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Group B Saponins in Soy Products in the U.S. Department of Agriculture—Iowa State University Isoflavone Database and Their Comparison with Isoflavone Contents

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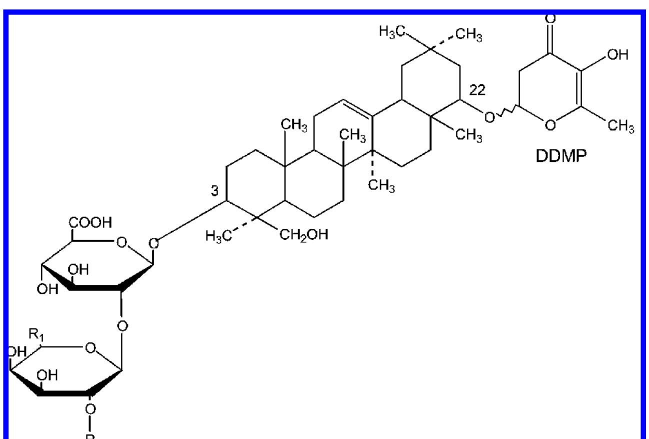
Isoflavones in soy protein foods are thought to contribute to the cholesterol-lowering effect observed when these products are fed to humans. The group B saponins are another ethanol-soluble phytochemical fraction associated with soy proteins and isoflavones and have also been associated with cholesterol-lowering abilities. We measured the group B soyasaponin concentrations in a variety of soy foods and ingredients in the U.S. Department of Agriculture—Iowa State University Isoflavone Database. We compared the isoflavone and soy saponin concentrations and distributions in intact soybeans, soy ingredients, and retail soy foods. Group B saponins occur in six predominant forms. There appears to be no correlation between saponin and isoflavone concentrations in intact soybeans ranging from 5 to 11 μmol isoflavones/g soybean and from 2 to 6 μmol saponin/g soybean. Depending upon the type of processing, soy ingredients have quite different saponins/isoflavones as compared to mature soybeans. In soy foods, the saponin:isoflavone ration ranges from 1:1 to 2:5, whereas in soy protein isolates, the ratio is ~5:3. Ethanol-washed ingredients have very low saponins and isoflavones. These very different distributions of saponins and isoflavones in soy products may affect how we view the outcome of feeding trials examining a variety of protective effects associated with soy consumption.

KEYWORDS: Soyasaponins; group B saponins; isoflavones; soy foods; soy ingredients

INTRODUCTION

There has been much attention given to the potential health effects of soy food consumption. The FDA approved a health claim for soy protein describing the relationship between soy protein consumption and reduced risk of heart disease as part of a diet low in saturated fat and cholesterol (*1*). A recent review concludes that soy protein and its associated phytochemicals are implicated in improving cardiovascular health (*2*). These authors reported a dose response between isoflavone concentrations in soy protein fed and plasma cholesterol concentrations. Messina and Bennink (*3*) and Messina (*4*) reviewed the roles of soy in colon and breast cancer, respectively. The isoflavones are only one class among several soy components linked with plasma cholesterol lowering effects, bone health, and cancer prevention and control. Other soybean components that have purported health effects are saponins, the Bowman—Birk protease inhibitor, sphingolipids, phenolic acids, and phytates.

Saponins are ethanol-soluble triterpenoid or steroid glycosides naturally occurring in plants. Relatively high concentrations of saponins have been reported in soybeans and soy products (*5–14*). Soyasaponin groups A and B are found in soybeans. Berhow et al. (*8*) suggest that soyasaponin group E are artifacts of group B soyasaponins formed during extraction and analysis when the 22-hydroxyl group is oxidized to a ketone. The group B soyasaponins were the primary soyasaponins present in soybeans



Saponin	-DDMP	R1	R2
Soy saponin βg	I	CH_2OH	α-L-Rhamnose
Soy saponin βa	II	H	α-L-Rhamnose
Soy saponin γg	III	CH_2OH	H
Soy saponin γa	IV	H	H
Soy saponin αg	V	CH_2OH	β-D-Glucose

Figure 1. Structures of group B saponins in soybeans.

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Table 1. Group B Soyasaponin Contents of Soymilk (μmol/g Wet Weight)

soymilk	% M[a]	saponin V	I	II	αg	βg	βa	total
aseptically processed								
DC[b]	86	0.03 A	0.25 C	0.10 B	0	0.03 A	0.01 C	0.42 C
HO	90	0.01 BC	0.24 C	0.08 B	0	0.04 A	0.01 B	0.38 C
TA	86	0.02 DC	0.33 A	0.11 A	0	0.04 A	0.01 AB	0.51 A
KC	86	0.01 DC	0.33 A	0.11 A	0	0.04 A	0.02 A	0.50 A
DN	86	0.02 DC	0.28 B	0.09 B	0	0.03 A	0.01 B	0.44 B
NY	89	0 D	0.24 C	0.09 B	0	0.01 B	0 D	0.34 C
DC composite	89	0.03 AB	0.22 C	0.09 B	0	0.01 B	0.01 C	0.37 C
LSD[d]	0.01	0.02	0.02			0.01		0.04
pasteurized[c]								
DN[d]	89	0.01 B	0.17 B	0.06 B	0	0.01 A	0.01	0.26 B
HO	91	0.03 A	0.14 C	0.06 B	0	0.01 B	0.01	0.24 C
KC	91	0.03 A	0.19 A	0.08 A	0	0.01 AB	0.01	0.32 A
DC	90	0.03 A	0.12 D	0.05 C	0	0.01 AB	0.01	0.21 D
TA	92	0.03 A	0.13 CD	0.04 C	0	0.01 B	0	0.21 D
LSD		0.01	0.02	0.01		0.011	0.003	0.02

[a] Moisture. [b] Aseptically processed soy milk, Original Eden Soy Beverage; DC = Giant Foods, Washington, DC; HO = Kroger, Houston; TA = Publix, Tampa; KC = Price Chopper; DN = Albertsons, Denver; and NY = Walbaum. [c] Pasteurized soy milk, White Wave Silk Dairyless Soy Beverage; TA = Nature's Food Patch, Tampa; KC = Wild Oats, Kansas City; HO = Whole Foods, Houston; DC = Fresh Fields, Washington, DC; and DN = Vitamin Cottage. [d] Values in a column with different letters are different at α ≤ 0.05; LSD = least significant difference; each sample was analyzed in duplicate.

Table 2. Group B Soyasaponin Contents of Tofu (μmol/g Wet Weight)

tofu	% M[a]	saponin V	I	II	αg	βg	βa	total saponin
raw								
tofu B [b]	86	0 B	0.32 D	0.12 C	0.01 BC	0.08 D	0.02 E	0.55 D
tofu D	86	0.06 A	0.33 C	0.14 B	0.01 A	0.13 A	0.04 A	0.73 B
tofu I	85	0 B	0.39 B	0.14 B	0.01 B	0.12 B	0.03 C	0.70 C
tofu J	85	0 B	0.39 B	0.15 B	0.01 BC	0.10 C	0.03 B	0.68 C
tofu K	82	0 B	0.44 A	0.21 A	0.01 C	0.08 D	0.03 D	0.77 A
LSD[c]		0.002	0.01	0.02	0.002	0.002	0.0008	0.02
cooked								
tofu C	84	0	0.43 A	0.15 B	0.01	0.11	0.03 A	0.72
tofu E	82	0	0.40 B	0.17 A	0.01	0.10	0.03 B	0.71
LSD			0.03	0.02			0.0009	

[a] Moisture. [b] Letters correspond to identity in ref *27*; B = Azumaya, extrafirm, raw; C = Azumaya, extrafirm, cooked; D = Azumaya, Albertsons, firm, raw; E = Azumaya, Albertsons, firm, cooked; I = Hinoichi regular; J = Hinoichi firm, two city composite; and K = Naysoya, firm, four-city composite. According to package labels, all tofus were coagulated with nigari except Naysoya (calcium sulfate). [c] See footnote [d] in **Table 1**.

(*Glycine max*) (*6*) (**Figure 1**). Group A saponins typically are glycosylated at the 3- and 22-hyroxyl groups. Gu et al. (*9*) reported 87 mol % group B saponins and 13 mol % group A saponins in soybean flour. When data are expressed on a weight %, the group B saponins are underestimated by several %. Soy germ or hypocotyls contained 71 mol % group B and 29 mol % group A saponins. Degerminated soybean cotyledons contained 100% group B saponins, suggesting that the hypocotyls are the source of group A saponins in soy products. Kudou et al. (*15*) reported that the 2,3-dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)-conjugated soyasaponins αg, βg, and βa were the genuine group B saponins present in soybeans and their non-DDMP counterparts, due to loss of the DDMP group from the 22-hydroxyl, and soyasaponin V, I, and II were products formed during heat treatment of soy foods and ingredients.

Table 3. Group B Soyasaponin Contents of Fermented Soyfoods (μmol/g Wet Weight)

food[a]	% M[b]	saponin V	I	II	αg	βg	βa	total saponin
tempeh								
A[c]	61	0.15	0.94	0.47	0.01	0.26	0.10	1.93
B	61	0.14	0.96	0.45	0.01	0.27	0.10	1.93
miso								
A	53	0.07 B	0.45	0.25	0.01	0.12	0.05	0.94 A
B	53	0.09 A	0.45	0.21	0.01	0.11	0.05	0.93 B
LSD[d]	0.003		0.001					
natto								
sumibi	<5	0.74	4.55	2.01	0	0	0	7.31 B
puchi	<5	0.84	4.83	2.45	0	0	0	8.12 A
LSD		0.24	1.61	1.73				0.37

[a] *n* = 4. [b] Moisture. [c] Tempeh A = raw and tempeh B = cooked; both five-city composite, White Wave; miso A = shiro (white) and miso B = aka (red); each six-bag composite from supplier; and natto supplied by Beecher and Nagano. [d] See footnote [d] in **Table 1**.

Table 4. Group B Soyasaponin Contents of Retail Soy Meat Analogues Raw vs Cooked (μmol/g Wet Basis)

food[a]	% M[b]	saponin V	I	II	αg	βg	βa	total saponins
chicken[c]								
raw	74	0.09	0.60 B[e]	0.28 B	0	0.01 A	0.01 A	1.00 B
cooked	70	0.08	0.71 A	0.33 A	0.003	0.01 B	0.01 B	1.14 A
LSD[d]		0.04	0.05	0.05		0.004	0.0018	0.08
frank								
raw	59	0	0.03 B	0 B	0	0	0	0.02 B
cooked	58	0	0.05 A	0.05 A	0	0	0	0.10 A
LSD			0.03	0.01				0.03
Harvest burger[d]								
raw	64	0.07	0.28 B	0.13 B	0	0	0	0.48 B
cooked	58	0.08	0.36 A	0.18 A	0	0.01	0.01	0.61 A
LSD		0.03	0.02	0.00				0.04

[a] *n* = 2. [b] Moisture. [c] Chicken = Worthington Foods FriChik, five cities, two cans/city composite; and frank = Loma Linda meatless franks, five cities, two cans/city composite. [d] Burger = Harvest burger, *n* = 6. [e] See footnote [d] in **Table 1**.

Soybean saponins may be an active component contributing to the cholesterol-lowering effect of soy products (*16–18*). There are a number of studies that show that soybean saponins inhibit different stages of cancer development or progression, in vivo and in vitro (*19–23*).

Therefore, determining the relationship between isoflavones and saponins in soy foods is of great interest. What is the correlation between saponins and isoflavones in a wide array of soy foods, ingredients, and nutraceuticals? We were able to analyze the soyasaponin content of many foods used in the development of the U.S. Department of Agriculture—Iowa State University Isoflavone Database (*24*), which enables us to determine if there is a correlation between isoflavone and saponin concentrations.

MATERIALS AND METHODS

Foods. The foods collected for the U.S. Department of Agriculture—Iowa State University Isoflavone Database (*24*) were analyzed for group B saponins in this study. The database food selection, collection, preparation, preservation, storage, and identity were reported in Murphy et al. (*25–29*). Products analyzed for group B saponins included the following: soymilks, Original Eden Soy beverage and White Wave Silk

Table 5. Group B Soyasaponin Contents of Retail Soy Meat Analogues by Location (μmol/g Wet Basis)

		saponin						
food[a]	% M[b]	V	I	II	αg	βg	βa	total saponins
raw[c]								
burger A	64	0 B	0.20 AB	0.11	0	0 B	0	0.31 AB
burger B	63	0 B	0.11 B	0.07	0	0 B	0	0.18 B
burger C	64	0.07 A	0.28 A	0.13	0	0 A	0	0.48 A
LSD[d]		0.01	0.10	0.07		0.00		0.18
cooked								
burger A	57	0.12 A	0.42 A	0.21 A	0	0.01 A	0.01 A	0.77 A
burger B	60	0.11 A	0.41 B	0.21 A	0	0 B	0 B	0.73 B
burger C	58	0.08 B	0.36 C	0.18 B	0	0 B	0 B	0.61 C
LSD		0.02	0.01	0.02		0.00	0.00	0.03

[a] $n = 2$. [b] Moisture. [c] Burger A = Harvest burger, west; burger B = Harvest burger, midwest composite; and burger C = Harvest burger, east composite. [d] See footnote [d] in **Table 1**.

from national sampling in Washington, DC, Houston, Tampa, Kansas City, Denver, New York, and a composite of Washington DC; tofus Azumaya, Hinoichi, and Nayasoya from national sampling; White Wave shiro and aka misos as composites of national sampling; White Wave tempehs; Worthington Foods chicken analogue, FriChik; Loma Linda meatless franks; and Harvest burgers; the latter three were from multicity composites. The details of sample selection, preparation, purchasing, compositing, and preservation are in ref *27*. The sample names in **Tables 1–5** are the same as in ref *27* so direct comparisons can be made between saponin and isoflavone concentrations. Additionally, commercially processed frozen edamame was purchased locally. Edamame products, in pods and without pods, were purchased to evaluate saponin and isoflavone concentrations. Natto was provided by Drs. Beecher and Nagano through the U.S. Department of Agriculture Food Composition Laboratory (Beltsville, MD). Some of the soy flours, soy protein isolates, soy protein concentrates, texturized soy proteins, and soy protein isolates (+ and −) were provided by Archer Daniels Midland Co. (ADM), Protein Technology International (PTI) now Solae, Dr. Connie Weaver (Purdue University), and Dr. Lee Alekel (Iowa State University).

Quantification of Group B Soyasaponin Content in Soy Products by High-Performance Liquid Chromatography (HPLC). Group B soyasaponin standards, V, I, II, αg, βg, and βa, were purified from soybeans and soy germ (*7*). The standard curves were obtained by plotting the soyasaponin concentration as a function of peak area obtained from HPLC at 205 nm.

Four grams of dried, finely ground soy samples was extracted with 100 mL of 70% aqueous ethanol with stirring for 2.5 h at room temperature in duplicate. Formononetin was used as an internal standard, and all data were corrected with an internal standard curve as in Hu et al. (*7*). Samples were analyzed by HPLC with an YMC-ODS-AM-303 column (RP-18, 5 μm, 4.6 mm × 250 mm, YMC, Inc., Wilmington, NC). The mobile phases were 0.05% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B) with gradient elution: solvent B increased from 37 to 40% in 12 min; then solvent B increased to 48% in 25 min; finally, solvent B increased to 100% in 1 min and was held at 100% for 2 min. The injection volume was 50 μL. The flow rate was 1 mL/min. The UV absorbance was monitored from 190 to 350 nm (*7*).

Quantification of Isoflavone Content in Soybeans and Soy Products. The isoflavone concentrations of all of the soybeans and soy products not previously reported elsewhere (*25–27*) were analyzed in duplicate according to Murphy et al. (*27*). These included edamamae soybeans and soy protein isolates N−S.

Statistical Analysis of Saponin Data. The samples were analyzed for soyasaponin concentrations in triplicate. Statistical analysis was conducted using the general linear models procedures. Analysis of variance was performed on means to determine differences that were significant at $\alpha = 0.5$ using the SAS program (version 6.03, 1995, Cary, NC).

RESULTS AND DISCUSSION

We were fortunate to have access to most of the soy foods analyzed as part of the U.S. Department of Agriculture−Iowa State University Isoflavone Database. These foods were in freeze-dried form and stored in sealed coded containers at −5 °C until analysis. This allows us to make direct comparisons between the isoflavones and the saponins in these foods.

All of the soymilk samples analyzed for the U.S. Department of Agriculture−Iowa State University Isoflavone Database (*27*) were available for saponin analysis. Both aseptically processed and pasteurized soy milks contained total saponins in similar proportion to isoflavones with aseptically packaged soymilk having about 2× the total saponins as pasteurized soy milks (**Table 1**). The highest saponin-containing soymilk was not the highest isoflavone-containing milk (*27*). The effects of heat processing of both products were evident by the lower concentrations of the DDMP-containing saponins, soyasaponins βg, βa, and αg, and higher proportions of soyasaponins I, II, and V, with saponin I the predominant form, as compared to a raw soy product (*7*).

Not all tofu samples analyzed for isoflavones in Murphy et al. (*27*) were still available for saponin assay. The distribution of the soyasaponin forms was slightly different than observed in soymilk samples (**Table 2**). Although tofu production involves heat processing similar to soymilk processing, somewhat higher proportions of soyasaponin βg survived as a mol % of the total saponins as compared to soy milks shown in **Table 1**. The distribution of the saponin forms is similar to the distribution of the heat-sensitive isoflavone forms reported in Murphy et al. (*27*). The heat-labile isoflavone-malonyl-β-glucosides were retained to a larger extent in tofus than they were in soymilks. The mild cooking of these tofus by steaming did not cause conversion of additional DDMP forms to more heat-stable saponins I, II, and V. In fact, the concentrations are somewhat higher since the cooking process reduced the % moisture.

The tofu and soymilks contain saponin concentrations very similar to whole soybeans at 3−5 μmol/g DW. Hu et al. (*7*) reported average saponin totals of 4.04 μmol/g with a range from 2.50 to 5.85 μmol/g on an "as is" basis. This conservation of saponin mole mass during soymilk and tofu production is similar to that reported by Wang and Murphy (*28*) for isoflavone mole mass.

Fermented foods, tempeh, miso, and natto, contain mixtures of saponin forms reflecting their heat-processing history (**Table 3**). The tempeh contains saponin levels similar to soybeans, while the misos' lower levels probably reflect the use of rice or wheat as the other major ingredient in miso manufacture. Tempeh soybeans are initially autoclaved to hydrate and sanitize the soybeans, which results in conversion of a significant portion of the DDMP saponins. The cooking of the tempeh resulted in no additional change in the distribution of the saponin forms. Miso production involves an initial autoclaving step prior to inoculation with miso mixed microorganism culture and a 2 month fermentation. The two types of miso were slightly different in saponin contents due to a difference in saponin V concentration. The natto products apparently had been completely dried with a high temperature heat source since no DDMP saponins were detected.

Meat analogue saponin levels seem to reflect the starting concentrations of saponins in the soy ingredients used to produce these foods (**Table 4**). The Frichick product, a soy-based chicken analogue, may be produced with a water-washed soy protein concentrate, while the franks were derived from an ethanol-

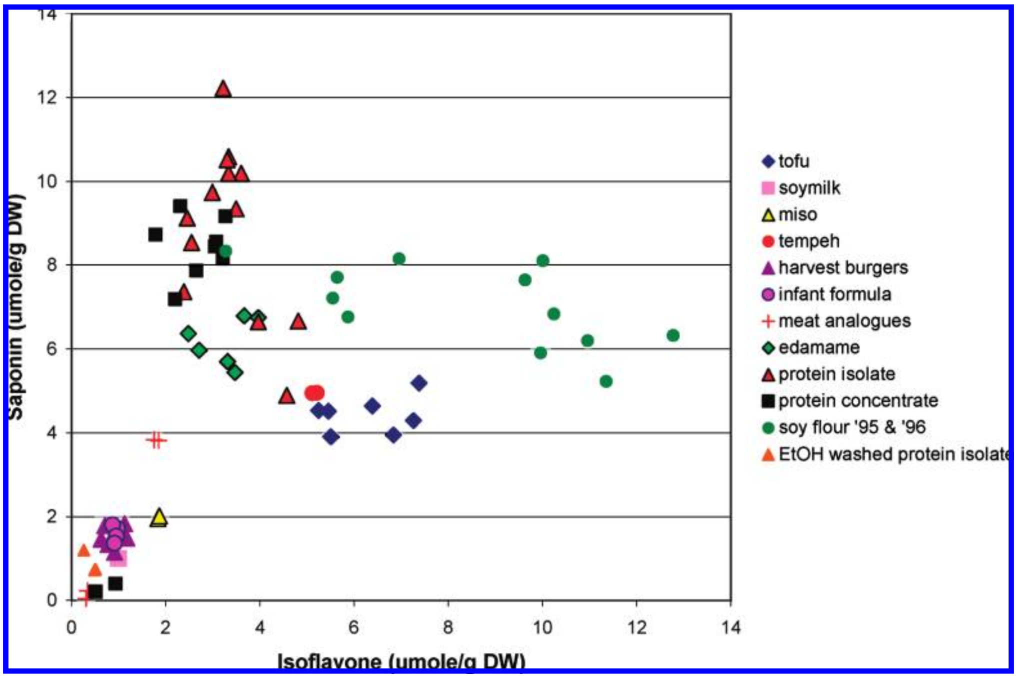

Figure 2. Comparison between isoflavones and group B soyasaponin contents of soybeans, soy ingredients, and soy foods (μmol/g). Data for isoflavone concentrations were taken from refs *24–29*.

Table 6. Group B Soyasaponin Contents of Soy Infant Formulas (Dry Concentrates) by Location of Purchase (μmol/g as Is Basis)

location	brand	saponin						total saponins
		V	I	II	αg	βg	βa	
east	Prosobee	0.09 AB	1.22 B	0.53 B	0.08 B	0.36 B	0.13 B	2.40 B
	Gerber	0.23 A	2.05 A	0.94 A	0.10 A	0.54 A	0.24 A	4.10 A
	Isomil	0.05 B	0.47 D	0.20 D	0.00 D	0.11 D	0.04 D	0.87 D
	Nursoy	0.08 AB	1.18 B	0.53 B	0.00 D	0.00 E	0.01 E	1.79 C
	Enfamil[a]	--						
	Alsoy	0.15 AB	0.83 C	0.39 C	0.02 C	0.16 C	0.09 C	1.65 C
	LSD	0.15	0.11	0.07	0.01	0.04	0.02	0.27
midwest	Prosobee	0.19 A	0.99 A	0.48 A	0.02 A	0.08 A	0.07 A	1.83 A
	Nursoy	0.09 C	0.95 AB	0.42 B	0.02 A	0.09 A	0.06 A	1.64 BC
	Enfamil	0.13 AB	1.01 A	0.40 B	0.01 B	0.03 B	0.04 B	1.81 AB
	Alsoy	0.08 C	0.89 B	0.41 B	0.02 A	0.09 A	0.06 A	1.56 C
	LSD	0.09	0.09	0.08	0.01	0.05	0.02	0.18
west	Prosobee	0.04 D	0.96 CD	0.36 B	0.4 A	0.17 B	0.09 B	1.66 CD
	Gerber	0.00 E	0.73 D	0.31 B	0.2 B	0.08 E	0.06 D	1.21 E
	Isomil	0.06 B	1.08 AB	0.43 A	0.02 B	0.13 C	0.08 BC	1.80 BC
	Nursoy[b]	0.20 A	1.02 BC	0.48 A	0.00 C	0.05 F	0.06 D	1.74 BCD
	Enfamil	0.08 B	1.17 A	0.46 A	0.02 B	0.11 CD	0.08 BC	1.93 AB
	Alsoy	0.21 A	1.03 BC	0.45 A	0.04 A	0.26 A	0.11 A	2.11 A
	LSD[c]	0.13	0.10	0.07	0.01	0.03	0.01	0.22

[a] Not available. [b] Only available as liquid concentrate; therefore, freeze-dried and assayed from two different lot numbers. [c] Values within a location in a column with different letters are different at ≤0.05; LSD = least significant difference; each sample was analyzed in duplicate. The moisture contents were as follows: Isomil, 2.50%; Prosobee, 3.00%; Nursoy, 2.00%; Enfamil, 3.30%; and Gerber, 2.00%; taken from U.S. Department of Agriculture National Nutrient Database for Standard Reference, release 20.

washed soy protein concentrate. Harvest burgers, produced with water-washed soy protein concentrates, contain modest saponin levels. Saponins I, II, and V were the predominant saponin forms reflecting major heat treatment in producing these soy analogues. Cooking these products results in higher concentrations of the non-DDMP saponin forms, but the increase was probably due to loss of moisture upon cooking (**Table 5**). School lunch program and U.S. Army hamburgers supplemented with soy protein contained no detectable saponins, although they were reported to contain very low but detectable amounts of isoflavones (*27*).

Soy infant formulas contain 0.87−4.10 μmol/g "as is basis" saponins principally as saponins I, II, and V (**Table 6**). The levels reflect the amount of soy protein isolate used in the formula's manufacture. Except for one infant formula in the east coast purchase lot, most of the soy infant formulas had relatively similar saponin distributions across the national distribution. The same trend was observed in isoflavone concentrations for these formulas (*27*). The reconstituted soy infant formulas would have about 0.10−0.40 μmol/mL based on package instructions for preparation of these formulas.

Edamame, or soy peas, contain saponin concentrations similar to soybeans of 5−7 μmol/g DW or 4−7 μmol/g wet weight (**Table 7**). These concentrations are essentially equivalent to mature soybeans levels (*7*) but somewhat lower than reported here for commercial soybean flours, which are ground mature soybeans (**Table 8**). The variation in saponin content in soybeans probably varies by variety and growing environment as reported

Table 7. Group B Soyasaponin Contents of Edamame (μmol/g Dry Weight Basis)

	saponin						
	V	I	II	αg	βg	βa	total saponins
raw bean							
A[a]	0.29 BC	0.70 F	0.23 DE	0.28 A	3.43 C	0.76 E	5.70 D
B[b]	0.29 BC	0.61 FG	0.21 E	0.23 AB	3.35 C	0.75 E	5.44 E
C[c]	0.30 B	0.42 H	0.20 E	0.08 DE	2.41 H	0.93 D	4.34 F
D[d]	0.17 D	0.52 GH	0.16 E	0.12 DE	2.32 H	1.20 C	4.49 F
microwave bean							
A	0.41 A	1.16 C	0.49 AB	0.22 B	2.93 F	0.66 G	5.85 CD
B	0.38 AB	1.28 B	0.53 A	0.16 CD	2.71 G	0.62 H	5.69 D
C	0.30 B	0.86 E	0.35 C	0.19 BC	3.74 A	1.36 A	6.79 A
boiled bean							
A	0.41 A	1.39 A	0.46 AB	0.22 B	3.20 D	0.70 F	6.37 B
B	0.37 AB	1.19 BC	0.42 BC	0.23 AB	3.06 E	0.70 F	5.97 C
C	0.33 AB	0.98 D	0.36 C	0.18 BC	3.59 B	1.31 B	6.75 A
microwave shells							
C[e]	0.19 CD	0.84 E	0.46 AB	0.02 F	0.33 I	0.20 J	2.04 G
boiling shells							
C	0.16 D	0.91 DE	0.33 CD	0.03 F	0.36 I	0.26 I	2.04 G
LSD[f]	0.10	0.11	0.10	0.06	0.10	0.03	0.24

[a] Raw frozen commercial beans, no pods, brand A. [b] Raw frozen commercial beans, no pods, brand B. [c] Raw frozen commercial beans, in pod, brand C, data for beans only. [d] Raw frozen commercial beans, in pods, brand D, data for beans only. [e] Inedible empty shells after cooking. [f] Values in a column with different letters are different at $\alpha < 0.05$; LSD = least significant difference; each sample was analyzed in duplicate. Edamame beans without seed pods averaged 66.38% moisture, while beans in pods averaged 74.30% moisture.

for isoflavone levels (*29*). The raw edemame products were probably blanched prior to freezing resulting in generation of non-DDMP forms. The saponin forms found in edamame are characteristic of minimally heat-processed products with saponins αg, βg, and βa as the predominant forms. Cooking by boiling or microwaving the edamame resulted in no loss of saponins. The edamame pods, the inedible portion of edamame, contained much lower saponin concentrations than the beans, in contrast to isoflavones where concentrations in pod and bean are very similar (data not shown).

Commercial defatted soybean flours, from the U.S. Department of Agriculture—Iowa State University Isoflavone Database set, contained 5−8 μmol/g DW (**Table 8**). These flours were derived from soybeans grown in 1995 and 1996. Several soy flours (A and I−L) appear to be heat-treated or toasted since these contain higher concentrations of saponins I, II, and V than raw or minimally heat-processed beans.

Soy protein concentrates occur as either water-washed or ethanol-washed products. The ethanol-washed concentrates are almost devoid of saponins at 0.20−0.40 μmol/g as they are of isoflavones (*26*). The ethanol-wasted soy protein concentrate saponins are all non-DDMP forms resulting from drying operations in production of this product. The water-washed concentrates contain 7.0−9.5 μmol/g DW. These water-washed protein concentrates are somewhat higher in saponin concentration due to losses of oligosaccharides and lipid (which are extracted prior to water washing commercially). The saponin forms in soy protein concentrate are principally the heat-generated saponins I, II, and V with reduced levels of the DDMP saponins as compared to the group B saponin distribution in soy flours. The redistribution of saponin forms was a result of the heating required in the final drying step during soy protein concentrate production. The water-washed soy protein concentrates are designed for food products, such as Harvest burgers,

Table 8. Group B Soyasaponin Contents of Commercial Soy Ingredients (μmol/g as Is Basis)

	saponin						
	V	I	II	αg	βg	βa	total saponins
soy flours							
A[a]	0.00	2.12	0.95	0.00	2.60	0.53	6.20
B[a]	0.00	0.24	0.12	0.13	5.41	0.86	6.77
C[a]	0.00	0.24	0.11	0.23	5.65	0.99	7.22
D[a]	0.00	0.00	0.08	0.26	6.10	1.03	8.15
E[a]	0.00	0.43	0.16	0.13	6.34	1.10	8.34
F[a]	0.00	0.39	0.14	0.15	6.50	1.15	8.34
G	0.00	0.72	0.33	0.00	5.90	1.17	8.11
H	0.00	0.49	0.30	0.24	4.83	0.99	7.65
I	0.00	1.01	0.52	0.22	4.87	1.02	7.65
J	0.00	1.59	0.72	0.08	2.43	0.42	5.91
K	0.00	1.53	0.78	0.09	2.69	0.57	5.91
L	0.48	2.70	1.31	0.09	1.45	0.53	6.32
soy defatted flakes							
A[a]	0.00	0.77	0.43	0.19	2.52	0.80	4.71
B[a]	0.00	0.49	0.31	0.21	3.50	1.15	5.66
soy protein concentrate							
A[a]	0.00	2.54	1.34	0.14	4.25	0.90	9.17
B[a]	0.00	2.41	1.05	0.19	4.90	0.86	9.41
C[a]	0.00	2.87	1.16	0.16	3.85	0.69	8.73
D[a]	0.00	2.67	1.11	0.13	3.36	0.60	7.87
E[a]	0.00	2.73	1.14	0.10	2.70	0.53	7.19
F[a]	0.00	0.22	0.18	0.00	0.00	0.00	0.40
G[a]	0.00	0.80	0.12	0.00	0.00	0.00	0.20
H[a]	0.05	0.10	0.07	0.00	0.00	0.00	0.21
I[a]	0.36	2.96	1.52	0.10	2.67	0.85	8.44
J[a]	0.48	3.11	1.40	0.10	2.23	0.83	8.16
K[a]	0.51	3.67	1.72	0.11	1.71	0.84	8.56
soy protein isolate							
A[a]	0.00	3.83	1.92	0.07	2.91	0.71	9.44
B[a]	0.65	5.10	2.58	0.03	1.10	0.72	10.19
C[a]	0.57	4.61	2.62	0.01	1.25	0.63	10.59
D[a]	0.60	5.45	2.89	0.08	1.50	0.65	10.59
E[a]	0.90	4.34	2.37	0.04	1.18	0.61	10.19
F[a]	0.00	5.29	2.40	0.06	2.05	0.71	10.52
G[a]	0.00	5.00	2.03	0.01	1.56	0.51	9.12
H[a]	0.31	3.68	1.87	0.00	0.59	0.21	6.65
I[a]	0.52	4.53	2.29	0.03	0.88	0.29	8.54
J[a]	0.00	4.14	2.12	0.03	0.80	0.28	7.37
K[b]	0.76	4.44	2.00	0.00	1.78	0.36	9.34
L[b]	0.78	4.99	2.08	0.00	1.78	0.36	9.72
M[b]	0.46	4.00	2.03	0.00	4.18	1.00	12.22
N[c]	0.59	3.30	1.43	0.07	0.93	0.34	6.67
O[c]	0.62	3.17	1.55	0.08	0.90	0.33	6.65
P[c]	0	0.48	0.18	0.08	0.01	0.04	0.72
Q[d]	0	0.49	0.19	0	0.02	0.04	0.74
R[d]	0.51	2.59	1.07	0.03	0.05	0.19	4.89
S[d]	0.32	0.49	0.34	0.01	0.01	0.04	1.20
texturized vegetable protein (TVP)							
A[a]	0.36	1.87	0.88	0.16	2.30	0.74	6.32

[a] ADM. [b] PTI = Solae. [c] Provided by Connie Weaver, Purdue University: N = Soy + AB, O = Soy + HP, and P = Soy-AB, Q-Soy-HP. [d] Provided by Lee Alekel, Iowa State University: R = SPI+, and S = SPI−.

so that isoflavones will be present in these products with a relatively insoluble form of soy protein.

Two types of soy protein isolate were available for analysis (**Table 8**). Traditional soy protein isolates, produced by acid precipitation of the proteins from a water extract of defatted soy flakes or flour, contain mostly the non-DDMP saponins, saponins I, II, and V, at total concentrations between 5 and 12 μmol/g DW. The non-DDMP saponins were probably generated during spray drying of the soy protein isolates (*10*). The heat-labile isoflavone malonyl-β-glucosides follow a similar pattern in these products (*10*). The second type of soy protein isolates is used in research feeding studies typically and is called "soy −" or "soy minus", indicating that these isolates have been extracted with ethanol to remove isoflavones. The three soy minus protein isolates evaluated here have much reduced saponin levels (soy protein isolates P, Q, and S). The DDMP saponin forms are present in much lower proportions as compared to the non-DDMP saponins as compared to traditional soy protein isolate saponins. The protein isolates analyzed here represent the major isolate-producing companies in the United States.

Rickert et al. (*10*) reported saponin fractionation during soy storage protein fractionation. Saponins preferentially fractionated with the β-conglycinin fraction and a denatured intermediate protein fraction, and little saponins were lost to soy whey. Isoflavones were partially lost to soy whey. Saponin concentrations averaged around 4 μmol/g in the β-conglycinin protein fraction and around 12.80 μmol/g in the denatured intermediate fraction. The intermediate fraction of Rickert et al. (*10*) is most similar to a commercial soy protein isolate. Lin et al. (*13*) reported saponins with process variations in soy isolate production with about 6 μmol saponin/g totals, which is somewhat lower than that reported here for commercial soy protein isolates. These authors reported that temperature changes for protein precipitation did not affect saponin concentrations in contrast to what they observed for isoflavone partitioning. Isoflavones were partially lost to washing water and whey.

Correlations between Group B Soyasaponins and Isoflavones. There was no apparent correlation between the whole mature soybeans' group B saponins and isoflavones nor in commercial soy flours (**Figure 2**). These data are in agreement with Rupasinghe et al. (*14*) for Canadian soybean varieties and Hubert et al. (*12*) for French- and U.S.-grown soybeans. However, the soy ingredients, soy protein isolates, and water-washed soy protein concentrates contained 2−5× more saponins than isoflavones. The soy foods examined approximated a 1:1 to 2:1 ratio of saponins to isoflavones. Edamame soybeans were more similar to other soy foods than mature soybeans in their saponin:isoflavone ratio. Clearly, the ingredients used in many cholesterol-lowering studies reported to date, soy protein isolates, do not compare equally in their saponin:isoflavone ratios as compared to other soy foods. Lin et al. (*13*) reported a saponin:isoflavone ratio of 1.65:1 (converted to μmol basis) for soy protein isolates that they produced. Rickert et al. (*10*) reported saponin:isoflavone ratios of fractionated soy storage proteins of 3.65:1 to 5.46:1 for β-conglycinin, 4.5−5.0:1 for their intermediate fraction, 0.20:1 for glycinin, and 0.07:1 for soy whey. Nutraceutical products such as Novasoy or soy hypocotyls have group B saponin:isoflavone ratios of 1:9 to 1:20, which are quite different than any soy food ratio (*7*). The more hydrophobic saponins appear to preferentially fractionate with the soy protein fraction as compared to the isoflavones (**Figure 1**) (*11*). Interpretation of the cholesterol-lowering ability of soy-associated isoflavones may need to be re-examined given the different relationships observed here for soy foods' and soy ingredients' saponin/isoflavone correlations.

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