performed on a Agilent 6890 system (Agilent Co., Palo Alto, CA) equipped with a FID by using a HP-Innowax capillary (cross-linked polyethylene glycol) column (0.25 μm × 30 m). The initial temperature of 150 °C was raised to the final temperature of 280 °C at a rate of 4 °C min^{-1}. Carrier gas was nitrogen at a flow rate of 10 mL min^{-1}. During analysis the temperatures of the inlet and detector were maintained at 250 and 300 °C, respectively. The standard FAME mix (C14−C22) was obtained from Supelco (Bellefonte, USA).

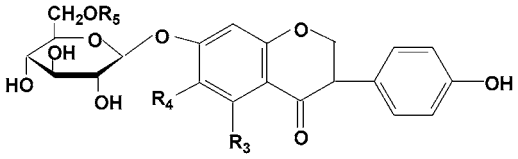
Table 2. Isoflavone Concentration (μg/g) in Korean Soybean Cultivars 'Sojinkong' and 'Daepungkong' during the Reproductive Stages

		aglycone			glucoside			malonylglucoside			acetylglucoside		
cultivars	RS[a]	daidzein	genistein	glycitein	daidzin	genistin	glycitin	daidzin	genistin	glycitin	daidzin	genistin	total
Sojinkong	R5	6.2 ± 0.3[c]	5.7 ± 0.1	4.7 ± 0.0	47.2 ± 3.2	32.7 ± 6.5	20.5 ± 1.9	82.5 ± 4.6	161.4 ± 1.7	36.5 ± 0.5	14.1 ± 2.1	3.5 ± 0.1	415.0 ± 20.8
	R6	5.1 ± 0.1	4.8 ± 0.0	4.1 ± 0.1	150.1 ± 12.1	331.5 ± 11.7	117.3 ± 10.7	265.2 ± 25.5	681.4 ± 20.5	127.1 ± 3.6	62.9 ± 6.0	5.1 ± 0.1	1754. 6 ± 90.1
	R7	4.2 ± 0.0	4.1 ± 0.0	3.2 ± 0.1	266.4 ± 18.6	623.4 ± 38.6	103.6 ± 5.1	535.9 ± 37.5	1086. 6 ± 54.4	94.1 ± 5.5	105.5 ± 7.3	6.9 ± 0.0	2833. 9 ± 166.9
	R8	2.5 ± 0.0	3.5 ± 0.0	2.9 ± 0.2	416.3 ± 23.3	609.0 ± 25.4	98.2 ± 3.2	811.6 ± 41.2	1499. 4 ± 87.6	73.2 ± 6.3	100.1 ± 5.2	6.1 ± 0.0	3622. 8 ± 192.4
Daepungkong	R5	7.7 ± 0.2	7.3 ± 0.1	6.0 ± 0.1	88.2 ± 4.5	55.9 ± 2.1	100.7 ± 8.4	197.1 ± 19.3	210.4 ± 9.1	66.2 ± 6.1	59.8 ± 2.1	10.9 ± 0.1	810.2 ± 51.9
	R6	7.2 ± 0.1	54.6 ± 3.1	4.9 ± 0.1	205.5 ± 9.9	482.1 ± 18.9	191.8 ± 15.1	308.0 ± 21.3	841.6 ± 23.9	79.9 ± 6.2	93.2 ± 5.9	13.7 ± 1.1	2282. 5 ± 105.6
	R7	6.2 ± 0.0	49.5 ± 3.3	5.1 ± 0.2	433.2 ± 20.6	931.3 ± 55.1	164.6 ± 22.3	988.4 ± 33.9	1542. 9 ± 107.3	133.0 ± 10.1	97.6 ± 5.3	33.6 ± 2.1	4385. 4 ± 260.0
	R8	5.3 ± 0.0	52.8 ± 2.8	-	588.3 ± 32.1	952.7 ± 36.3	131.5 ± 17.6	1125. 7 ± 110.1	2244. 2 ± 186.8	182.8 ± 15.1	123.6 ± 9.6	15.4 ± 0.5	5422. 3 ± 411.0

[a] RS: reproductive stage. [b] Not detected. [c] Mean ± SD, $n = 3$.



aglycons	R_1	R_2
daidzein	H	H
genistein	OH	H
glycitein	H	OCH_3



glucosides	R_3	R_4	R_5
daidzin	H	H	H
genistin	OH	H	H
glycitin	H	OCH_3	H
6"-*O*-malonyldaidzin	H	H	$COCH_2COOH$
6"-*O*-malonylgenistin	OH	H	$COCH_2COOH$
6"-*O*-malonylglycitin	H	OCH_3	$COCH_2COOH$
6"-*O*-acetyldaidzin	H	H	$COCH_3$
6"-*O*-acetylgenistin	OH	H	$COCH_3$
6"-*O*-acetylglycitin	H	OCH_3	$COCH_3$

Figure 2. Chemical structures of the 12 isoflavones found in the soybean.

Free sugars were analyzed using a 5 μM YMC-Pack Polyamine II column (4.6 × 250 mm; YMC Co., Ltd., Kyoto, Japan). Twenty milliliters of 20% ethanol solution was added to 1.0 g samples, shaken for 60 min at 35 °C in a water bath, and then centrifuged at 5000*g* for 5 min. The collected supernatant was filtered with a Sep-Pak NH_2 solid-phase extraction cartridge (Waters, Milford, MA), and 1.0 mL of filtrate was evaporated to dryness at 50 °C in a dry bath by blowing with N_2 gas. The residue was then dissolved in 0.2 mL of water, and 20 μL was injected into a high-performance liquid chromatography (HPLC) system equipped with a Waters 510 Pump, Waters 410 R.I. Detector, and Waters 746 Integrator. The operating conditions were as follows: column temperature 35 °C; detector temperature 39 °C; mobile phase acetonitrile:water (65:35, v/v); flow rate 0.7 mL/min. The mono- and oligosaccharides were obtained from Sigma (St. Louis, MI).

Isoflavone Analysis. For analysis of the 12 isoflavones, 0.5 g of freeze-dried finely ground soybean samples was put into a test tube, into which 10 mL of 50% acetonitrile was added. The solution was then mixed overnight with a vortex mixer at room temperature. Extracts were filtered through Whatman No. 42 filter paper and washed twice with 10 mL volumes of extraction solution. Samples were condensed to approximately 1 mL using a vacuum rotary evaporator (Labo Rota S-300; Resona, Zürich, Switzerland) in a water bath at 30 °C. The dried material was redissolved in a mixture of methanol:water (80:20, v/v) to a final volume of 3 mL and filtered through a 0.45 μm PTFE syringe filter (Waters, Milford, MA) prior to HPLC analysis. Analysis of isoflavones was conducted with a reverse-phase HPLC system equipped with a YMC-Pack ODS-AM303 (4.6 × 250 mm; YMC Inc., Wilmington, NC) with a guard column packed with a μBonda C_{18} Waters guard-Pak (Waters, Milford, MA). The mobile phases for HPLC consisted of solvent A, 0.1% acetic acid in water, and solvent B, 0.1% acetic acid in acetonitrile. The solvent gradient was as follows: solvent B was increased from 15% to 25% over 35 min, increased to 26.5% over the next 12 min, and finally increased to 50% over the next 50 s. It was then held at that percentage for the next 14.5 min. The flow rate was 1.0 mL/min up to 48 min and increased to 1.3 mL/min from 48.5 to 63 min. The injection was 20 μL of sample, and the eluted isoflavones were detected at 254 nm using a Waters 2487 dual λ absorbance detector. All HPLC analyses were performed at ambient temperature (24 °C).

Six isoflavone (aglycones and glucosides) standards—daidzein, genistein, glycitein, daidzin, genistin, and glycitin—were purchased from Fujicco Co. Ltd. (Kobe, Japan). Six isoflavone malonyl and acetyl glucosides—malonyldaidzin, malonylgenistin, malonylglycitin, acetyldaidzin, acetylgenistin, and acetylglycitin—were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan) and PKC Pharmaceuticals Inc. (Woburn, MA), respectively.

Soyasaponin Analysis. For quantitative soyasaponin analysis, 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 1−2 h. An aliquot was removed from the vial and filtered through a 0.45 μm nylon filter (Waters, Milford, MA) for HPLC analysis. Soyasaponins were analyzed using an Agilent 2690 HPLC system equipped with a Waters 2996 photodiode array detection system (Waters). 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/min. The effluent was monitored at 210 nm on a 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. The soyasaponin HPLC elution profile was determined by identification of the eluting peaks and examination of a series of purified standards by LC-MS in the Peoria Laboratory (*22*, *28*). Before the samples were run, a linear standard curve for the soyasaponins was determined based on mAbs units vs nmolar concentration injected. This curve was prepared from a dilution series of a pure soyasaponin I standard that had been purified by the USDA-ARS (Peoria, IL) (*28*). This standard curve for soyasaponin I was also used to quantitate the group A soyasaponins and the DDMP group B soyasaponins, as pure standards for those compounds were not available, with the appropriate molecular weight factor in the calculation.

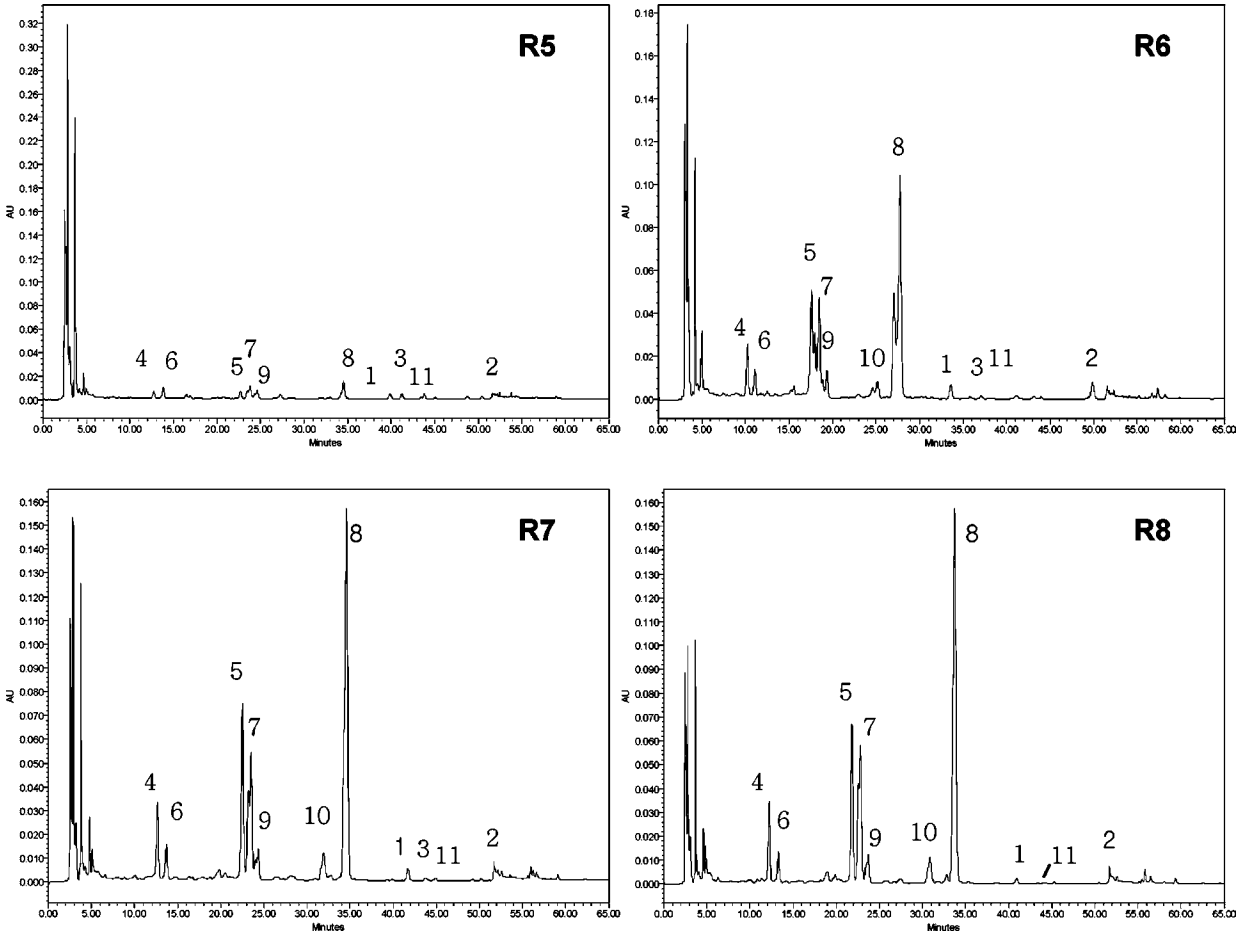


Figure 3. Changing profiles of free isoflavone concentration in the Korean soybean cultivar 'Daepungkong' during the reproductive stages monitored at 260 nm: 1, daidzein; 2, genistein; 3, glycitein; 4, daidzin; 5, genistin; 6, glycitin; 7, malonyldaidzin; 8, malonylgenistin; 9, malonylglycitin; 10, acetyldadzin; 11, acetylgenistin; 12, acetylglycitin.

Statistical Analysis. In all experiments, triplicate measurements were made with three independent samples using a randomized complete block design. Analysis of correlation coefficients among isoflavones, soyasaponins concentrations, and major chemical components was performed using the SAS program (SAS Institute, Inc., 2000) (*29*).

RESULTS AND DISCUSSION

Seed Development. Seed dry weights, lengths, and widths were examined to define the development patterns of soybean seed (**Table 1**).

The seed length of these two soybean cultivars increased from the R5 stage to the R7 stage and then decreased slightly by the R8 stage. The seed widths of the two soybean cultivars increased until the R8 stage.

The green soybeans turned greenish-yellow at the R7 stage (40−45 days after anthesis) (**Figure 1**). In general, soybean seeds begin to develop when soybean plants are at the R5 stage (*10*). At the R6 stage, soybean seeds were green with the seed filling the pod cavity. At the R7 stage, the seeds commence to mature and some pods were physiologically mature. At the final R8 stage, soybean seeds are fully mature and more than 95% of the pods show a mature pod color (*10*). As shown in **Table 1** and **Figure 1**, the decreases in seed moisture content continued at a faster rate until the latter stages of maturation and were accompanied by increases in seed dry weight. When seed weight was expressed on a dry matter basis, it was found to increase rapidly from stage R5 to R8.

Protein, Lipids, and Free Sugars. Protein, lipid, and free sugar contents in developing soybean seeds are shown in **Table 1**. Protein accumulation was rapid during reproductive stages and reached the highest content at the R8 stage, while the lipid content showed a relatively moderate increase and the highest content was observed at the R7 stage. It then decreased slightly by the R8 stage.

The free sugars found in the developing seeds were fructose, glucose, maltose, sucrose, raffinose, stachyose, trisaccharide, hexasaccharide, and heptasaccharide. Sucrose and stachyose are the major saccharides in soybean seeds (data not shown). As maturation progresses, the free sugar levels increase, and the highest levels were observed at the R6 stage in 'Sojinkong' and at the R7 stage in 'Daepungkong'. Soybean carbohydrates make up approximately 35% of soybean seed dry matter. One-half of these carbohydrates are nonstructural in nature and include low molecular weight sugars, oligosaccharides, and small amounts of starch, while the other half are structural polysaccharides (*30*−*33*).

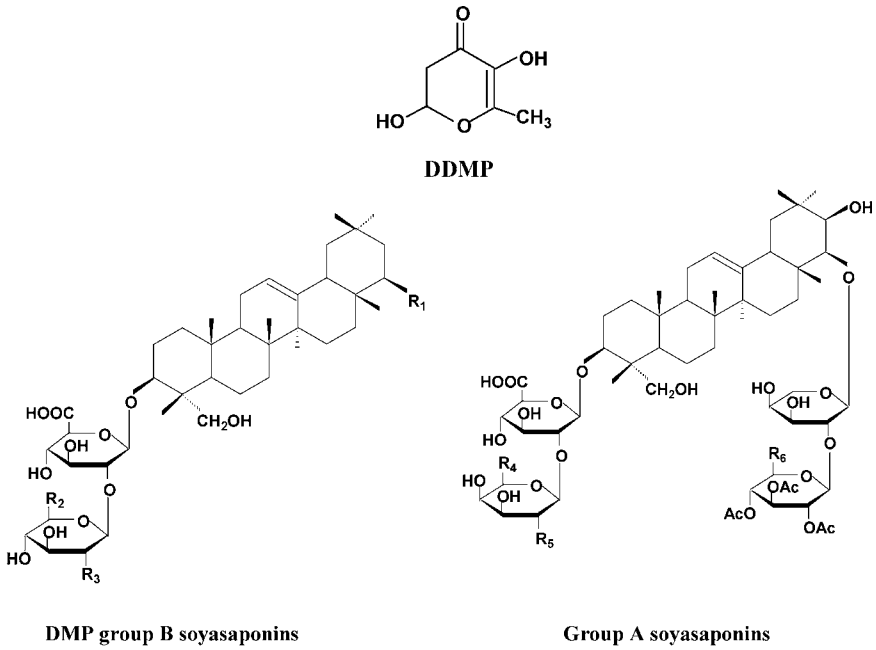
In general, young soybean plants undergo many changes before reaching maturity. A major feature of these changes is the accumulation of key seed chemical components such as proteins, lipids, and sugars. As a result, there is an overall increase in dry matter (*34*−*36*). From the results presented here, we have shown that the free sugars and lipids reach their maximal concentration levels at a relatively early stage of seed development and the level remains constant as compared with other chemical components as the seed matures.

Isoflavones and Soyasaponins. Accumulation of isoflavones daidzein, genistein, and glycitein and their corresponding

Table 3. Soyasaponin Concentration (μg/g) in Korean Soybean Cultivars 'Sojinkong' and 'Daepungkong' during the Reproductive Stages

		group B soyasapon in						
cultivars	RS[a]	A_1	I	II + III	V	αg	βg	total
Sojinkong	R5	512.2 ± 36.1[a]	2102.6 ± 108.9	196.8 ± 11.5	305.5 ± 26.6	866.2 ± 53.7	2511.7 ± 99.3	6495.0 ± 335.8
	R6	527.8 ± 41.1	1854.3 ± 91.3	188.5 ± 7.3	335.8 ± 28.3	663.9 ± 27.6	2415.2 ± 102.1	5985.5 ± 296.5
	R7	238.6 ± 13.5	1051.7 ± 63.2	122.6 ± 9.8	715.0 ± 51.3	505.2 ± 28.3	2387.9 ± 95.4	5021.0 ± 261.0
	R8	157.2 ± 6.7	853.6 ± 41.3	97.3 ± 8.8	462.6 ± 35.2	73.3 ± 2.1	2352.6 ± 95.3	3996.6 ± 189.1
Daepungkong	R5	587.1 ± 27.4	809.2 ± 38.2	206.3 ± 15.3	426.1 ± 33.3	1573.6 ± 10.6	2215.6 ± 101.3	5817.9 ± 226.1
	R6	652.2 ± 25.3	812.9 ± 31.2	173.1 ± 12.7	1733.3 ± 90.3	477.2 ± 22.3	1558.5 ± 69.3	5407.2 ± 250.9
	R7	401.5 ± 11.9	732.1 ± 30.3	250.4 ± 12.3	996.3 ± 50.7	543.7 ± 25.5	1523.6 ± 92.1	4447.6 ± 223.0
	R8	392.0 ± 13.5	668.7 ± 21.7	230.6 ± 16.1	641.5 ± 26.3	585.6 ± 16.8	1631.3 ± 112.3	4149.6 ± 206.9

[a] RS: reproductive stage. [b] Mean ± SD, $n = 3$.



group B soyasaponins	R_1	R_2	R_3
soyasaponin I	OH	CH_2 OH	*O*-β-D-glucose
soyasaponin II	OH	H	*O*-α-L-rhamnose
soyasaponin III	OH	CH_2 OH	OH
soyasaponin IV	OH	H	OH
soyasaponin V	OH	CH_2 OH	*O*-α-L-rhamnose
soyasaponin βg	*O*-DDMP	CH_2 OH	*O*-β-D-glucose
soyasaponin αg	*O*-DDMP	CH_2 OH	*O*-α-L-rhamnose
group A acetyl-saponins	**R_4**	**R_5**	**R_6**
soyasaponin A_1	CH_2 OH	*O*-β-D-glucose	CH_2 OAc

Figure 4. Structures and nomenclature of the soybean saponins. The nomenclature of the group A and B soyasaponins used in this paper is that of Berhow et al. (*23*).

conjugated forms (**Figure 2**) in the R5−R8 stage of maturing soybean seeds is shown in **Figure 3** and **Table 2**.

In this study the concentration of the individual forms of the isoflavones varies greatly among the growth stages and soybean cultivars. As the seed matures, the total isoflavone concentration increases until the R8 stage, and the total isoflavone content is higher in 'Daepungkong' than in 'Sojinkong'. The malonylglucosides are the predominant isoflavone form, followed by glucoside, acetylglucoside, and aglycone forms. The general theory is that the acetyl forms are degradative products formed from the malonate forms, either in the seed or during the course of extraction and processing. It is possible that all isoflavones accumulated in the seed were at one time in the malonyl form. The approximate composition rates of conjugated isoflavones throughout all seed development stages were malonylglucosides (62.5%), glucosides (33.4%), acetylglucosides (3.7%), and aglycones (0.6%).

Recent work suggests that although seeds are the principal site of isoflavone synthesis, some accumulation may be due to transport from other plant organs (*37*) Jung et al. (*38*) and Subramanian et al. (*39*) reported that isoflavone synthase has been shown to be expressed only in embryos and seed coats, not in the developing cotyledons, suggesting that the majority of isoflavones in the cotyledons are transported from other

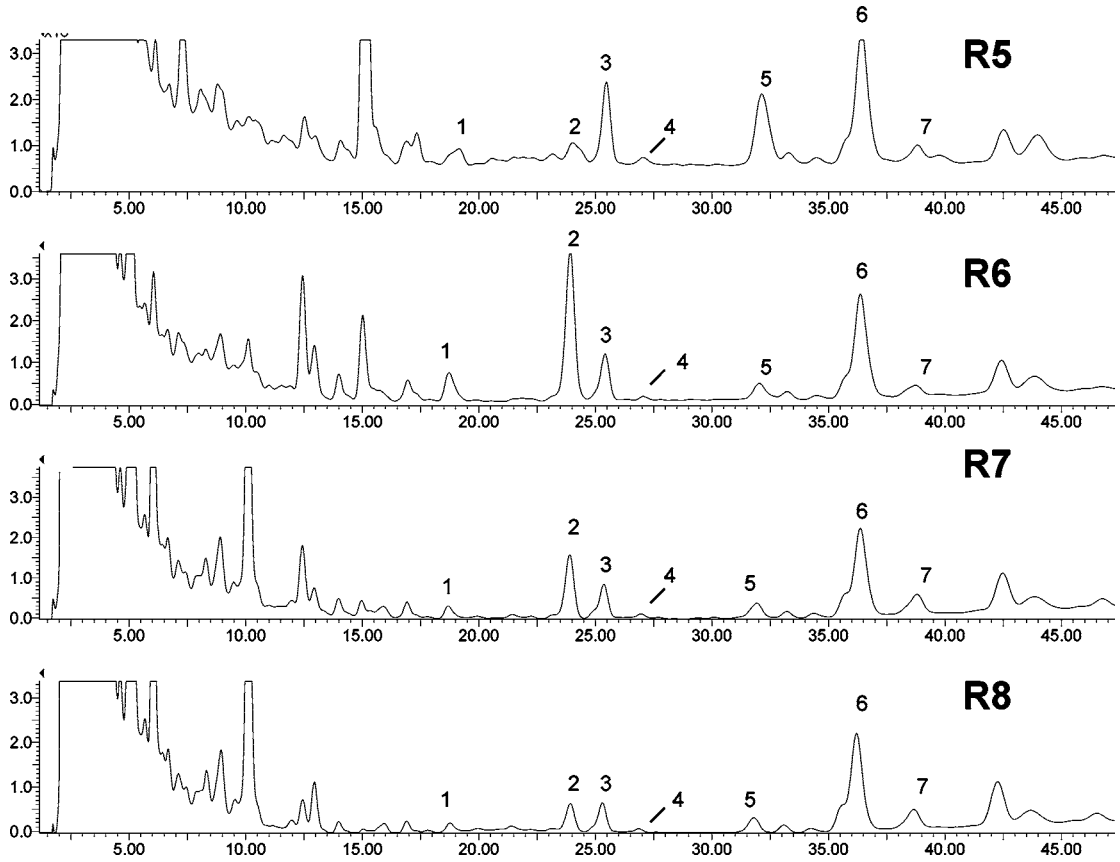


Figure 5. Changing profiles of soyasaponin concentration in the Korean soybean cultivar 'Daepungkong' during the reproductive stages monitored at 210 nm: 1, saponin A_1; 2, saponin I; 3, saponin II + III; 4, saponin V; 5, saponin αg; 6, saponin βg; 7, DDMP moiety.

tissues. However, accumulation of isoflavones in soybean is cultivar dependent and influenced by environmental conditions during the seed filling stage (*4*−*9*).

Results from this study show that the concentration of malonylgenistin, malonyldaidzin, and genistin increases throughout the entire seed development period, whereas the concentration of glycitein, malonylglycitin, acetylglycitin, and acetylgenistin showed little change or decreased slightly during the late stages of seed development (**Table 2**).

Soyasaponin A_1, the DDMP-conjugated group B soyasaponins αg and βg, and the B group soyasaponins, soyasaponin I, II + III, and V **(Figure 4)** were quantified in developing soybean seeds (**Table 3** and **Figure 5**). As soybean seed matures, the total soyasaponin concentration was constantly decreased until the R8 stage. It was noted that soyasaponin βg was the most abundant soyasaponin, followed by soyasaponin I and soyasaponin A_1. The approximate composition of soyasaponins throughout all seed development stages was DDMP-conjugated soyasaponins (53.0%), B group soyasaponins (38.6%), and soyasaponin A_1 (8.4%).

Between the two Korean soybean varieties tested, the soyasaponin concentration of 'Sojinkong was slightly higher than that of 'Daepungkong'.

We observed the ratio of total isoflavones to total soyasaponins in developing soybean seeds during maturity. The ratio increased from 0.06 at the R5 stage to 0.91 at the R8 stage in 'Sojinkong' and from 0.14 at the R5 stage to 1.31 at the R8 stage in 'Daepungkong' (**Figure 6**). This difference is mainly considered to be due to genentic characteristics of the different varieties. 'Sojinkong' has a relatively higher soyasaponin concentration and a lower isoflavone concentration than 'Daepungkong'.

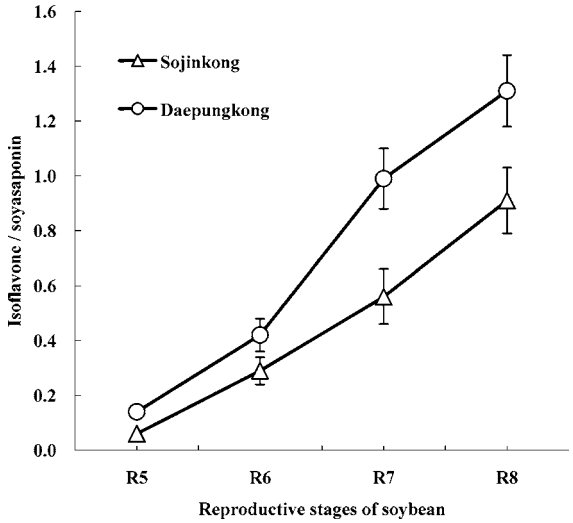


Figure 6. Ratio of total isoflavones to total soyasaponins in developing soybean seeds.

Hu et al. (*40*) reported that isoflavone concentrations were slightly higher than saponin concentrations in raw soybean seeds and that the ratio of total isoflavones to total soyasaponins in the 46 varieties of soybeans tested ranged from 0.9 to 2.9 on a molar basis. The authors also demonstrated that the ratio of total isoflavones to soyasaponins in commercial soy products ranged from 0.2 to 20.4 on a molar basis. Production processes for the type of soy product apparently affects the ratio of isoflavones to soyasaponins, indicating that the changes of isoflavone and soyasaponin contents are independent of each other. We conclude from our results that the variations in the soyasaponin concentration and composition in soybean and soybean ingre-

Table 4. Relationship Among Protein, Lipid, Free Sugar, Isoflavone, and Soyasaponin Concentration in Maturing Soybean Seeds

	isoflavone					soyasoponin		
	aglycone	glucoside	malnoylglucoside	acetylglucoside	T-ISF[d]	A_1	group B	T-SAP[e]
protein	0.461[a]	0.846[b]	0.806[b]	0.887[b]	0.826[b]	–0.852[b]	–0.291[c]	–0.176[c]
lipid	–0.490[a]	0.698[b]	0.681[b]	0.626[b]	0.686[b]	–0.415[c]	–0.714[b]	–0.693[b]
sugar	–0.094[ns]	0.752[b]	0.69[b]	0.731[b]	0.718[b]	–0.449[a]	–0.391[c]	–0.335[c]

[a] Significant at $p < 0.05$ level. [b] Significant at $p < 0.01$ level. [c] Not significant. [d] T-ISF: total isoflavone. [e] T-SAP: total saponin.

dients may depend on the variety of soybean. Therefore, a database of soyasaponin content in soybean seeds would be valuable for evaluating the biological activities of these compounds and clarifying the health-promoting effects of soy products.

Relationships between the Chemical Components in Isoflavones and Soyasaponins. On the basis of these results, we tried to develop an understanding of the ratio relationships among the protein, lipid, and free sugars, isoflavones, and soyasaponins in developing soybean seeds.

As shown in **Table 4**, the protein and lipid contents in maturing soybean seeds showed significant positive correlations with conjugated isoflavones and total isoflavone contents, while lipid contents showed a negative correlation with the isoflavone aglycone ($r = -0.490$, $p < 0.05$).

Free sugar contents had a positive correlation with the isoflavone glucosides, malonylglucosides, and acetylglucosides as well as with total isoflavone concentration ($r = 0.752$, 0.696, 0.731, and 0.718 at $p < 0.01$, respectively). However, the aglycones did not have a significant correlation with isoflavones ($r = -0.094$).

Statistical analyses of correlations among protein, lipids, and free sugars with isoflavones and soyasaponins in the developing soybean were conducted to determine whether these components affected the relative content of soyasaponins. Results represented here show that protein, lipid, and free sugar contents showed a negative correlation with soyasaponin A, B, and total soyasaponins. However, it was noted that in the relationship between free sugars and soyasaponins, only soyasaponin A was significantly negatively correlated with free sugars. This suggests that the free sugar content in developing soybean seeds affects the isoflavone content to a greater extent than total soyasaponins.

Hu et al. (*40*) found no statistically significant correlation between total isoflavone concentration and six group B saponins in 46 cultivars of soybeans ($r = 0.1285$, $P > 0.05$). Similarly, Rupasinghe et al. (*41*) found no apparent relationship ($r^2 = 0.057$) between the distribution of isoflavone and soyasaponins in the 10 cultivars tested. In the present study, the isoflavone concentrations in developing soybean seeds were compared with soyasaponin concentrations to determine whether there was any correlation. As shown in **Figure 7**, isoflavone concentration was negatively correlated with soyasaponins ($r = -0.828$, $p < 0.01$). However, biosynthesis of isoflavone and soyasaponin occurs through two distinct metabolic pathways: the phenylpropanoid pathway (*38*) and the isoprenoid pathway (*42*). Therefore, there is probably not any close metabolic coordination between the two biosynthetic pathways for these two groups of secondary metabolites.

In order to elucidate the physiological role of saponins in plants, it is necessary to understand their biosynthetic pathways and identify the regulatory mechanism of their metabolism. Information about the biosynthesis of triterpene has been provided (*41*), but little is known about the sugar transferase reactions for triterpene and sugar-chain biosynthesis. From the obtained results in this study, it is suggested that an accurate characterization of the relationship between isoflavone and soyasaponin biosynthesis in maturing soybean seeds is important to understand the interactions concerned as well as improve soybean quality and utilization.

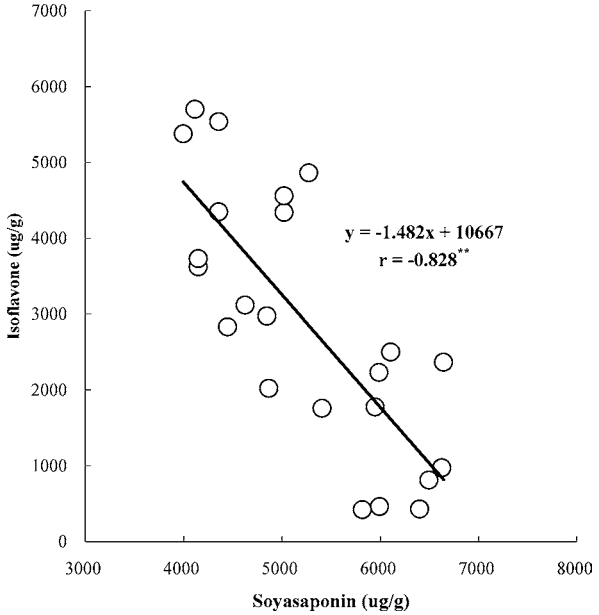


Figure 7. Relationship between isoflavone and soyasaponin concentration in developing soybean seeds.

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