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Soyasapogenol A and B Distribution in Soybean (*Glycine max* L. Merr.) in Relation to Seed Physiology, Genetic Variability, and Growing Location

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An efficient analytical method utilizing high-performance liquid chromatography (HPLC)/evaporative light scattering detector (ELSD) was developed to isolate and quantify the two major soyasaponin aglycones or precursors in soybeans, triterpene soyasapogenol A and B. Soaking of seeds in water up to 15 h did not change the content of soyasapogenols. Seed germination had no influence on soyasapogenol A content but increased the accumulation of soyasapogenol B. Soyasapogenols were mainly concentrated in the axis of the seeds as compared with the cotyledons and seed coat. In the seedling, the root (radicle) contained the highest concentration of soyasapogenol A, while the plumule had the greatest amounts of soyasapogenol B. In 10 advanced food-grade soybean cultivars grown in four locations in Ontario, total soyasapogenol content in soybeans was 2 ± 0.3 mg/g. Soyasapogenol B content (1.5 ± 0.27 mg/g) was 2.5−4.5-fold higher than soyasapogenol A content (0.49 ± 0.1 mg/g). A significant variation in soyasapogenol content was observed among cultivars and growing locations. There was no significant correlation between the content of soyasapogenols and the total isoflavone aglycones.

KEYWORDS: Soybean; *Glycine max*; soyasaponin; soyasapogenol; triterpene aglycones; HPLC/ELSD; isoflavones; germination; soaking

INTRODUCTION

The presence of saponins in soybean has attracted considerable interest because of both their health benefits and adverse sensory characteristics. Soybean saponins are triterpenoid glycosides and comprise a hydrophobic aglycone (triterpenoid soyasapogenol) linked to one or more hydrophilic mono- or oligosaccharide moieties (*1*). Soyasaponins are classified into two major groups, soyasaponin A and B (**Figure 1**) (*2*). Group A acetylated saponins, present in soybean, are implicated as the phytochemicals mostly responsible for undesirable bitter and astringent taste (*3*, *4*). However, group B saponins, including 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP)-conjugated saponins, possess several health benefits (*5*, *6*). They appear to possess inhibitory activity against infection by human immunodeficiency virus (HIV) (*7*) and the activation of Epstein−Barr virus early antigen (*8*). Soyasaponin B_1 in particular is a potent inhibitor of HIV infection in vitro and offers great potential in the treatment of retroviral infections (*7*). Recent in vitro studies suggest that group B saponins also possess hypocholesterolemic, immunostimulatory, anticarcinogenic, antioxidative, antitumor, antivirus, antihepatitic, antidiabetic, and hepatoprotective properties (*9*). Dietary saponins of soybean are beneficial in preventing hypercholesterolemia and aortic atherosclerosis in rats (*10*).

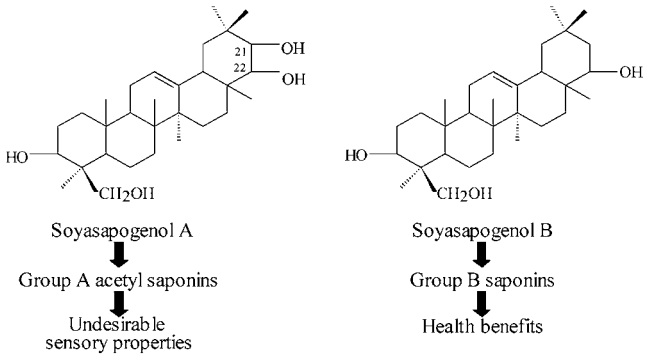

Figure 1. Chemical structures of soyasaponin precursors soyasapogenol A and B.

Group A saponins appear to be a naturally occurring form, and Shiraiwa et al. (*11*) identified six different group A saponins, designated as Aa, Ab, Ac, Ad, Ae, and Af, according to their

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elution order from high-performance liquid chromatography (HPLC). However, acid hydrolysis of all six saponin A compounds yielded the common aglycone, soyasapogenol A. Characteristically, in all of these saponins, the terminal sugar of the oligosaccharide chain, attached to the C-22 position of the soyasapogenol A, is acetylated (*11*). Ireland and Dziedzic (*12*) proposed that soyasapogenols C, D, and E are formed as artifacts during acid hydrolysis. In contrast, Tsukamoto et al. (*13*) recognized soyasapogenol E as the aglycone of the third type of saponin, saponins Bd and Be. However, Bd and Be saponins are heat labile and thus are presumed to be transformed into soyasapogenol B during acid hydrolysis (*5*). The other soyasaponins, which have been isolated and designated as soyasaponins I, II, III, and IV, contain soyasapogenol B as the common aglycone (*14*). Structurally, soyasapogenol A possesses only an additional hydroxyl group at the C-21 position as compared to soyasapogenol B (**Figure 1**).

The chemical structures of more than 20 saponins from soybeans and various soy products have been determined (*15*). However, it is now apparent that many of these reported soyasaponins are artifacts formed due to alteration of the chemical structures of the naturally occurring saponins during extraction and analysis. Despite a great deal of analytical research on soyasaponins, distinguishing and quantifying the two major groups of soyasaponins (group A and B saponins) has been hampered by the complex nature of the analytical procedures due to the lack of intact glucoside forms of soyasaponins (especially group A saponins) for use as standards for quantitative determination. Therefore, in this study, we have developed a rapid analytical technique to quantify the precursors of the group A and B saponins, soyasapogenol A and B, respectively. The total soyasaponin content is approximately twice the total soyasapogenol content (*16*).

Seeds of soybean contain about 0.5% of soyasaponins (dry weight basis) depending on the variety, cultivation year, location grown, and degree of maturity (*11*, *17*). Germination has been suggested to enhance the overall nutritional value of the seeds and the contents of health-promoting phytochemicals, including saponins (*18*), ascorbic acid, and riboflavin (*19*). To further characterize the major groups of soyasaponins in soybeans, we developed a rapid analytical technique to quantitate soyasapogenols A and B and determined their distribution (i) in seeds and seedlings, (ii) during seed soaking and germination, and (iii) among advanced food-grade soybean cultivars.

MATERIALS AND METHODS

Plant Materials. For the histological and germination studies, three advanced food-grade soybean cultivars of the 2001 harvest were selected from a commercial soybean breeder (First Line Seeds Ltd., Guelph, Ontario, Canada). Cultivar 41102-A has a higher sugar content and the ability to produce better tasting soyfoods such as soymilk. Cultivar 41102-B has large white seeds of excellent quality, high protein, and ability to produce firm, smooth, white tofu. Cultivar 41102-C has a very high protein content (approximately 49% on dry weight basis).

For the comparison of soybean cultivars, 10 advanced food-grade ones (S20-F8, CL970321, 7025308, 9910, 2004, AC X790P, Harovinton, AC Vin-Pro, AC Hime, and 9305) were selected. They were grown at four different geographical locations (Chatham, Malden, Tilbury, and Woodslee) in Ontario, Canada, during the 1999 season. Standard three replicate randomized complete block yield trails were grown at each location following normal agronomic practices.

Chemicals. Concentrated hydrochloric acid, glacial acetic acid, HPLC-grade acetonitrile, and methanol were purchased from Caledon (Mississauga, Ontario, Canada). 1-Propanol was a product of Fisher Scientific, and 95% ethanol was purchased from Commercial Alcohol Inc., Toronto, Ontario, Canada. Deionized water was generated from a Milli-Q analytical deionization system.

Seed Soaking and Germination. Seed soaking and germination studies were conducted in triplicate, each replicate containing approximately 3 g of seeds. Each replicate was soaked separately in a beaker at room temperature with 200 mL of distilled water, with aeration. For the germination studies, seeds were soaked for 2 h before being transferred into glass Petri dishes (15 cm diameter) with prewetted, sterilized cotton. Seeds were spread evenly, and distilled water was added periodically and incubated at 25 ± 1 °C.

After the designated soaking or germination period, excess water was drained, and the seeds were immersed in liquid nitrogen and then stored at −70 °C until used for freeze-drying. Samples were freeze-dried for 48 h using a Labconco freeze-dry system (10×10^{-3} mbar, −40 °C) (Caltec Scientific Ltd., Toronto, Ontario, Canada) and ground immediately into a fine powder with a mortar and pestle. The ground powder was stored in a desiccator at −70 °C until used for the HPLC analysis.

To determine the distribution of soyasapogenols within seeds and seedlings, approximately 15 g lots of seeds (in triplicate) were soaked in distilled water for 10 h with aeration before being separated into axis, seed coat, and cotyledons. For seedling parts, seeds were germinated and grown for 120 h before dissection. The seeds and seedling parts were freeze-dried and ground into a fine powder as described before.

Extraction of Saponins and Analysis of Soyasapogenols A and B. The isolation of saponins from soybean was accomplished by a modification of the method described by Daveby et al. (*20*). Finely ground soybean powder (0.2 g) was dissolved in 30 mL of 80% (v/v) aqueous ethanol in a round-bottom flask (125 mL) with consistent mixing in a horizontal water bath/shaker (SW22 model, Julabo Inc., Allentown, PA) at 50 °C for 2 h. The residue was removed by centrifuging the extract at 3000 rpm for 10 min using a bench centrifuge at room temperature and decanting the clear supernatant. Fifteen milliliters of the supernatant was dried under reduced pressure using a rotary vacuum evaporator system (Buchi B-481 model, Brinkmann Instruments, Palo Alto, CA), and the remaining residue was redissolved in 8 mL of 1 N HCl in anhydrous methanol. The resuspension was transferred to a screw-capped glass vial and was subjected to acid hydrolysis at 75 °C for 2.5 h in a horizontal water bath/shaker to release the aglycones (soyasapogenols) from soyasaponins. The soyasapogenols produced were isolated by solid phase extraction using a Vac 6CC 500 mg C-18 Sep-Pak cartridge (Waters, Marlborough, MA) by eluting the 8 mL of solution through the cartridge, washing the cartridge with 3 mL of water, and eluting the soyasapogenols with 100% methanol. The eluent was filtered through a 0.2 μm nylon filter (Waters Chromatography, Mississauga, ON) and a 25 μL aliquot separated by reverse-phase (RP) HPLC using a Shimadzu 10AD HPLC system consisting a SCL-10A system controller, SIL-10A autoinjector (Shimadzu Scientific Instruments, Columbia, MD) and an evaporative light scattering detector (ELSD) (Alltech ELSD 2000 model, Alltech Assoc., Deerfield, IL).

Soyasapogenols A and B were well-resolved using a 250 mm long × 4.6 mm i.d. ODS C_{18} column with a C_{18} guard column (Phenomenex, Torrance, CA) at a flow rate of 0.9 mL/min pumped isocratically with a mobile phase consisting of acetonitrile:1-propanol:water:0.1% acetic acid (80:6:13.9:0.1). The detection of soyasapogenols was performed using the ELSD, which was set to the drift tube temperature of 70 °C, and the nebulizer nitrogen gas flow was adjusted to 2 mL/min. Soyasapogenol A and B standards (ChromaDex, Santa Ana, CA) were initially dissolved in 100% methanol (1000 μg/mL) and stored at −70 °C in a desiccator. Standard curves were obtained by plotting standard concentrations (soyasapogenol A, 12.5, 25, 50, 75, 100, and 150 μg/mL; soyasapogenol B, 37.5, 75, 150, 225, 300, and 450 μg/mL) as a function of peak area in HPLC chromatograms. Quadratic calibration curves ($r^2 > 0.98$) were generated by the Shimadzu Class VP software. The quantification of soyasapogenol A ($R_t = 5.9$ min) and soyasapogenol B ($R_t = 9.9$ min) was performed on the basis of the peak area of chromatograms created by ELSD in comparison to the standard curves of authentic external standards of soyasapogenols A and B.

HPLC Analysis of Three Isoflavone Aglycones. Analysis of daidzein, glycitein, and genistein was performed by a modification of

Table 1. Efficiency of Different Extraction Methods on the Recovery of Soyasapogenol A and B

extraction[a]	acid hydrolysis[b]	detector response (10^{-3} × area under the curve) soyasapogenol A	soyasapogenol B	artifacts (unknowns)
100% methanol	1 N HCl	45 ± 0.5	164 ± 12	11 ± 3
80% ethanol	1 N HCl	46 ± 0.4	224 ± 16	151 ± 0.9
80% ethanol	evaporate extract, add 1 N HCl in anhydrous methanol	85 ± 0.7	310 ± 12	0
80% ethanol	evaporate extract, add 1 N H_2SO_4 in anhydrous methanol	71 ± 0.3	240 ± 13	0
80% ethanol	evaporate extract, add 1 N HCl in dioxane	65 ± 0.4	227 ± 11	0

[a,b] All of the extractions (50 °C for 2 h) and acid hydrolysis (75 °C for 2.5 h) were as described in the Materials and Methods.

the method described by Franke et al. (*21*). Approximately 0.5 g of finely ground soybean powder was added into 50 mL glass vials. Simultaneous extraction of isoflavones and acid hydrolysis to their respective aglycones was carried out by mixing with 12 mL of 2 N HCl in 100% ethanol and incubating at 125 °C for 2 h. After acid hydrolysis, the samples were centrifuged at 3000 rpm for 10 min in a bench centrifuge at room temperature. An aliquot of the clear supernatant was filtered through a 0.45 mm nylon filter before being introduced into the HPLC consisting of a Waters 600E multisolvent delivery system, 717 plus auto sampler, 996 photodiode array detector monitoring at 200−350 nm or a Waters 486 tunable absorbance UV detector set at 254 nm and Millennium (version 2.10) chromatography software. The column used was a 250 mm long × 3.9 mm i.d. Waters Nova Pak C-18 with a C-18 guard column. All HPLC analyses were performed at ambient temperature. A Hewlett-Packard 8452A diode array spectrophotometer was used to verify the concentrations of stock standards (daidzein, glycitein, and genistein) (Indofine Chemical, Somerville, NJ). The mobile phases for HPLC consisted of solvent (A) 4.0% acetic acid in filtered MilliQ water and (B) 100% methanol. The solvent gradient was as follows: solvent B was increased from 40 to 65% over 10 min, then run constantly for the next 11 min, and finally decreased back to 40% within 9 min and held for another 5 min before the next injection. The flow rate was 1 mL/min. The minimum detectable concentrations for genistein and daidzein were 100 and 185 ng/mL, respectively. UV spectra were recorded, and area responses were integrated by Waters software.

Statistical Analysis. Statistical analysis of variance in a complete randomized design was performed using PROC GLM procedure of the SAS System version 8e for Windows. Mean separations were examined using Tukey's Studentized range test (*t*-test).

RESULTS AND DISCUSSION

Isolation and Determination of Soyasaponins. A primary goal of the research was to develop an analytical technique to determine the two major saponin components (soyasapogenols A and B) in soybeans. In the literature, data on the quantification of saponin precursors (soyasapogenols) are contradictory because of the formation of artifacts (soyasapogenols C, D, E, and F) due to acid hydrolysis during their preparation. In previous studies, UV detection at the wavelength of 204 nm was commonly used, but this is inefficient due to the lack of prominent chromophores for soyasapogenols. Quantification of the two major groups of soyasaponins (group A and B saponins) has been hampered also by the complex nature of the analytical procedures and the lack of intact glucoside forms of soyasaponins (especially group A saponins) to use as standards for quantitave determination. Recently, Gurfinkel and Rao (*2*) proposed a rapid analytical method to quantify total soyasaponins based on direct densitometry, but this method is not able to distinguish the two major groups of saponins.

In the present study, the alternative use of ELSD was successfully employed to characterize and quantitate soyasapogenols A and B with very high resolution and high sensitivity. ELSD is based on the scattering of light by the nebulized solutes without absorption by the analytes. This technique has been used successfully in the determination of other saponins (*22*).

On the basis of previously reported extraction methods for saponins, five extraction protocols were compared for the extraction recovery of soyasapogenols (**Table 1**). Extraction in 80% aqueous ethanol produced the highest recovery of soyasapogenols when compared with 100% methanol (**Table 1**). The amount of soyasapogenols obtained from soybean extracts (200 mg of soybean in 30 mL of 80% ethanol) was optimized by incubating them for 2 h at 50 °C. Multiple extractions were performed to determine the extraction efficiency for soybeans, and the third successive reextraction yielded undetectable amounts of soyasapogenols A and B. A single extraction was able to extract over 96% of total soyasapogenols from soybean powder. Before acid hydrolysis of soyasaponins to remove the sugar moieties, the ethanolic extract was evaporated under reduced pressure at 40 °C, and the residue was dissolved in 1 N HCl in anhydrous methanol before hydrolysis at 75 °C for 2.5 h. This procedure yielded the highest recovery of soyasapogenols A and B without forming artifacts. Performing acid hydrolysis with the same strength of H_2SO_4 produced less soyasapogenols. HCl−dioxane has been used successfully for acid hydrolysis of soybeans (*23*) and other legume (*Pueraria lobata* L.) triterpene saponins (*24*), but it was less efficient for hydrolysis of soyasaponins than HCl−methanol.

Similarly to the present finding, Daveby et al. (*20*) observed higher and more stable extraction of DDMP-conjugated soyasaponin I from dehulled peas (*Pisum sativum* L.) using 80% ethanol, as compared to 100% methanol. Most of the previously reported methods for extractions of saponins included defatting of soybean powder for at least 36 h in methanol using a Soxhlet apparatus (*3*, *14*). However, we found that defatting soybean powder using a Soxhlet extraction (140 °C in hexane for 1 h) prior to saponin extraction reduced the recovery of soyasapogenols by about 25% as compared to nondefatted samples.

Tsukamoto et al. (*13*) classified soyasapogenol E as a third aglycone moiety of soyasaponins, Bd and Be. In the present study, while ELSD could detect the authentic soyasapogenol E, neither this nor any other related compound was detected in the extracts of soybeans. Soyasaponins Bd and Be are heat labile and could have been transformed into soyasapogenol B during acid hydrolysis (*5*). However, when soyasapogenol E was subjected to acid hydrolysis, it was not converted to soyasapogenol B or degraded. Therefore, under the present extraction and hydrolysis conditions, intact soyasaponin E could be converted to soyasaponin B and then hydrolyzed into soyasapogenol B. Alternatively, under the previously reported rigorous defatting, extraction, and acid hydrolysis conditions (*3*, *14*), soyasapogenol E may have formed due to the pinacol rear-

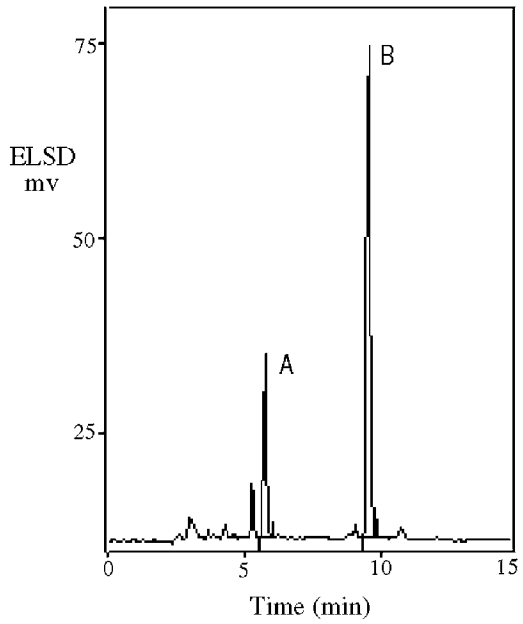

Figure 2. Representative HPLC/ELSD chromatogram of extract from the soybean. Peaks: A, soyasapogenol A; B, soyasapogenol B.

rangement of soyasapogenol A (Robert Lange, University of Guelph, personal communication). The present results supported the notion that soyasapogenols C, D, E, and F were artifacts of the extraction and hydrolysis procedure employed (*12*).

The two soyasapogenols (A and B) were well-resolved with the retention time difference of 4 min (**Figure 2**). The retention time and relative standard deviation (RSD) values for measured soyasapogenols A and B were 5.9 (RSD = 1.2%, $n = 18$) and 9.9 min (RSD = 1.8%, $n = 18$), respectively, reflecting an acceptable precision. The within day RSD values for detection of soyasapogenols A and B were 5.6 ($n = 6$) and 7.4 ($n = 6$), respectively. The peak identity was determined by spiking soybean extracts with known amounts of authentic analytes and observing the peak overlap of the resultant chromatograms. For validating analytical accuracy, 200 mg portions of a reference sample ("control" soybean powder) were spiked with three different concentrations of authentic soyasapogenol A and soyasapogenol B for the calculation of percent recovery. The overall mean recoveries of soyasapogenols A and B were 104.7 and 102.7%, respectively. The limits of quantitation for soyasapogenols A and B were 0.06 and 0.08 mg/g, respectively. Thus, the method described here can be used as a rapid analytical tool to determine soyasapogenols A and B content and to estimate approximate total saponin content in commercial soybean food products such as soya protein extracts and soybean feed.

Effect of Soaking and Germination on Soyasapogenol Content. The total soyasapogenol content was significantly different between the seeds of the three soybean cultivars employed. The seed size of cultivar 41102-B was larger than the other two, but it contained the lowest concentration of total soyasapogenols on a whole seed basis.

Soyasapogenols A and B content was not affected by soaking seeds in water for up to 15 h in the three cultivars tested (**Table 2**). Thus, hydration of seeds does not result in any net biosynthesis of soyasapogenols nor a net leaching of soyasapogenols from seeds. Similarly, Ruiz et al. (*25*) reported no influence of soaking on soyasaponin VI content of two other legumes, chickpeas (*Cicer arietinum* L.) and lentils (*Lens culinaris* L.).

Table 2. Effect of Soaking Soybean Seeds on Their Soyasapogenol A and B Content

duration of soaking (h)	cultivars		
	41102-A	41102-B	41102-C
	soyasapogenol A (mg/g dry weight)		
0	0.33 ± 0.14	0.21 ± 0.02	0.73 ± 0.15
5	0.32 ± 0.08	0.24 ± 0.05	0.74 ± 0.07
10	0.36 ± 0.06	0.28 ± 0.04	0.75 ± 0.08
15	0.39 ± 0.03	0.25 ± 0.02	0.71 ± 0.05
	soyasapogenol B (mg/g dry weight)		
0	1.18 ± 0.05	0.68 ± 0.19	1.5 ± 0.27
5	1.19 ± 0.11	0.87 ± 0.39	1.55 ± 0.05
10	1.21 ± 0.17	0.82 ± 0.13	1.55 ± 0.19
15	1.29 ± 0.19	0.92 ± 0.15	1.54 ± 0.07

Table 3. Effect of Early Seedling Growth on the Concentration of Soyasapogenol A and B

duration of imbibition (h)	cultivars		
	41102-A	41102-B	41102-C
	soyasapogenol A (mg/g dry weight)		
0	0.33 ± 0.14	0.21 ± 0.02	0.73 ± 0.15
20	0.32 ± 0.11	0.24 ± 0.01	0.78 ± 0.14
40	0.34 ± 0.03	0.26 ± 0.04	0.72 ± 0.12
60	0.39 ± 0.03	0.22 ± 0.08	0.73 ± 0.08
	soyasapogenol B (mg/g dry weight)		
0	1.22 ± 0.19	0.78 ± 0.04	1.5 ± 0.28
20	1.27 ± 0.25	0.88 ± 0.07	1.47 ± 0.22
40	1.50 ± 0.16	1.14 ± 0.28	1.71 ± 0.21
60	1.57 ± 0.12	1.23 ± 0.46	1.81 ± 0.04

Seed germination and subsequent seedling growth have a marked effect on the chemical composition, biochemical constituents, antinutritional factors, and functional properties of soybean (*26*). Development of food products from germinated seed has been suggested as a way to increase the versatility and utility of soybean through the in situ modification of certain specific biologically active compounds including saponins, phytoestrogens, lecithin, and phytosterols (*18*). Our findings indicate that during seedling growth there is a differential effect on soyasapogenols A and B content. Soyasapogenol A content was not affected for up to 60 h from the start of imbibition, but soyasapogenol B content increased after 60 h by 32% (**Table 3**). Germination was completed relatively faster in cultivar 41102-C (after approximately 20 h) as compared to 36 h in the other cultivars, but there was no statistically significant interaction of soyasapogenol content between cultivar and germination period.

Shimoyamada and Okubo (*27*) found that germination and growth of soybean for 8 days increased seed soyasaponin content 8-fold. Amaranthus (*Amaranthus cruentus* L.) seeds contain 0.09−0.1% (dry matter basis) triterpene saponins, and an increase to 0.18% was observed after 4 days of seedling growth (*28*). In contrast, Duhan et al. (*29*) found that soaking for up to 18 h and germination/seedling growth for up to 48 h reduced the saponin content in four cultivars of pigeon pea (*Cajanus cajan* L.), another grain legume. Loss of saponin during soaking and germination of this legume species was due to leaching out of saponin into the soaking water solution through diffusion (*29*).

Soyasapogenol Distribution in Seed and Seedling Parts. The ratio of soyasapogenol A in axis:cotyledon was 40:1, whereas that of soyasapogenol B was 9:1 (**Table 4**). The total soyasapogenol concentration was 14-fold greater in the axis (15.6 mg/g dw) as compared to that in cotyledons (1.1 mg/g

Table 4. Distribution of Soyasapogenol A and B in the Seed Parts of Three Soybean Cultivars

cultivar	seed part	dry weight (g)	dry weight ratio (%)	concentration mean ± SD (mg/g dry weight)		total amount (mg)		% distribution among seed parts	
				soyasapogenol A	soyasapogenol B	soyasapogenol A	soyasapogenol B	soyasapogenol A	soyasapogenol B
41102-A	axis	0.35	7.0	8.0 ± 0.13	8.4 ± 0.65	2.8	2.94	83.6	47.1
	seed coat	1.08	21.7	0.045 ± 0.001	0.083 ± 0.011	0.049	0.089	1.5	1.4
	cotyledons	3.55	71.3	0.14 ± 0.004	0.9 ± 0.097	0.497	3.195	14.9	51.5
	total	4.98	100	8.185	9.383	3.346	6.225	100	100
41102-B	axis	0.32	7.6	7.3 ± 0.2	5.3 ± 0.58	2.336	1.696	86.6	45.7
	seed coat	0.99	23.6	0.015 ± 0.002	0.046 ± 0.016	0.015	0.045	0.6	1.2
	cotyledons	2.89	68.8	0.12 ± 0.002	0.68 ± 0.001	0.347	1.965	12.8	53.1
	total	4.2	100	7.435	6.026	2.698	3.706	100	100
41102-C	axis	0.42	8.9	7.6 ± 0.98	10.2 ± 0.59	3.192	4.284	75.9	55.5
	seed coat	1.18	25.2	0.016 ± 0.001	0.013 ± 0.002	0.019	0.0153	0.5	0.2
	cotyledons	3.08	65.9	0.32 ± 0.03	1.1 ± 0.129	0.986	3.388	23.6	44.3
	total	4.68	100	7.936	11.313	4.196	7.687	100	100

Table 5. Distribution of Soyasapogenol A and B in the Radicle, Plumule, and Cotyledons of 120 h old Seedlings in Three Soybean Cultivars

cultivar	seedling part	dry weight (g)	dry weight ratio (%)	concentration mean ± SD (mg/g dry weight)		total amount (mg)		% distribution among seedling parts	
				soyasapogenol A	soyasapogenol B	soyasapogenol A	soyasapogenol B	soyasapogenol A	soyasapogenol B
41102-A	radicle	1.64	26.3	1.45 ± 0.05	3.22 ± 0.06	2.378	5.281	65.1	26.9
	plumule	0.21	3.4	0.22 ± 0.006	11.22 ± 1.38	0.046	2.356	1.3	12.0
	cotyledons	4.39	70.3	0.28 ± 0.01	2.73 ± 0.16	1.229	11.985	33.6	61.1
	total	6.24	100	1.95	17.17	3.653	19.622	100	100
41102-B	radicle	1.56	26.8	1.49 ± 0.07	3.13 ± 0.17	2.324	4.883	70.1	28.7
	plumule	0.29	5.0	0.15 ± 0.008	10.92 ± 0.28	0.044	3.167	1.3	18.6
	cotyledons	3.96	68.2	0.24 ± 0.027	2.26 ± 0.02	0.95	8.95	28.6	52.7
	total	5.81	100	1.88	16.31	3.318	17.0	100	100
41102-C	radicle	1.67	25.5	2.17 ± 0.057	4.23 ± 0.14	3.624	7.664	55.8	30.7
	plumule	0.38	5.8	0.22 ± 0.023	10.53 ± 1.29	0.084	4.001	1.3	16.0
	cotyledons	4.49	68.7	0.62 ± 0.027	2.96 ± 0.043	2.784	13.29	42.9	53.3
	total	6.54	100	3.01	17.72	6.492	24.955	100	100

dw) (**Table 4**). However, once the dry weight ratios of seed part to total seed weight were taken into consideration, the percentage distribution of total soyasapogenols among axis, seed coat, and cotyledons was 62, 0.8, and 37.2%, respectively.

Similar to the present finding, Shimoyamada et al. (*30*) reported that two metabolites of soyasapogenol A, acetyl-soyasaponins A_1 and A_4, occur only in immature hypocotyls of soybean seed. Using HPLC of fluorescent derivatives of the saponins, Tani et al. (*31*) reported that soyasaponins, except soyasaponin II, are located predominantly in the axis of seed (plumule, hypocotyl, and radicle), with the content of total soyasaponins in the axis being about six times higher than that in the cotyledons. In the above study, soyasaponins were not detected in the seed coat but the axis accounted for about 90% of the whole grain weight (*31*). It is evident that soyasapogenols are synthesized in the physiologically active axis of the seed and perhaps some of them translocate to the cotyledons. Kudou et al. (*23*) reported that soyasaponin αg is present only in hypocotyls, soyasaponin βa in the cotyledons, and soyasaponin βg in both parts. In addition, ginsenoside, the predominant saponin compound in ginseng (*Panax ginseng* L.), is also localized in specific tissues in roots (*31*). Unlike saponins, 80−90% of total seed isoflavones are located in the cotyledons, with the reminder in the hypocotyls (*32*).

Dehulling of soaked soybean seeds is a common practice during soybean processing for soymilk and other soy food manufacturing. However, the present results suggest that such a practice could affect the nutritional value of soy foods since important health-promoting phytochemicals including saponins are located in the axis, which is removed through dehulling.

Distribution of soyasapogenols A and B was also determined in the radicle, plumule, and cotyledons in 120 h old seedlings (**Table 5**). Soyasapogenol concentration in cotyledons of seedlings was greater as compared to that in seeds due to the reduced dry matter content, which resulted from mobilization and utilization of stored carbohydrates, proteins, and oils from cotyledons by the embryonic axis during the seedling development (*19*). A distinctly different distribution of soyasapogenols A and B in the radicle and plumule was noted. Soyasapogenol A concentration was about 9-fold higher in the radicle as compared to that in the plumule. In contrast, soyasapogenol B concentration was about 3-fold higher in the plumule than the radicle. With regard to the total soyasapogenol distribution within the seedling, they were more abundant (approximately 2-fold) in the radicle than in the plumule (**Table 5**).

Distribution of Soyasapogenol among Food-Grade Soybean Cultivars. The concentrations of soyasapogenols A and B were determined in 10 selected food-grade soybean cultivars, which were grown in four locations (**Table 6**). The total soyasapogenol content in food-grade soybeans ranged from 1.43 to 2.65 mg/g, or an average of 0.2% "as is" weight basis. The concentration of soyasapogenol B (1.5 ± 0.27 mg/g) was 2.5−4.5-fold higher than the concentration of soyasapogenol A (0.49 ± 0.1 mg/g) in all of the cultivars tested. The analysis of variance compared the 10 cultivars in four locations. The concentrations of soyasapogenols A and B were influenced by both cultivars ($p < 0.001$) and by location ($p < 0.001$). Mean soyasapogenol A content among cultivars ranged from 0.34 to 0.60 mg/g, while mean soyasapogenol B content ranged from 1.19 to 1.88 mg/g (**Table 6**). Hu et al. (*33*) found that the total

Table 6. Distribution of Soyasapogenol A and B in Ten Food-Grade Soybean Cultivars Grown in Four Locations of Ontario, Canada[a]

cultivars	growing location				mean ± SD[a]
	Chatham	Malden	Tilbury	Woodslee	
	soyasapogenol A (mg/g)				
S20-F8	0.36	0.31	0.40	0.28	0.34 ± 0.05^{d}
CL970321	0.55	0.51	0.75	0.53	0.59 ± 0.11^{ab}
7025308	0.28	0.36	0.50	0.36	0.38 ± 0.09^{cd}
9910	0.29	0.51	0.69	0.42	0.48 ± 0.17^{abc}
2004	0.47	0.59	0.50	0.47	0.51 ± 0.06^{ab}
ACX790P	0.50	0.54	0.54	0.43	0.50 ± 0.05^{ab}
Harovinton	0.38	0.46	0.60	0.45	0.47 ± 0.10^{bc}
AC Vin-Pro	0.43	0.44	0.63	0.41	0.48 ± 0.10^{bc}
AC Hime	0.64	0.59	0.65	0.41	0.60 ± 0.07^{a}
9305	0.54	0.47	0.62	0.47	0.53 ± 0.07^{ab}
	soyasapogenol B (mg/g)				
S20-F8	1.57	1.38	1.39	1.70	1.51 ± 0.15^{bcd}
CL970321	1.50	1.40	1.56	1.80	1.57 ± 0.17^{bc}
7025308	1.33	1.23	1.48	1.73	1.44 ± 0.22^{bcde}
9910	1.24	1.63	1.66	1.54	1.52 ± 0.19^{bcd}
2004	1.82	1.91	1.69	2.09	1.88 ± 0.17^{a}
ACX790P	1.28	1.38	1.25	1.48	1.35 ± 0.11^{cde}
Harovinton	1.22	0.98	1.18	1.40	1.19 ± 0.17^{e}
AC Vin-Pro	1.34	1.01	1.22	1.42	1.25 ± 0.18^{de}
AC Hime	1.56	1.41	1.45	2.07	1.62 ± 0.3^{abc}
9305	1.72	1.37	1.48	2.18	1.69 ± 0.36^{ab}

[a] Different superscripts in the column indicate significant differences in the mean at $\alpha = 0.05$.

content of three major group B saponins and their non-DDMP counterparts ranged from 2.5 to 5.85 μmol/g among 46 cultivars of soybean. Genetically modified "Round-up ready" soybeans have significantly ($p < 0.05$) lower amounts of group B soyasaponin than those of conventional cultivars (*33*), perhaps because these soybeans suffer less environmental stress when other plants are removed by the herbicide (*34*). Similarly, Tsukamoto et al. (*35*) found that soyasaponin concentrations in cultivated soybeans are 2-fold less than in wild soybeans.

Relationship between Soyasapogenols and Isoflavones. Isoflavones in soybean have been credited with performing several health-promoting functions including prevention of cardiovascular diseases, hormone-related cancers such as breast and prostate cancers, and menopausal symptoms (*36*−*38*). Therefore, attempts have been made to enhance the isoflavone content of soybean cultivars and soy foods (*39*). We determined the content of three isoflavone aglycones, genistein, daidzein, and glycitein, in the 10 soybean cultivars grown in four locations and compared them with the content of soyasaponin aglycones, soyasapogenols A and B.

The concentration of total isoflavone aglycones ranged from 0.91 to 2.45 mg/g. The ratio of genistein, daidzein, and glycitein in the 10 cultivars was approximately 49:46:5. Total soyasapogenols A and B content (**Table 6**) and total isoflavone aglycone content (data not presented) were the highest in soybean cultivars grown in Woodslee. There was no apparent relationship ($r^2 = 0.057$) between the distribution of isoflavone and soyasapogenols in the 10 cultivars tested (**Figure 3**). Similarly, Hu et al. (*33*) found no statistically significant correlation between total isoflavone concentrations and six group B saponins in 46 cultivars of soybeans. This relationship needs to be further analyzed using a larger number of more genetically diverse soybean cultivars. It is also important to study the effect of growing conditions such as total heat units and soil moisture and nutrient factors on the accumulation of these health-promoting secondary metabolites in soybean. Among the 10 cultivars, the ratio of total isoflavone aglycones to total

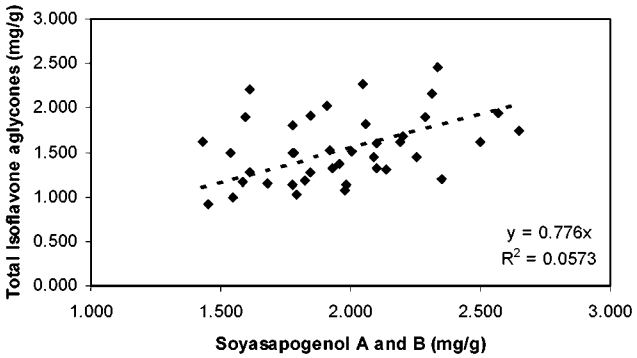

Figure 3. Relationship between the concentrations of soyasapogenol A and B and those of three isoflavone aglycones (daidzein, glycitein, and genistein) in 10 food-grade soybean cultivars grown in four different locations in Ontario.

soyasapogenols ranged from 0.5 to 1.37 on mg/g basis. Biosynthesis of sapogenols and isoflavones occurs through two distinct metabolic pathways, the isoprenoid pathway and the phenylpropanoid pathway, respectively, and there is not any close metabolic coordination between the biosynthetic pathways for these two groups of secondary metabolites.

Identification and development of food-grade cultivars with enhanced health-promoting phytochemicals are important for the value-added food industry. Enhancement of beneficial group B saponins and isoflavones, together with the elimination of group A saponins, will contribute to improvement of the quality of soybean and their food products and better public acceptance.

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