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Isoflavone Aglycon and Glucoconjugate Content of High- and Low-Soy U.K. Foods Used in Nutritional Studies

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The isoflavone aglycon and glucoconjugate content of commercially prepared and "home-prepared" high- and low-soy foods selected for use in an on-going nutritional study were measured by LC-MS. The daidzin, daidzein, 6′′-*O*-malonyldaidzin, 6′′-*O*-acetyldaidzin, genistein, genistin, 6′′-*O*-malonylgenistin, 6′′-*O*-acetylgenistin, glycitin, glycitein, 6′′-*O*-malonylglycitin, and 6′′-*O*-acetylglycitin content are expressed in terms of individual isoflavones, total isoflavone equivalents, and milligrams of isoflavones per portion served. Soybeans (774 mg kg^{-1} total isoflavones) and soybean-containing foods had the highest isoflavone content of the foods examined. The low-soy foods all contained very low concentrations (<8 mg kg^{-1} total isoflavones) of the isoflavone aglycons and glucoconjugates. High- and low-soy 11 day rotating menus were constructed from the analyzed foods to deliver 100.0 and 0.5 mg of isoflavones per day, respectively.

KEYWORDS: Phytoestrogens; genistein; daidzein; isoflavones; soy foods; glucoconjugates; LC-MS; food ingredients

INTRODUCTION

Phytoestrogens are being extensively investigated to determine their beneficial health role and therapeutic potential, particularly in disease prevention (*1*–*4*). The soy isoflavone phytoestrogens genistein, daidzein, and glycitein are plant-derived nonsteroidal estrogen mimics (*1*–*4*). Epidemiological studies in Far Eastern counties, where there is an abundance of soy phytoestrogens in the diet, have indicated a protective effect of soy consumption against hormone-dependent cancer of the breast and prostate (*1*), and it is likely that soy phytoestrogens also protect against bowel cancer, heart disease, menopausal symptoms, and osteoporosis (*2*, *3*). Isoflavones are ligands for both the ERα and ERβ estrogen receptors, with genistein showing preferential binding to ERβ (*5*). The predominant isoflavones genistein, daidzein, and glycitein occur mainly as glucoconjugates in soybeans and consequently in a wide range of soy-derived foods and to a lesser extent in other legumes (*6*, *7*). Traditional soy foods are made from soybeans and include both fermented and nonfermented foods. Nonfermented soy foods contain isoflavones primarily as β-glucosides, some of which are further esterified with malonic or acetic acids. Fermented soy foods such as miso or tempeh contain mostly the unconjugated isoflavone aglycons (*8*).

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The genistein and daidzein content of vegetables and vegetable products in the United Kingdom has recently been reported (*9*). Foods derived from soybeans contained the highest concentration of genistein and daidzein, followed by the legume group and finally other vegetables. In addition, the daidzein and genistein content of fruits and nuts has also been quantified, and currants and raisins were found to be the richest sources (*10*). Recent work has also shown that there are other significant nonsoy sources of isoflavones, including sprouted beans, herbs, and spice mixes, that have been excluded from many studies as they contribute an insignificant mass to the daily diet. Some of these individual items can contain >500 mg of genistein kg^{-1}. These minor dietary components can, when considered together, contribute to a significant intake of isoflavones.

Previous studies have generally quantified the isoflavone content of foods, in terms of the genistein and daidzein content (*7*, *11*), following an acid or enzymatic hydrolysis step. More information is clearly needed regarding the isoflavone glycoconjugate and aglycon content of both high- and low-soy foods and also regarding their rates of absorption, metabolism, excretion, and overall bioavailability. The isoflavone content of soy foods depends primarily on the isoflavone content of the soybean source. The total isoflavone aglycon and glucoside content of soybeans is generally in the range of 1000–4000 mg kg^{-1}. The USDA–Iowa State University isoflavone database suggests that typical raw, full-fat soy flour contains 712, 968, and 162 mg kg^{-1}, respectively, of daidzein, genistein, and

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glycitein aglycon equivalents (*12*). Distribution of the glucoside forms within this total is even more variable; Japanese soy can contain a 10-fold higher proportion of the malonylglucosides to glucosides than do U.S. soy products (*13*). Acetylglucosides, originally isolated from toasted defatted soyflakes (*14*), are generally found at low levels in the intact minimally processed soybean. It is accepted that the malonyl esters are stable in foods but unstable in solution at high temperatures, where they will undergo decarboxylation to the corresponding acetyl ester, and that this degradation is the source of most measured acetylglucosides in soy foods (*15*). At room temperature the rate of degradation slows to an acceptable level, whereas heat processing, enzymatic hydrolysis, and fermentation all alter the distribution of isoflavone forms.

The aim of this study was to measure the daidzin, daidzein, 6″-*O*-malonyldaidzin, 6″-*O*-acetyldaidzin, genistein, genistin, 6″-*O*-malonylgenistin, 6″-*O*-acetylgenistin, glycitin, glycitein, 6″-*O*-malonylglycitin, and 6″-*O*-acetylglycitin content of selected high- and low-soy foods. Foods were analyzed without hydrolysis to enable quantification of the individual isoflavone glucoconjugates and aglycons. The high- and low-soy foods were required for a controlled dietary intervention study of chronic soy consumption in healthy human subjects, for the purpose of studying isoflavone metabolism and bioavailability. The foods were used to construct high- and low-soy isoflavone diets on an 11-day rotating menu.

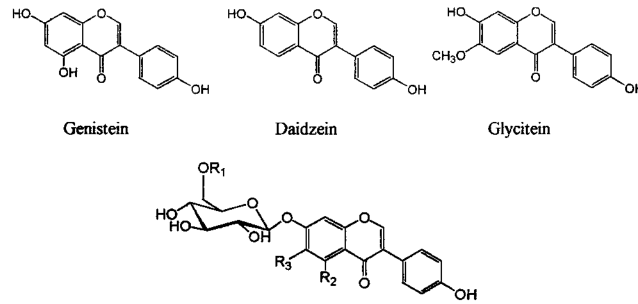
MATERIALS AND METHODS

Food Preparation. The commercially prepared high- and low-soy foods were purchased from supermarkets in London, U.K. The "home-prepared" high- and low-soy foods were prepared in the metabolic kitchen in the Department of Nutrition and Dietetics, King's College London, U.K. The food samples were freeze-dried to constant weight, powdered, and stored at −70 °C prior to analysis for aglycon and conjugate content.

Reference Compounds. Daidzein, daidzin, genistein, genistin, glycitein, glycitin, biochanin A, formononetin, and coumestrol were purchased from Apin Chemicals (Abingdon, U.K.). Genistein-4′-glucoside was purchased from Plantech (Reading, U.K.). [$^2H_4$]Daidzein was supplied by Dr. N. Botting (St. Andrews University, U.K.). The reference compounds were stored at −18 °C. Stock solutions were prepared in ethanol and stored at 4 °C. The purity of standards was assessed by HPLC-UV, and any containing >1% of contaminants or byproducts were discarded. The purity of daidzein and genistein analytical standards was further assessed by an interlaboratory trial in which five European laboratories provided extinction coefficient data on separately sourced standards. UV measurements were made in ethanol at 262 nm.

Extraction. Sample extraction was an adaptation of the method of Coward (*8*). Single replicates of freeze-dried food (0.5 g) and 5 μg of [$^2H_4$]daidzein (internal standard) were dispersed by sonication and extracted with 80% aqueous ethanol (5 mL) by stirring for 1 h at 60 °C. The mixture was cooled and centrifuged at 8000*g* for 5 min, the solvent extract aspirated off, and the residue pellet resuspended in further aqueous ethanol (2 × 2.5 mL). The combined extract was reduced in volume (5 mL), and lipids were removed by partitioning into and discarding a hexane wash (4 × 20 mL). The extract was dried under a nitrogen stream and the residue dissolved in 50% aqueous methanol (10 mL). An aliquot (2 mL) of the extract was filtered (0.45 μm) prior to HPLC analysis.

Chromatographic Conditions. Analysis was carried out on an LC-UV-MS system comprising an Agilent quaternary HP1100 pump and solvent degasser (Waldbronn, Germany), a Gilson 231 XL autosampler, with a 20 μL sample loop (Anachem, Luton, U.K.), a column oven (Jones Chromatography, U.K.), a Spectroflow 757 UV detector (Kratos, Ramsey, NJ), and a platform benchtop mass spectrometer and a MassLynx data handling system (Micromass, Manchester, U.K.). The UV signal was collected as an analogue input into the MassLynx data handling system. Reversed phase separation was carried out at 30 °C using a 250 × 3.2 mm i.d., 5 μm YMC ODSAM 250 AS C_{18} column fitted with a 30 × 3.2 mm guard column of identical phase. Elution was at 0.5 mL min^{-1} with the solvent systems A = water/acetic acid (0.5%) and B = acetonitrile/acetic acid (0.5%): 15% B in A (v/v) linearly to 22% B at 30 min, 35% at 50 min, 45% at 52 min, 46% at 53 min, 70% at 56 min, 80% at 60 min, 85% at 62 min, and 15% at 65 min. The system was equilibrated for a further 7 min between injections. Analytes were monitored at 262 nm through the UV detector and then passed into the MS source. Mass spectra were run in +APcI mode as an SIM program. The source temperature was 140 °C, and the probe temperature was 500 °C; the cone voltage was 10 V with a 5 V skimmer lens offset. A blank food that was free of isoflavones was used to prepare matrix-matched calibration standards. This blank was also spiked with standards and used to calculate experimental recovery and to correct for experimental losses. Concentrations of glucoside and aglycon isoflavones were calculated from the LC-MS standard curves of authentic isoflavone standards normalized to the amount of [$^2H_4$]-daidzein added to each sample. The $[M + H]^+$ ions of the 12 daidzein, genistein, and glycitein soy aglycon and glucoconjugates, biochanin A, formononetin, coumestrol, and [$^2H_4$]daidzein were monitored as an SIM program. The deuterated internal standard in each extract was used as a retention time normalization point. Concentrations of acetyl- and malonylglucoside were semiquantified using UV molar equivalents of the appropriate glucoside after peak identification by LC-MS (*16*). Concentrations were expressed as milligrams per kilogram of whole food (ppm).



R1	R2	R3	compound	molecular weight	aglycone equivalents
-	-	-	genistein	270	1.000
H	OH	H	genistin	432	0.625
$COCH_3$	OH	H	6''-*O*-acetylgenistin	474	0.570
$COCH_2COOH$	OH	H	6''-*O*-malonylgenistin	518	0.521
-	-	-	daidzein	254	1.000
H	H	H	daidzin	416	0.613
$COCH_3$	H	H	6''-*O*-acetyldaidzin	458	0.555
$COCH_2COOH$	H	H	6''-*O*-malonyldaidzin	502	0.506
-	-	-	glycitein	284	1.000
H	H	OCH_3	glycitin	446	0.637
$COCH_3$	H	OCH_3	6''-*O*-acetylglycitin	488	0.582
$COCH_2COOH$	H	OCH_3	6''-*O*-malonylglycitin	532	0.534

Figure 1. Chemical structures, molecular weights, and aglycon molecular equivalents of the soy isoflavones.

RESULTS AND DISCUSSION

Standard Solutions. Molar extinction coefficients for the daidzein, daidzin, genistein, and genistin standards used in this work (**Figure 1**) were found to be in good agreement (within 5%) with the published values (*17*). A further interlaboratory trial (supported by the Food Standards Agency Phytoestrogen Research Program) demonstrated that when the solvent choice and wavelength are standardized at 100% ethanol and 262 nm, respectively, the between-laboratory coefficient of variation in such purity measurements is 1−2% (**Table 1**).

Isoflavone Content of Foods. The individual isoflavone content of the high-soy foods is shown on a milligrams per kilogram wet weight of food basis in **Table 2**. Meals 3 and 7 were found to contain a further phytoestrogen, coumestrol, at 4

Table 1. Measurement of Isoflavone Extinction Coefficients by Interlaboratory Trial[a]

laboratory	genistein (M^{-1} cm^{-1})	daidzein (M^{-1} cm^{-1})
1	35751	24864
2	36481	24406
3	35585	25629
4	36001	24044
5	35394	24751
mean ± SD	35842 ± 421	24739 ± 592
CV %	1.2	2.4

[a] Measurements conducted in 100% ethanol at 262 nm.

and 1 mg kg^{-1}, respectively. Formononetin and biochanin A can co-occur in soy products but were not detected in this work. A limit of quantitation of 0.1 mg kg^{-1} was generally achieved; data are rounded to whole ppm for presentation. Of the commercially prepared soy foods, the highest concentrations of isoflavones were found in a strawberry milk drink (food 13, i.e., 121 mg kg^{-1} daidzin and 132 mg kg^{-1} genistin). Of the home-prepared foods the mixed soybeans and baked beans (food 5) had the highest overall isoflavone content (i.e., 256 mg kg^{-1} daidzin, 67 mg kg^{-1} 6-malonyldaidzin, 213 mg kg^{-1} genistin, and 88 mg kg^{-1} 6-malonylgenistin). This is likely to have come from the soybean content. Soybeans (food 23) were found to contain approximately twice the isoflavone content of the final dish (457 mg kg^{-1} daidzin, 117 mg kg^{-1} 6-malonyldaidzin, 436 mg kg^{-1} genistin, and 156 mg kg^{-1} 6-malonylgenistin). The high-soy spaghetti bolognaise dish (food 1) contained textured soy protein (TSP) as the soy source and was found to contain just 14 mg kg^{-1} daidzin, 10 mg kg^{-1} daidzein, 10 mg kg^{-1} 6-acetyldaidzin, 23 mg kg^{-1} genistin, 20 mg kg^{-1} genistein, 8 mg kg^{-1} 6-malonylgenistin, and 18 mg kg^{-1} 6-acetylgenistin. The results show that in all of these soy foods the isoflavones were predominantly in the glucoside, malonyl, and acetyl conjugated forms. An increase in the ratio of acetyl to malonyl glucosides gives an indication of which foods have been subject to aggressive processing or preparation conditions. Soy milks and soy milk desserts had the lowest ratio, whereas "home-prepared" foods with extensive cooking times such as casseroles (e.g., turkey and soybean) had undergone extensive conjugate decarboxylation. The conjugate profiles observed in the current work are consistent with the soy products containing American rather than Japanese soybean varieties.Triplicate 50 mg replicates of a quality assurance soy flour sample were analyzed in each analytical batch. The between-batch variation associated with these measurements was <30%; within-batch variation was generally <10% where standards were available. The quality assurance soy flour sample matched the American soybean profile with glucose 52, malonylglucose 38, acetylglucose 9, and aglycon <1, as percent total actual genistein, daidzein, and glycitein aglycon and conjugate concentrations.

Unexpectedly low concentrations of isoflavones were found in two commercially prepared soy foods [soy burgers (food 22) and soy meatballs with spaghetti (food 8)]. This is presumably a result of alcohol extraction of the TSP or a similar procedure, which is commonplace in the food industry, to increase the digestibility and palatability of the soy protein.

Table 3 shows the isoflavone content of the low-soy foods on a milligrams per kilogram wet weight of food basis, and these were mostly below or very close to the limit of determination. **Tables 4** and **5** show the total daidzein, genistein, and glycitein equivalents and the total isoflavone equivalents present in the high- and low-soy foods, respectively, on a milligrams per kilogram wet weight basis and the total isoflavone equivalents per portion served. The strawberry soy milk drink (food 13) provided the most isoflavones per portion of the soy milk items tested (52 mg of total isoflavones). A portion of the turkey and soybean casserole (food 9) provided a similar amount of isoflavones (69 mg of total isoflavones) as a portion of the lamb

Table 2. Isoflavone Aglycon and Glucoside Content of High-Soy Food Samples (Milligrams per Kilogram w/w)

food no.	food item	% moisture	daidzin	daidzein	6-malonyl-daidzin	6-acetyl-daidzin	genistin	genistein	6-malonyl-genistin	6-acetyl-genistin	glycitin	glycitein	6-malonyl-glycitin	6-acetyl-glycitin
1	spaghetti bolognaise	78	14	10	5	10	23	20	8	18	2	1	1	nd[a]
2	lamb stew	74	43	6	29	7	43	9	41	5	8	nd	4	nd
3	turkey curry	74	25	12	6	12	32	26	11	19	6	1	nd	2
4	turkey chilli (with soybeans and red kidney beans)	66	140	3	41	10	120	4	50	12	nd	nd	8	1
5	mixed soybeans and baked beans	66	256	4	67	15	213	6	88	22	nd	nd	10	2
6	mixed soybeans, red kidney beans, and vegetables	65	160	3	41	9	131	3	53	14	nd	nd	6	1
7	soy sausage and batter dish "toad in the hole"	48	24	4	16	8	32	nd	26	18	nd	1	5	4
8	soy "meatballs" and spaghetti	75	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
9	turkey and soybean casserole	73	70	14	10	14	68	24	15	24	nd	1	nd	2
10	hazelnut soy milk pudding	77	56	8	7	1	72	15	18	2	nd	nd	nd	nd
11	chocolate soy milk pudding	73	40	3	nd	nd	58	2	7	2	nd	nd	nd	nd
12	chocolate soy milk drink	83	67	3	nd	nd	75	2	4	1	nd	nd	nd	nd
13	strawberry soy milk drink	85	121	12	13	1	132	17	30	3	nd	nd	nd	nd
14	banana soy milk drink	82	69	4	nd	nd	83	4	5	1	nd	nd	nd	nd
15	plain soy milk	93	50	4	8	1	83	6	19	2	nd	nd	nd	nd
16	vanilla soy milk yogurt	83	66	13	19	1	128	20	37	1	nd	nd	nd	nd
17	cherry soy milk yogurt	80	55	10	18	1	109	18	36	1	nd	nd	nd	nd
18	vanilla soy milk dessert	75	43	1	7	1	76	nd	13	3	nd	nd	nd	nd
19	custard (with soy milk)	81	63	2	15	1	86	1	27	3	nd	nd	nd	nd
20	banana cake (with soy flour)	31	39	21	19	2	57	35	32	7	6	2	nd	nd
21	soy sausages (1)	41	40	7	21	18	78	4	48	51	16	2	5	6
22	soy burgers	55	5	nd	nd	4	7	nd	nd	6	nd	nd	nd	nd
23	soybeans	62	457	5	117	18	436	3	156	37	50	1	15	2
24	soy sausages (2)	41	74	9	28	20	93	16	47	46	30	2	8	6

[a] nd, not detected.

Table 3. Isoflavone Aglycon and Glucoside Content of Low-Soy Food Samples (Milligrams per Kilogram w/w)

food no.	food item	% moisture	daidzin	daidzein	6-malonyl-daidzin	6-acetyl-daidzin	genistin	genistein	6-malonyl-genistin	6-acetyl-genistin	glycitin	glycitein	6-malonyl-glycitin	6-acetyl-glycitin
25	spaghetti bolognaise	75	nd[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
26	lamb stew	75	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
27	turkey curry	72	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
28	turkey chilli	73	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
29	baked beans	69	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
30	mixed red kidney beans, baked beans, and vegetables	81	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
31	pork sausage and batter dish "toad in the hole"	49	2	1	nd	nd	2	2	4	nd	nd	nd	nd	nd
32	turkey and red kidney bean casserole	73	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
33	vegetable lasagne	72	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
34	wholemeal bread	39	nd	1	1	nd	nd	nd	1	nd	nd	nd	nd	nd
35	white bread	36	1	1	nd	nd	nd	5	nd	nd	nd	nd	nd	nd
36	apple pie	22	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
37	sponge cake	16	2	nd	nd	nd	2	1	nd	nd	nd	nd	nd	nd
38	custard	77	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
39	currant bun	30	2	nd	2	nd	1	nd	2	nd	nd	nd	nd	nd

[a] nd, not detected.

Table 4. Total Isoflavone Equivalents (Milligrams per Kilogram w/w) and Isoflavones per Serving (Grams) of High-Soy Foods

meal no.	food item	soy source	total daidzein equiv	total genistein equiv	total glycitein equiv	total isoflavone equiv	portion size (g)	total isoflavones per serving (mg)
1	spaghetti bolognaise	textured soy protein	26	49	3	78	305	24
2	lamb stew	soy flour	50	60	8	118	610	72
3	turkey curry	textured soy protein	37	63	6	106	250	26
4	turkey chilli (soybeans and red kidney beans)	soybeans	113	112	5	231	191	44
5	mixed soybeans and baked beans	soybeans	203	198	6	407	80	32
6	mixed soybeans, red kidney beans, and vegetables	soybeans	125	120	4	249	270	67
7	soy sausage and batter dish "toad in the hole"	C[a]	31	44	6	81	190	15
8	soy "meatballs" and spaghetti	C	nd[b]	nd	nd	nd	350	nd
9	turkey and soybean casserole	soybeans	69	88	2	159	435	69
10	hazelnut soy milk pudding	C	46	71	nd	117	125	15
11	chocolate soy milk pudding	C	27	42	nd	69	125	9
12	chocolate milk drink	C	44	52	nd	96	250	24
13	strawberry milk drink	C	93	116	nd	209	250	52
14	banana milk drink	C	46	59	nd	105	250	26
15	plain milk	C	39	69	nd	108	280	30
16	vanilla soy milk yogurt	C	63	120	nd	183	125	23
17	cherry soy milk yogurt	C	53	106	nd	159	125	20
18	vanilla soy milk pudding	C	32	56	nd	88	125	11
19	custard	soy milk	49	71	nd	120	125	15
20	banana cake	soy flour	56	91	5	152	77	12
21	soy sausages (1)	C	52	106	18	176	60	11
22	soy burgers	C	6	8	nd	14	60	0.8
23	soybeans	C	354	378	42	774	104	80
24	soy sausages (2)	C	80	125	28	233	60	14

[a] C, commercially prepared. [b] nd, not detected.

stew (food 2, 72 mg of total isoflavones; soy flour was added here as the soy source).

Most previous studies of the isoflavone content of soybeans and soy foods report values for genistein and daidzein following acid hydrolysis (*7*) or enzymatic hydrolysis (*9*) of the glycosidic bond. The isoflavone content of the soy milks (foods 12−15) reported here and expressed in terms of genistein and daidzein equivalents (**Table 4**) are broadly in agreement with previous studies (*7*), as are our values for the isoflavone content of soybeans (food 23) (*7*, *9*). One previous study measured the concentrations of genistin and daidzin but not the concentrations of the glucoconjugates (*8*).

The extraction profiles of minimally processed soy products ("first-generation" soy products) are well documented, and HPLC coupled with modern photodiode array detection (PDA) software continues to be successfully applied to the resolution and quantitation of isoflavone glucoconjugates. In soy products where small proportions of soy are incorporated into complex food items ("second-generation" soy products), coextractable interference can make peak identification by UV alone questionable. Mass spectrometric (MS) detection is the only technique able to correctly identify soy isoflavones in complex matrices and to identify other nonsoy isoflavone glycoconjugates in foods. Failure to monitor for the presence of other isoflavone conjugates can lead to an underestimation of the aglycon equivalent content relative to actual hydrolysis and analysis. As soy was the predominant isoflavone source in this work, the nonglycolytic procedure was considered to be appropriate. The extraction

Table 5. Total Isoflavone Equivalents (Milligrams per Kilogram w/w) and Isoflavones per Serving (Grams) of Low-Soy Foods

meal no.	food item	soy source	total daidzein equiv	total genistein equiv	total glycitein equiv	total isoflavone equiv	portion size (g)	total isoflavones per serving (mg)
25	spaghetti bolognaise	nil	nd[a]	nd	nd	nd	145	nd
26	lamb stew	nil	nd	nd	nd	nd	509	nd
27	turkey curry	nil	nd	nd	nd	nd	104	nd
28	turkey chilli	nil	nd	nd	nd	nd	275	nd
29	baked beans	C[b]	nd	nd	nd	nd	50	nd
30	mixed red kidney bean, baked beans, and vegetables	nil	nd	nd	nd	nd	338	nd
31	pork sausage and batter dish "toad in the hole"	C	2	6	nd	8	120	1.0
32	turkey and red kidney bean casserole	nil	nd	nd	nd	nd	248	nd
33	vegetable lasagne	C	nd	nd	nd	nd	300	nd
34	wholemeal bread	C	2	1	nd	3	70	0.2
35	white bread	C	2	5	nd	7	70	0.5
36	apple pie	C	nd	nd	nd	nd	65	nd
37	sponge cake	C	1	1	nd	2	40	0.1
38	custard	C	nd	nd	nd	nd	125	nd
39	currant bun	C	2	2	nd	4	34	0.1

[a] nd, not detected [b] C, commercially prepared.

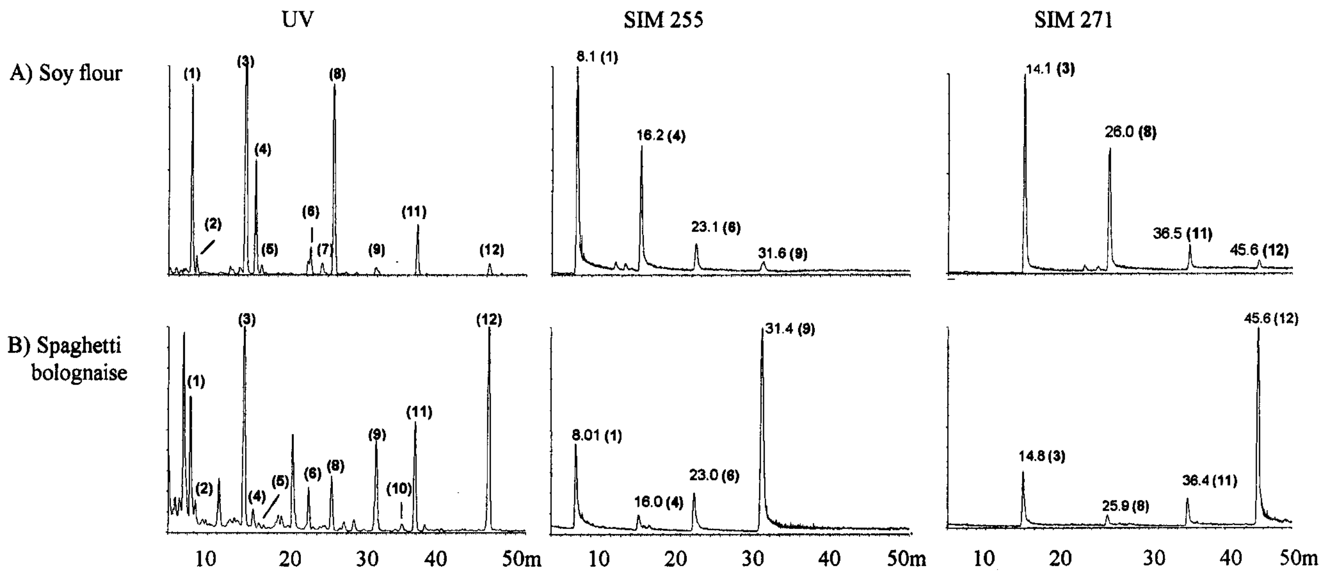


Figure 2. UV and SIM chromatograms of (A) soy flour standard and (B) high-soy (HS) spaghetti bolognaise (with textured soy protein). Peak elution order and identification: (1) daidzin, (2) glycitin, (3) genistin, (4) malonyldaidzin, (5) malonylglycitin, (6) acetyldaidzin, (7) acetylglycitin, (8) malonylgenistin, (9) daidzein, (10) glycitein, (11) acetylgenistin, and (12) genistein. APcI $[M + H]^+$ SIM ions of mass 255 and 271 relate to molecules containing daidzein and genistein.

profile of a spaghetti bolognaise containing TSP (food 1) illustrates the changes in conjugation observed after the extrusion and cooking processes (**Figure 2**). The majority of the genistin and malonylgenistin has been degraded to the free aglycon genistein. As the majority of soy-containing foods are highly processed and the different isoflavone forms may exhibit different rates of absorption from the gut following consumption, it is important to have information on the conjugation profile of the soy foods consumed when the data obtained from studies of soy isoflavone metabolism are interpreted.

It is relatively straightforward to monitor the presence of further isoflavone conjugates; these appear as components of similar mass but with different retention characteristics. Only one isoflavone conjugate from a nonsoy source was identified in significant amounts, sophoricoside, genistein-4′-*O*-glucoside; this was observed at a low milligrams per kilogram level in the low-soy turkey curry meal. This identification was confirmed by comparison with an authentic standard and was traced back to the use of the spice blend garam masala in this "home-prepared" meal. This is the first reported occurrence of genistein-4′-*O*-glucoside in a food item.

Daily intake figures for isoflavones are difficult to determine. Unpublished results (D. B. Clarke et al.) suggest that the isoflavone content in foods consumed in the United Kingdom has increased from the <1 mg/day estimated in the late 1980s (*18*). Japanese consumers have been assigned intake values from 25 mg day^{-1} (*19*) to 150−200 mg day^{-1} of total isoflavones (*20*), although a figure of 50 mg day^{-1} is probably more realistic (*21*).

Isoflavone glucosides have been shown to have similar relative estrogenic potencies to the corresponding aglycons (*22*). No data are available on the estrogenicity of acetyl- and malonylglucosides, but it is expected that they will have activities similar to those of both the parent aglycons and glucosides. The absorption rates and bioavailabilities of the aglycons and glucosides differ, as do the rates between pure components and

Table 6. Daily Menu and Daily Total Isoflavone Equivalents Intake (Milligrams per Day) of High-Soy Foods

	meal position in 11 day cycle[a]										
	1	2	3	4	5	6	7	8	9	10	11
meal items	16	10	11	11	13	11	11	10	11	18	10
	12	12	13	10	4	12	6	12	13	13	13
	1	2	9	5	19	3	18	7	5	7	5
	11	11		14	20	10	12	5	10		
					11						
isoflavones per serving (mg)	23	15	9	9	52	9	9	15	9	11	15
	24	24	52	15	44	24	67	24	52	52	52
	24	72	69	32	15	26	11	15	32	15	32
	9	9		26	12	15	24	32	15		
					9						
daily total (mg)	80	120	130	82	132	74	111	86	108	78	99

[a] Mean isoflavone intake over the 11 day cycle was 100.0 ± 21.4 mg.

foods. Industrial processing can alter the expected distribution of isoflavones. Soybeans generally contain 10% glycitein, which can increase up to 40% of the total isoflavones in products that themselves can have several times the isoflavone content of soy flour. Soy germ has a daidzein/genistein/glycitein ratio of 4:1:3, containing three times more glycitein than genistein (*23*–*25*). Soy flour can be produced from the hypocotyl portion of the soybean; we find this material has a daidzein/genistein/glycitein ratio of 2:1:1 (4290, 1930, and 2050 mg kg^{-1} daidzein, genistein, and glycitein aglycons). This was a 3-fold increase in isoflavone content relative to a standard soy flour milled from the same batch of beans (0.7:1:0.1: 1000, 1530, and 180 mg kg^{-1}). Daidzein and glycitein are concentrated in the hypocotyl portion of the soybean, whereas genistein is more evenly distributed throughout. Such concentrated isoflavone sources are being developed for the rapidly expanding nutraceuticals market. An increasing proportion of second-generation soy products can be expected to deliver higher ratios of glycitein in the future. As the relative estrogenic potency of the soy isoflavone aglycons is glycitein > genistein > daidzein (*26*, *27*), glycitein has the greater contribution to the overall estrogenic potency of some foods and dietary supplements. When attempts are made to assess the effects of dietary intervention, it is essential to have considered the distribution and ratio of the various conjugated forms in a particular food matrix as consumed. It is even more critical to have measured all of the isoflavones, including glycitein, which has often been overlooked.

The high-soy rotating diet, in use in our on-going dietary intervention study, was constructed to provide an average of 100.0 mg of total isoflavones day^{-1} (**Table 6**). The corresponding low-soy diet was constructed to provide an average of 0.5 mg of total isoflavones day^{-1}. High-soy foods were thus selected for this study with an aim of providing an average of 100 mg total isoflavones day^{-1}, which is an exposure level considered to be appropriate to the studies on the gut microflora being undertaken. The food items analyzed (**Tables 2** and **3**) made up the bulk of the subjects' diets; filler foods were used to complete the diets. Both high- and low-soy diets had a balanced energy intake of 1550 kcal/day.

In conclusion, the results reported here contribute new data on the isoflavone aglycon and glucoconjugate content of a selection of high-soy foods compared to low-soy foods.

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