Validation of a High-performance Liquid Chromatography–Ultraviolet Method to Quantify Soy Sapogenols A and B in Soy Germs from Different Cultivars and in Soy Isoflavone-Enriched Supplements

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ABSTRACT: An increasing number of studies investigating the health effects of soy phytochemicals has led to the commercialization of many soy-based products. Due to its particularly high concentration in many secondary metabolites, the use of soy germ as raw material is emerging for the processing of soy dietary supplements. However, the soybean seeds and germs do not exhibit the same major phytochemical profiles. This is particularly the case for soy saponins. Due to their structural diversity, the analysis of each individual soy saponin remains difficult. In this study, the total amount of these health-protective phytochemicals was determined through the quantification of their aglucon precursors, soy sapogenols A and B. A simple and rapid analytical method was developed using high-performance liquid chromatography coupled with an ultraviolet detection. The within-day and between-day variabilities of total soy sapogenol concentration were 7.3% and 10.9% in the whole seed and 3.3% and 4.7% in the germ, respectively. The total soy sapogenol contents investigated among the germs from 43 cultivars ranged from 32.8 μmol/g to 63.1 μmol/g. High amounts of soy saponins were also observed in several soy-based dietary supplements, from 5.5 μmol/g to 107.8 μmol/g, with an A/B ratio varying from 0.3 to 8.6, showing large differences between the raw materials and concentration process used. These results indicate that these compounds have to be clearly determined when discussing the biological activity of dietary supplements issued from soy.

Keywords: soybean, soyasapogenols, soyasaponin, HPLC/UV detection, dietary supplements

Introduction

Soy-based products have gained considerable interest due to the presence of several health-promoting components that provide potential effects against several diseases afflicting humans, including anticancer effects (Kwon and others 1998). Soy isoflavones have been investigated for the past 2 decades and are widely recognized for their antiestrogenic activity (Adlercreutz and others 1986), for their contribution in reducing the risk of cardiovascular diseases (Anthony and Clarkson 1996), lowering rates of prostate, breast, and colon cancers (Peterson and others 1998), and improving bone protection (Barham and others 1996). However, other components of soybean may be acting as well. In soy germ and whole soybean seed extracts, saponins are present in significant amounts, sometimes higher than the levels of isoflavones (Okubo and others 1994). Many studies have been conducted on the biological properties of soy saponins, including anti-hepatotoxicity (Miyao and others 1998; Rowlands and others 2002) and reduction of the intestinal absorption of cholesterol (Oakenful 2001). According to Malinow and others (1981) and Sidhu and others (1986), soy saponins seem to prevent the development of colon cancer and show an inhibiting effect on human carcinoma (HCT-15) cells, with significant reduction in their viability (Rao and Sung 1995). Even if these compounds have been reported to possess several health benefits, they are not well absorbed (Yoshikoshi and others 1995). However, after ingestion, saponins are present in high concentrations in the gastrointestinal (GI) tract (Fang and Yu 2004), and thus could be biologically active for the GI tract balance by acting indirectly through other mediators. The biological value of soy saponins is closely related to their chemical structure, which determines their polarity, hydrophobicity, and acidity. Saponins are glycosidic compounds of triterpenoid (C_{30}) sapogenin nucleus with 1 or more side chains of carbohydrates (Figure 1). They are divided into 2 major groups, A and B. Group B soy saponins are monodesmosidic, having just 1 glycosilation site, whereas group A soy saponins have 1 more hydroxyl group at C-21 and are either bisdesmosides at C-3 and C-22 or monodesmosides. Shiraiwa and Kudou (1991) identified 6 different group A saponins, with a characteristic acetyl group on the terminal sugar of the oligosaccharide chain, attached to the C-22 position. Group A acetylated saponins were initially regarded as undesirable metabolites, due to their bitter and astringent taste (Kitagawa and others 1988; Okubo and Ijima 1992), but they also show antioxidant properties (Jiang and others 1993). Moreover, specific effects of soy sapogenol A on the liver injury mediated by the immune response in concanavalin A-induced hepatitis have been studied in mice (Kuzuhara and others 2000). It was shown that soy sapogenol A reduced the number of infiltrating inflammatory cells in the liver and significantly lowered the elevated level of plasma tumor necrosis.

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On an equal weight basis, total soy saponins are more concentrated in the germ than in all other fractions of soybean seeds (Shimoyamada and Kudou 1990). The concentration of group A soy saponins in the cotyledons is quite low, but, on the contrary, it is particularly high in the germ. Because the germ represents only 2% of the whole soybean seed, soy saponins of group A are less well-studied than soy saponins B, which are present in both germ and cotyledons.

There is an increasing number of commercial products of isoflavone-enriched extracts from soy in the market of western countries. According to the process used during soy manufacturing, such as hydro-alcoholic extraction, these products could also be enriched in saponins. Thus, the soy materials used for their production need to be considered with attention. Some soy-based health supplements are prepared from soy germs, whereas others are prepared from whole seeds or cotyledons. Soy saponins exhibit different profiles and contents depending on the matrix used during the industrial process. The concentration of soy saponins in dietary supplements purchased for isoflavones has to be clarified when discussing the biological mode of action of these products.

The quantification of each individual soy saponin is difficult, due to the structural similarities between group A and group B saponins, which both possess labile forms that undergo structural changes under certain processing conditions (Rickert and Johnson 2004). However, acid hydrolysis of all A and B saponins yielded the corresponding aglucon forms, soy sapogenols A and B (Figure 1). Several methods have been reported for the quantification of sapogenols A and B, including solid phase extraction followed by high-performance liquid chromatography (HPLC) coupled with an evaporative light scattering detector (Rupasinghe and others 2003) or coupled with an electrospray ionization mass spectrometer (Gu and others 2002). Nevertheless, the use of mass spectrometric detection is too expensive for many laboratories. Consequently, development of effective and simple analytical procedures is needed. The most suitable method to have access to both group A and B soy saponins is to analyze their aglucon precursors. In our work, soy sapogenols were isolated from several soybean varieties and commercial soy-based products and subsequently quantified by HPLC coupled with ultraviolet (UV) detection without laborious purification and cleanup of the samples before assay. The rapidity, simplicity, and low cost of the method make feasible the analysis of a large number of samples. This study was undertaken to evaluate the variation of soy saponin contents in different cultivars. The distribution, recovery, and profiles of soy saponins in germs and cotyledons, and in commercial soy-based health supplements, were also evaluated.

Materials and Methods

Plant materials and chemicals

Seeds from 43 soybean varieties were obtained from the Euralis

Validation of a HPLC-UV Method to Quantify Soy Sapogenols

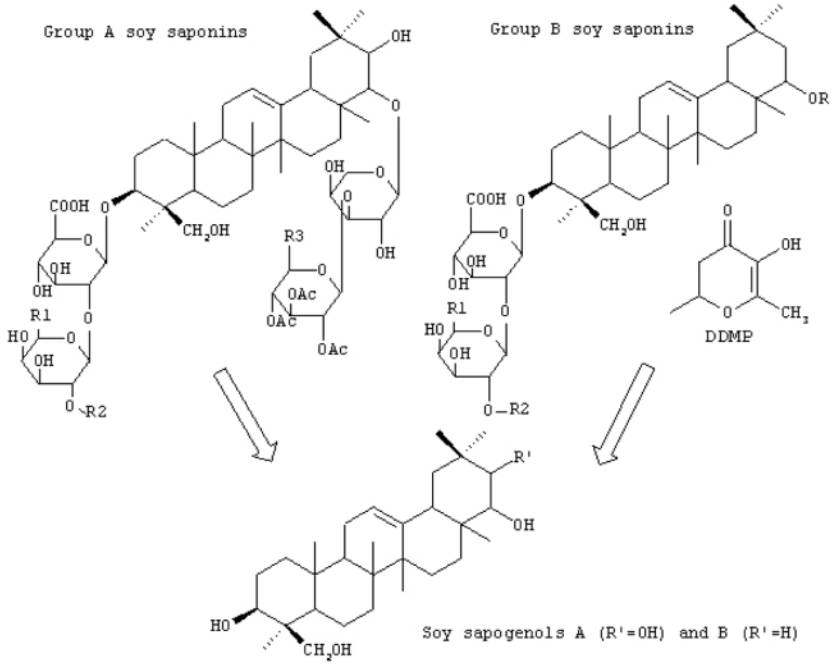

Figure 1—Structure of soy saponins A and B and their aglycone corresponding forms. Group B nomenclature is from Kitagawa and others (1998) with the nomenclature of Okubo and others (1993) in parentheses

Group A soy saponins			
	R1	R2	R3
acetyl A1(Ab)	CH_2OH	β-D-glu	CH_2OAc
acetyl A2(Af)	CH_2OH	H	CH_2OAc
acetyl A3(Ah)	H	H	CH_2OAc
acetyl A4(Aa)	CH_2OH	β-D-glu	H
acetyl A5(Ae)	CH_2OH	H	H
acetyl A6(Ag)	H	H	H

Group B soy saponins			
R=H	R=DDMP	R1	R2
I(Bb)	βg	CH_2OH	α-L-rham
II(Bc)	βa	H	α-L-rham
III(Bb')	γg	CH_2OH	H
IV(Bc')	γa	H	H
V(Ba)	αg	CH_2OH	β-D-glu

Table 1—Calibration data for soy sapogenols A and B

Calibration method	Soy sapogenol A			Soy sapogenol B		
	Response factor (μmol/mL)	R^2	LOD[a] (nmol)	Response factor (μmol/mL)	R^2	LOD[a] (nmol)
In pure methanol	1.040×10^7	0.9995	0.19	8.782×10^6	0.9999	0.55
In soy germ extract	1.043×10^7	0.9994	0.34	8.411×10^6	0.9999	1.21

[a]Limit of detection = 3 times the signal-to-noise ratio.

Society (Mondonville, France) in 2001. Germs were collected by hand from lyophilized seeds and ground with mortar and pestle. For the whole seed analysis, the lyophilized seeds were ground into a fine powder (particle size <200 μm) with a mill (IKA Labortechnik, Staufen, Germany). Dietary supplements were purchased locally. Soybean germ samples issued from an industrial load were provided by Genibio (Saint Girons, France) and used as control samples. All the samples were stored at –20 °C. Concentrated hydrochloric acid, acetonitrile, methanol, and 1-propanol with HPLC purity were purchased from SDS (Peypin, France). Deionized water was generated from a Milli-Q analytical deionization system (Millipore, Saint Quentin Yvelines, France). Purified standards of soy sapogenols A and B were provided by Chromadex (Santa Ana, Calif., U.S.A.).

Extraction and hydrolysis of soy saponins

Finely ground soy germ powders (0.2 g), whole seed powders (0.5 g), or dietary supplement powders (0.1 g) were dissolved in 5 mL of 80% (v/v) aqueous methanol and extracted for 2 h at room temperature. The residue was removed by centrifuging the extract at 12000 g for 10 min and decanting the clear supernatant. The extracts were filtered through 0.20 μm (Acrodisc, Pall, N.Y., U.S.A.), and 4 mL of the supernatant was transferred into a hemolysis tube. Five milliliter of 1 *N* HCl in methanol was added. The solution was subjected to acid hydrolysis at 85 °C for 6.5 h in a water bath to release the aglucons from the soy saponins. Two simultaneous extraction and hydrolysis were carried out on the same powder sample to ensure the unfolding during the hydrolysis step. The solutions were cooled at room temperature and an aliquot was analyzed on a reverse-phase HPLC with a P4000 pump controller, an AS3000 autosampler, and a UV2000 SpectraSystem detector (Spectra Physics Analytical Inc., Fremont, Calif., U.S.A.). The analytical column, 250 × 4.6 mm inner dia, 5 μm, Satisfaction RP-C_{18}-AB (Cluzeau, Sainte Foy La Grande, France), was kept at 30 °C in a thermal chamber. Solvent A consisted of acetonitrile:1-propanol:water:acetic acid (80/6/13/0.1) and solvent B was 100% acetonitrile. The gradient elution was accomplished by a modification of the method described by Rupasinghe and others (2003): solvent A was pumped isocratically for 15 min, then solvent B increased to 100% in 2 min and remaining at 100% for 2 min. The gradient was recycled back to the initial state of 100% solvent A in 3 min. The injection volume was 50 μL and the flow rate 0.9 mL/min. UV absorbance was monitored at 205 nm. Chromatograms were recorded and integrated with SpectraSystem PC1000 software.

Soy sapogenol calibration

Standards (1 mg) were initially dissolved in pure methanol (10 mL). After 10 min of homogenization, each stock solution was submitted to serial dilution (20, 50, 80, 200, 300, and 400 μg/mL) using 100% methanol. Standard curves were obtained by plotting the soy sapogenol concentrations as a function of peak area recorded from HPLC chromatograms. The calibration curves of soy sapogenols A and B were also established by serial dilutions of the stock solutions in plant matrix. Six extracts of soy germ were prepared as described previously. Each of these 6 extracts was separated into 50 μL aliquots, each in which 0, 50, 100, 150, 200, and 250 μL of the stock solution of purified standards were respectively added. The solutions were filled up to 300 μL with 80% aqueous methanol. The response factors of soy sapogenols A and B were calculated by linear regression. Their identity was confirmed by HPLC retention times. Two replications of the stock solution preparations were performed for each external standard of soy sapogenol A and B.

Kinetics of soy saponin hydrolysis

To ensure a complete hydrolysis step, soy sapogenols A and B were quantified as a function of hydrolysis time. Ten samples of soy germ powder and 10 samples of whole seed powder were extracted in 80% aqueous methanol as described previously. After adding the 1 *N* HCl solution, each extract was subjected to acid hydrolysis at 85 °C. Samples were removed periodically (every 30 min during the 1st 3 h, and every hour up to 9 h), refreshed in a cold bath, and analyzed for soy sapogenol distribution. The heating experiment was repeated twice over a month.

Evaluation of precision and accuracy

Two control samples were chosen for their characteristic soy saponin contents and distribution: isolated soy germs (Genibio, St Girons, France), exhibiting significant amounts of soy saponins A and B, and whole soybean seeds (cv Imari, Monsanto Seeds, Montbequi, France), almost exclusively containing saponins B, lyophilized, and stored at 4 °C. Ten replications of the extraction procedure followed by acid hydrolysis and HPLC assay were carried out successively on each control sample to evaluate the within-day variation. The assay was replicated 3 times over a month to determine the between-day variation. The means, standard deviations, and coefficients of variation of within-day and between-days assays were calculated for each soy sapogenol. A control sample was analyzed before the 1st sample each day and at least once per 20 samples.

Results and Discussion

Validation of the calibration method

Separate stock solutions were prepared from the commercially available standards of soy sapogenols A and B. The results obtained from linear regression of the calibration curves are presented in Table 1, including the response factor, correlation coefficient, and limit of detection (LOD) of each soy sapogenol, either diluted in pure methanol or in a soy germ extract. This last method was used to ensure the absence of interference with other molecules in the extract analyzed. All serial dilutions were duplicated. This method gave high reproducibility and was free from interference from the matrix. Linearity of the 2 standards was very high, with R2 values greater than 0.999 over the concentration range injected (10 to 200 μmol/mL) in the different calibration conditions. The LOD, defined as 3 times the signal-to-noise ratio (S/N), was 0.34 nmol for soy sapogenol A and 1.21 nmol for soy sapogenol B in soy germ extracts, which corresponds to 0.17 μmol/g and to 0.60 μmol/g, re-

Table 2—Variability of soy sapogenol analysis in soy germ and whole seed

	Germ			Whole seed		
	Means[a] ± SD (μmol/g DW)	CV within days[b] (%)	CV between days[c] (%)	Means[a] ± SD (μmol/g DW)	CV within days[b] (%)	CV between days[c] (%)
Sapogenol A	23.47 ± 0.67	2.9	7.4	0.80 ± 0.19	6.7	11.3
Sapogenol B	21.58 ± 0.96	4.4	3.7	2.36 ± 0.16	7.1	5.4
Total	45.05 ± 1.58	3.3	4.7	3.16 ± 0.29	7.3	10.9
A/B	1.09 ± 0.02	4.5	3.2	0.34 ± 0.03	7.2	9.2

[a] n = 30.
[b] 10 control samples were analyzed successively within a day.
[c] 10 control samples were analyzed 3 times over a month.

spectively, according to the dilution conditions. The noise was the signal width in blank injections realized between the sample injections. Noise peaks were integrated at the same time of elution as the soy sapogenols. Thus, the UV detection used here, at a very low wavelength, presented a high sensitivity. The 2 soy sapogenols were well resolved, with a retention time difference of 4 min (Figure 2). Soy sapogenols A and B were eluted at 6.7 ± 0.2 min (n = 20) and 10.3 ± 0.2 min (n = 20), respectively. The peak identities were confirmed by adding a known amount of the corresponding standard in a soy extract, overlapping the expected soy sapogenol peaks in the resultant chromatogram.

Validation of the analytical procedure

Multiple hydrolysis times were tested on the soy germ reference sample to determine the hydrolysis efficiency. The kinetics of soy sapogenol formation during acid hydrolysis are presented in Figure 3. The amount of soy sapogenols obtained from soybean extracts was optimized by incubating them for 6.5 h at 85 °C. Before this time, the formation of the 2 sapogenols is not quantitative, whereas a too long hydrolysis step promotes their alteration. The best compromise to produce the optimal quantity of sapogenols in our dilution conditions without inducing their degradation was to stop the reaction after 6.5 h of hydrolysis.

Soy saponins were extracted from 10 samples of soy germs and 10 samples of whole seeds, hydrolyzed in their aglucon forms and then quantified with the HPLC method described previously. The precision and within-day and between d variabilities of the method were evaluated and are reported in Table 2. The within-day variability of the total amount of sapogenols in the whole seed was 7.3% and 3.3% in the germ, which indicates a good repeatability of the sample preparation. The between-day variabilities were 10.9% in the whole seed and 4.7% in the germ. Because the germ presents higher quantities of sapogenols than the cotyledons, the analytical variation is lower in this fraction. This method demonstrates that purification and cleanup, as described previously (Ireland and others 1986), can be eliminated for the determination of soy sapogenols A and B in soybean or in complex soy-food matrices. The elimination of the evaporation step before hydrolysis reduces the duration of sample preparation.

Soy sapogenol distribution in soy germs from different cultivars

Previous studies have been conducted to determine the soy saponin variation among cultivars (Gu and others 2002; Hu and others 2002), but these studies were carried out on whole soybean seeds, and particularly focused on saponins from group B. Nevertheless, soy saponins have been reported to be mainly located in the germ fraction of soybean seeds, with a total concentration 6 times higher than that in the cotyledons (Tani and others 1985).

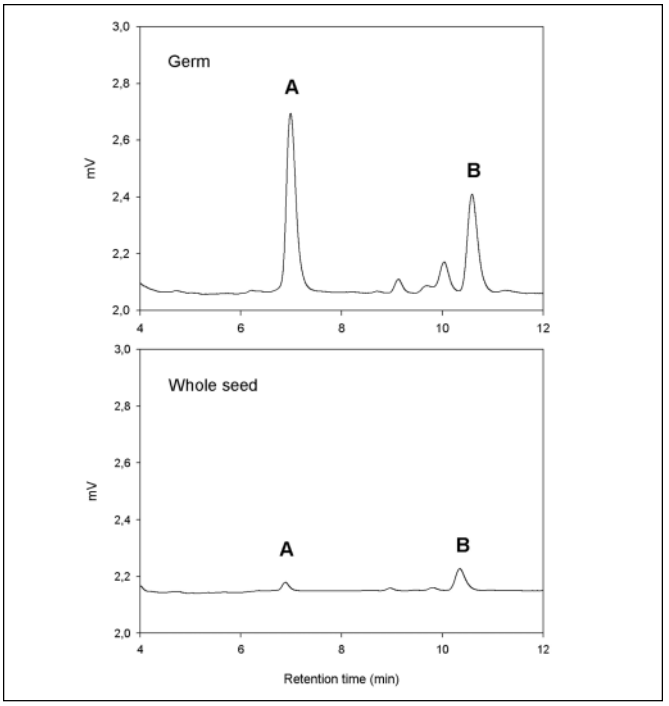

Figure 2—High-performance liquid chromatography (HPLC) profiles of soy sapogenols A and B in soybean germ and in the whole seed (UV = 205 nm)

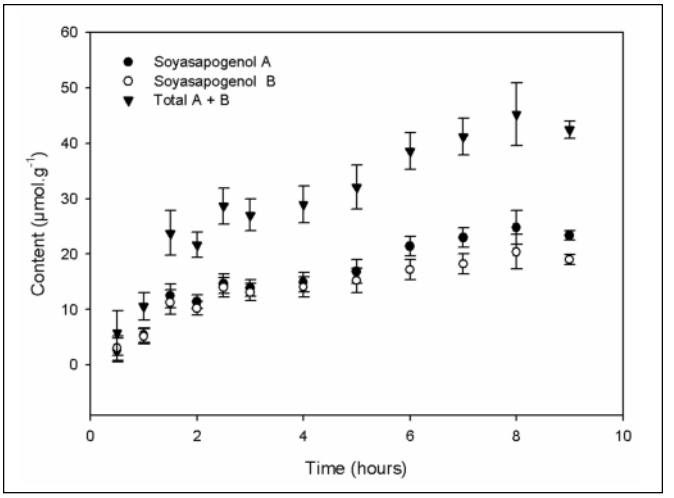

Figure 3—Kinetics of soy sapogenol formation during acid hydrolysis carried out on aliquots of a soy germ sample. Means ± SD.

Little information is available about the variability of soy saponin contents in soy germs from different cultivars, although the soy saponin profile in the germ can reflect the genetic characteristic of a variety (Tsukamoto and others 1994). In this study, soy saponins A and B were determined in the germs of 43 cultivars through the quantification of their corresponding aglucon precursors. The cultivars tested were field-grown in 2001 in 2 plots. There was a significant difference in total, but also in individual, soy sapogenol contents and soy sapogenol A/B ratio ($P < 0.001$) in soy germs from different genotypes; plot effect was not significant. The total soy sapogenol contents ranged from 32.8 μmol/g to 63.1 μmol/g (Table 3). The levels of individual soy sapogenol A and B varied from 15.0 μmol/g to 30.8 μmol/g, and from 14.7 μmol/g to 32.3 μmol/g, respectively. These quantities are slightly higher than those found by Kitagawa and others (1988) in the hypocotyls fraction, but are in agreement with those found in more recent studies (Tsukamoto and others 1994; Gu and others 2002). Our findings confirmed the very high concentrations of soy saponins in the germs compared with the whole seeds, which were found to contain, on average, 4.04 ± 0.91 μmol/g in different varieties in a previous study (Hu and others 2002). The ratio of group A to group B presents great variations, from 0.67 to 1.65, which indicates that the group A and B concentrations were not highly correlated ($r = 0.414$). Similarly, Rupasinghe and others (2003) reported differences in distribution of soy sapogenols A and B in the axes of 3 distinct seedling cultivars. Because the described method allows many analyses, it could be used to evaluate the environmental and genetic variability of soy sapogenols and thus the results obtained could supply basic information for breeding soybean cultivars with higher soy saponin A or soy saponin B contents.

Soy sapogenol determination in commercial soy isoflavone products

Many isoflavone extracts are sold as dietary supplements, and

Table 3—Sapogenol contents in the germs of 43 field-grown soybean cultivars[a]

Cultivar	Total (μmol/g)	Sapogenol A (μmol/g)	Sapogenol B (μmol/g)	A/B ratio
LOGAN	32.8 ± 2.2 A	17.6 ± 0.4 A → D	15.2 ± 1.9 A	1.16 ± 0.14 A → E
F88-1243	33.1 ± 3.7 A	17.0 ± 3.3 A B C	16.1 ± 1.0 A B	1.06 ± 0.19 A → E
YING NAN	33.5 ± 2.0 A	15.0 ± 2.2 A	18.5 ± 0.5 A B C	0.81 ± 0.13 A B
GENA	33.6 ± 0.3 A	18.8 ± 0.1 A → E	14.7 ± 0.2 A	1.28 ± 0.02 A → E
MACON	33.6 ± 5.6 A	18.9 ± 4.4 A → E	14.7 ± 1.5 A	1.28 ± 0.21 A → E
VICKERY	34.3 ± 0.9 A	15.5 ± 0.9 A B	18.8 ± 0.2 A B C	0.83 ± 0.05 A B C
SLOAN	34.4 ± 0.9 A	19.7 ± 0.7 A → F	14.7 ± 0.2 A	1.33 ± 0.03 A → E
ATLAS	34.8 ± 3.1 A B	19.8 ± 2.6 A → F	15.0 ± 1.0 A	1.32 ± 0.16 A → E
MIXER	35.4 ± 7.1 A B	20.4 ± 5.5 A → F	15.0 ± 2.1 A	1.36 ± 0.29 A → E
LINCOLN	35.9 ± 1.3 A B	20.6 ± 2.6 A → F	15.4 ± 2.2 A	1.37 ± 0.39 A → E
8729SE	36.6 ± 2.9 A B	20.0 ± 2.9 A → F	16.6 ± 5.1 A B	1.34 ± 0.58 A → E
MUKDEN	38.0 ± 2.1 A B C	18.4 ± 1.6 A → E	19.5 ± 1.3 A → E	0.95 ± 0.10 A → E
QUITO	38.6 ± 4.8 A → D	19.9 ± 4.0 A → F	18.6 ± 2.9 A B C	1.09 ± 0.26 A → E
CN5	38.6 ± 0.8 A → D	23.0 ± 0.2 A → H	15.6 ± 0.9 A B	1.47 ± 0.10 B → E
CENTURY	38.8 ± 4.1 A → D	19.1 ± 3.5 A → E	19.7 ± 0.6 A → E	0.97 ± 0.15 A → E
SAFRANA	39.1 ± 1.5 A → D	23.1 ± 2.8 A → H	16.0 ± 1.5 A B	1.47 ± 0.30 B → E
ALARIC	41.2 ± 1.9 A → E	18.6 ± 1.0 A → E	22.6 ± 0.9 A → F	0.82 ± 0.01 A B C
GIULETTA	41.2 ± 4.4 A → E	21.2 ± 3.3 A → G	20.0 ± 4.0 A → E	1.09 ± 0.27 A → E
J14	41.8 ± 3.1 A → F	18.4 ± 0.9 A → E	23.4 ± 2.3 A → G	0.79 ± 0.05 A B
MESSIDOR	41.8 ± 1.5 A → E	25.7 ± 0.9 C → H	16.1 ± 0.7 A B	1.60 ± 0.03 C D E
OPIUM	42.4 ± 0.2 A → F	24.0 ± 0.4 A → H	18.3 ± 0.6 A B C	1.31 ± 0.07 A → E
RPS815	42.7 ± 2.3 A → F	18.1 ± 2.2 A → D	24.6 ± 0.1 A → H	0.74 ± 0.09 A B
SONEL	42.8 ± 1.2 A → G	26.6 ± 0.9 C → H	16.2 ± 1.3 A B	1.65 ± 0.17 E
CN4	42.9 ± 3.2 A → G	17.7 ± 2.2 A → D	25.3 ± 1.7 B → H	0.70 ± 0.08 A B
WOLFSTHA	43.9 ± 3.7 A → G	22.0 ± 1.6 A → H	21.9 ± 2.1 A → F	1.01 ± 0.02 A → E
CN290	44.2 ± 10.5 A → G	20.5 ± 0.6 A → F	23.8 ± 9.9 A → H	0.94 ± 0.37 A → E
L1BC5	44.5 ± 6.0 A → G	26.5 ± 0.8 C → H	18.0 ± 6.9 A B C	1.61 ± 0.54 D E
BA YUE Z	45.0 ± 5.4 A → G	19.7 ± 1.8 A → F	25.3 ± 3.6 B → H	0.78 ± 0.04 A B
WEBER 84	45.0 ± 2.6 A → G	22.9 ± 4.3 A → H	22.1 ± 2.6 A → F	1.06 ± 0.29 A → E
MERIT	45.7 ± 2.4 A → G	22.5 ± 1.3 A → H	23.2 ± 1.1 A → G	0.97 ± 0.01 A → E
SPUTNIK	46.5 ± 5.8 A → H	24.9 ± 3.4 B → H	21.6 ± 2.4 A → F	1.15 ± 0.03 A → E
TRESOR	46.7 ± 14.5 A → H	27.5 ± 7.1 D → H	19.1 ± 7.5 A → D	1.48 ± 0.21 B → E
TOTEM	46.9 ± 14.2 A → H	26.8 ± 9.5 C → H	20.1 ± 4.7 A → E	1.31 ± 0.17 A → E
TF17A	48.9 ± 2.4 B → H	26.8 ± 0.5 C → H	22.1 ± 1.9 A → F	1.22 ± 0.08 A → E
CN2	49.1 ± 0.6 B → H	19.6 ± 2.1 A → F	29.5 ± 1.6 F G H	0.67 ± 0.11 A
ER HUANG	51.8 ± 6.5 C → I	24.5 ± 4.0 A → H	27.3 ± 2.5 C → H	0.89 ± 0.06 A → E
PANNONIA	52.4 ± 2.3 D → I	23.9 ± 1.4 A → H	28.5 ± 1.0 D → H	0.84 ± 0.03 A → D
GNOME	53.4 ± 2.4 E → I	27.1 ± 1.6 D → H	26.3 ± 3.6 C → H	1.05 ± 0.23 A → E
VNIIMK	55.6 ± 7.3 F → I	28.1 ± 4.3 E → H	27.5 ± 4.5 C → H	1.03 ± 0.17 A → E
TIAN E D	55.7 ± 1.8 F → I	29.3 ± 0.1 F G H	26.4 ± 1.9 C → H	1.11 ± 0.09 A → E
AGATA	56.5 ± 0.6 G H I	24.7 ± 2.1 B → H	31.8 ± 2.7 G H	0.78 ± 0.13 A B
DANUBIAN	58.8 ± 2.0 H I	30.2 ± 0.9 G H	28.6 ± 2.5 E → H	1.06 ± 0.12 A → E
S5606	63.1 ± 1.3 I	30.8 ± 0.8 H	32.3 ± 0.5 H	0.95 ± 0.01 A → E
CV[b]	9.8%	12.8%	13.8%	20.4%
LSD[b]	6.0	4.0	4.1	0.32

[a]For each cultivar, $n = 4$; duplicate samples from 2 plots. Mean values ± standard deviation. Letters indicate grouping after a Newman and Keuls test ($\alpha = 0.05$; A → F stands for means belonging to groups A B C D E F).
[b]Calculated with the analysis of variance (ANOVA) residual mean square.

Table 4—Soy saponin contents in commercial isoflavone-enriched supplements[a]

Soy product	Total (μmol/g)	Sapogenol A (μmol/g)	Sapogenol B (μmol/g)	A/B ratio	Mass equivalent saponin (%)
P1	59.4	12.0	47.5	0.25	5.5%
P2	50.5	12.9	37.6	0.34	4.7%
P3	36.0	9.2	26.7	0.35	3.3%
P4	20.7	6.8	14.0	0.49	1.9%
P5	12.7	4.2	8.5	0.49	1.2%
P6	25.7	8.9	16.7	0.54	2.4%
P7	10.6	4.0	6.6	0.61	1.0%
P8	107.8	43.0	64.8	0.66	10.0%
P9	39.5	19.6	19.9	0.99	3.7%
P10	42.0	21.9	20.0	1.09	3.9%
P11	14.0	7.5	6.5	1.15	1.3%
P12	74.9	41.2	33.7	1.23	7.0%
P13	6.8	3.6	3.2	1.24	0.6%
P14	90.5	51.5	39.0	1.32	8.5%
P15	6.5	3.5	3.1	1.62	0.6%
P16	53.8	33.6	20.2	1.66	5.0%
P17	46.8	32.2	14.6	2.25	4.4%
P18	54.7	38.2	16.5	2.32	5.1%
P19	21.8	15.5	6.4	2.43	2.0%
P20	23.8	20.8	2.9	7.10	2.2%
P21	21.9	19.6	2.3	8.64	2.1%
SE	2.2	1.3	1.2	0.31	

[a]Means from n = 3 analyses. Standard errors are calculated with the analysis of variance (ANOVA) residual mean square.

their consumption is increasing in many western countries. As reported previously, soybeans contain high levels of soy saponins, which could be either extracted simultaneously with isoflavones or removed during processing. This means that the nutritional value of soy-based products could be modified according to the process used. The potential presence of saponins in isoflavone-enriched supplements cannot be ignored. We investigated the soy saponin amounts in 21 commercial soy-based health supplements (Table 4). The method used in this study is particularly suitable for this type of samples: As the naturally occurring group A and B soy saponins are easily hydrolyzed during industrial processing, due to the pH and temperature conditions, it is appropriate to complete the hydrolysis of soy saponins, which is already partial in the products, and to quantify the total aglucon forms obtained. The total soy sapogenol concentrations in the soy isoflavone supplements ranged from 6.5 μmol/g to 107.8 μmol/g. The ratio of soy sapogenol A to B ranged from 0.25 to 8.64, reflecting the soy material used during processing. As noted earlier, a ratio higher than 0.6 is characteristic of a germ-based product, whereas a ratio under 0.5 is more characteristic of the soy sapogenol distribution in the whole seed. Thus, the products from P1 to P6 are probably prepared from whole soybean seeds. Considering that a 1:1 sapogenol:sugar ratio could be used to estimate the mass equivalent concentration of soy saponins (Ireland and others 1986), very high concentrations of soy saponins were detected in some products (7.0% in P12, 8.5% in P14, and 10.0% in P8). In contrast, some products, such as P4, contained a lower quantity of soy saponins (1.9%) than isoflavones (7.1% claimed, data not shown). Other products, such as P2, contained the same quantity of soy saponins (4.7%) as isoflavones (5% claimed). But the most surprising was when the amount of soyasaponins was very high compared with the amount of isoflavones. This was the case in product P14, which contained about 8.5% of soy saponins and only about 4% of isoflavones. These significant amounts of soy saponins in products which are supposed to be only isoflavone concentrates have to be taken in consideration. Soy saponins may interact with isoflavone biological activities or contribute to many of the health benefits attributed to isoflavones (Tongtong and others 2003).

Conclusions

We developed an easy and short method to quantify the 2 major groups of saponins present in soybeans. Although their distribution fluctuates among soy-based health products, their total concentration is often significant in soy isoflavone preparations. Their quantification is necessary in each commercial health supplement to provide better information concerning the bioactive compounds contained in these products.

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References

Adlercreutz H, Fotsis T, Banwart C, Wahala K, Makela T, Brunow G, Hase T. 1986. Determination of urinary lignans and phytoestrogen metabolites, potential antiestrogens and anticarcinogens, in urine of women on various habitual diets. J Steroid Biochem 25:791–7.

Anthony MS, Clarkson TB. 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 126(1):43–50.

Barham HA, Alekel L, Hollis BW, Amin D, Stacewicz-Sapuntzakis M, Guo P, Kukreja SC. 1996. Dietary soybean proteins prevent bone loss in an ovariectomized rat model of osteoporosis. J Nutr 126:43–50.

Fang NB, Yu SG. 2004. Comprehensive phytochemical profile of soy protein isolate. J Agric Food Chem 52(12):4012–4020.

Gu LW, Tao G, Gu W, Prior RL. 2002. Determination of soyasaponins in soy with LC-MS following structural unification by partial alkaline degradation. J Agric Food Chem 50(24):6951–9.

Hu J, Lee SO, Hendrich S. 2002. Quantification of the group B soyasaponins by high-performance liquid chromatography. J Agric Food Chem 50(9):2587–94.

Ireland P, Dziedzic SZ, Kearsley W. 1986. Saponin content of soya and some commercial soya products by means of high-performance liquid chromatography of the sapogenins. J Agric Food Chem 37:694–8.

Jiang Y, Zhong GG, Qi H, Ma XY. 1993. Antioxidative action of soyasaponin monomers I, A1, A2, and their inhibitory effects on action potential and beating of myocardiocytes in culture. Zhongguo Yao Li Xue Bao 4(3):269–73.

Kitagawa I, Taniyama T, Saito M. 1988. Structure of acetyl-soyasaponin A1, A2 and A3, astringent partially acetylated bisdesmoside of soyasapogenol A from American soybean, the seed of Glycine max L. Merrill. Chem Pharm Bull 36:2819–28.

Kuzuhara H, Nishiyama S, Minowa N, Sasaki K, Omoto S. 2000. Protective effects

of soyasapogenol A on liver injury mediated by immune response in a concanavalin A-induced hepatitis model. Eur J Pharmacol 391(1–2):175–81.
Kwon TW, Song YS, Kim JI, Honh JH. 1998. Current research on the bioactive functions of soyfoods in Korea. J Korean Soybean Dig 15:1–12.
Malinow MR, Connor WE, Mc Laughlin P, Stafford C, Lin DS, Livingston AL, Kohler GO, McNulty WP. 1981. Cholesterol and bile acid balance in *Macaca fascicularis*. Effects of alfalfa saponins. J Clin Invest 67:156–62.
Miyao H, Arao T, Udayama M, Kinjo J, Nohara T. 1998. Kaikasaponin III and soyasaponin I, major triterpene saponins of Abrus cantoniensis, act on GOT and GPT: influence on transaminase elevation of rat liver cells concomitantly exposed to CCl4 for 1h. Planta Med 64:5–7.
Oakenful D. 2001. Soy protein, saponins and plasma cholesterol. J Nutr 131(11):2971–2971.
Okubo K, Ijima M. 1992. Components responsible for the undesirable taste of soybean seeds. Biosci Biotechnol Biochem 56:99–103.
Okubo K, Kudou S, Uchida T, Yoshiki Y, Yoshikoshi M, Tonomura M. 1994. Soybean saponin and isoflavonoids. Structure and antiviral activity against human immunodeficiency virus in vitro. In: Rosen RT, editor. Food phytochemicals I: fruits and vegetables Washington, D.C.: American Chemical Society. p 330–9.
Peterson TG, Ji GP, Kirk M, Coward L, Falany CN, Barnes S. 1999. Metabolism of isoflavones genistein and biochanin A in human breast cancer cell lines. Am J Clin Nutr 68:1505-11S.
Rao AV, Sung MK. 1995. Saponins as anticarcinogens. J Nutr 125(3):717S–24S.
Rickert DA, Johnson LA. 2004. Improved fractionation of glycinin and beta-conglycinin and partitioning of phytochemicals. J Agric Food Chem 52(6):1726–34.
Rickert DA, Meyer MA, Murphy PA. 2004. Effect of extraction pH and temperature on isoflavone and saponin partitioning and profile during soy protein isolate production. J Food Sci 69:C623–31.
Rowlands JC, Berhow MA, Badger TM. 2002. Estrogenic and antiproliferative properties of soy sapogenols in human breast cancer cells in vitro. Food Chem Toxicol 40:1767–74.
Rupasinghe HPV, Jackson CJC, Poysa V, Berardo CD, Bewley JD, Jenkinson J. 2003. Soyasapogenol A and B distribution in soybean (*Glycine max* L. Merr.) in relation to seed physiology, genetic variability, and growing location. J Agric Food Chem 51(20):5888–94.
Shimoyamada M, Kudou S. 1990. Saponin distribution in soybean plants. Agric Biol Chem 54:77–81.
Shiraiwa M, Kudou S. 1991. Composition and structure of "group A saponin" in soybean seed. Agric Biol Chem 55(4):315–22.
Sidhu GS, Oakenful DG. A mechanism for the hypocholesterolemic activity of saponins. Br J Nutr 55:643–9.
Tani T, Katsuki T, Kubo M, Arichi S, Kitagawa I. 1985. Histochemistry. V. Soyasaponins in soybean (*Glycine max* L. Merr.) seed. Chem Pharm Bull 33:3829–33.
Tongtong S, Lee SO, Murphy PA, Hendrich S. 2003. Soy Protein with or without isoflavones, soy germ and soy germ extract, and daidzein lessen plasma cholesterol levels in golden Syrian hamsters. Exp Biol Med (Maywood) 228(9):1063–8.
Tsukamoto C, Kikuchi A, Kudou S, Harada K, Iwasaki T, Okubo K. 1994. Genetic improvement of saponin components in soybean. Food phytochemicals for cancer prevention. Washington D.C.: American Chem Soc. I. Fruits and vegetables. 31:373–9.
Yoshikoshi M, Kahara T, Yoshiki Y, Ito M, Furukawa Y, Okubo K, Amarowicz R. 1995. Metabolism and nonabsorption of soybean hypocotyls saponins in the rat model. Acta Aliment 24(4):355–64.