Daidzein and genistein contents of vegetables

J. Liggins[1], L. J. C. Bluck[2], S. Runswick[1], C. Atkinson[1], W. A. Coward[2] and S. A. Bingham[1]*

[1]*Medical Research Council, Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 2XY, UK*

[2]*Medical Research Council, Human Nutrition Research, Downhams Lane, Milton Road, Cambridge CB4 1XJ, UK*

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Food samples (*n* 114) were prepared from vegetables commonly eaten in Europe. The glycosidic forms of the phyto-oestrogens daidzein and genistein were extracted from the dried foods into aqueous methanol. The isoflavones were quantified by GC–MS after hydrolytic removal of any conjugated carbohydrate. Completeness of extraction and any procedural losses of the isoflavones were accounted for using synthetic daidzin (7-O-glucosyl-4′-hydroxyisoflavone) and genistin (7-O-glucosyl-4′5-dihydroxyisoflavone) as internal standards. Of the 114 foods assayed, at a limit of quantification of 0·1 μg/kg dry weight, forty-eight contained no detectable daidzein or genistein, forty-one contained less than 100 μg/kg of the two isoflavones combined and the remaining twenty-five contained more than this amount. Soyabean products contained between 470 and 1420 mg (average of 960 mg) daidzein and genistein combined per kg wet weight of food, and legumes contained between 20 and 5750 μg/kg wet weight of food, with an average of 620 μg/kg. Cooking by boiling in water caused a decrease in the daidzein and genistein content of food in twenty-four of twenty-eight foods. The extent of the decrease was variable and warrants further investigation. The present paper comprises the first comprehensive description of the content of daidzein and genistein in vegetables.

Phyto-oestrogens: Daidzein: Genistein

Phyto-oestrogens are a diverse group of naturally derived compounds that bear a structural resemblance to 17β-oestradiol (E_2) and which may protect against a wide range of conditions including breast, prostate, bowel and other forms of cancer, cardiovascular disease, osteoporosis and menopausal symptoms (Messina *et al.* 1994; Adlercreutz & Mazur, 1997; Brzezinski *et al.* 1997; Kurzer & Xu 1997; Bingham *et al.* 1998; Murkies *et al.* 1998). One of the proposed mechanisms for protection against these conditions is through their interaction with the oestrogen receptor (ER) and the subsequent perturbation of mechanisms controlled by E_2. Two oestrogen receptors (ERα and ERβ) have been identified in human subjects (Kuiper & Gustafsson, 1997). The concentration of these receptors differs between tissues (Enmark *et al.* 1997) and possibly differs at the point of cell differentiation (Arts *et al.* 1997). The effect of phyto-oestrogens bound to ER remains to be elucidated. *In vitro*, physiological concentrations of phyto-oestrogens have been shown to act both agonistically (Kuiper *et al.* 1998) and antagonistically (Barkhem *et al.* 1998) to the E_2-mediated transactivation of both ERα and ERβ.

The health effects of phyto-oestrogens are, however, far more wide ranging than can be explained by interactions with ER. Genistein has been shown to be a potent protein tyrosine kinase inhibitor (Akiyama *et al.* 1987). Since that discovery, work on cell lines devoid of ER has revealed that phyto-oestrogens affect DNA topoisomerase II and ribosomal S6 kinase, which could explain their observed effects on cell cycle, differentiation, proliferation and apoptosis (Kurzer & Xu, 1997). Phyto-oestrogens have also been proposed to offer antioxidant protection both directly (Ruiz Larrea *et al.* 1997) and indirectly by increasing the activity of antioxidant enzymes (Wei *et al.* 1995).

Soyabeans are a rich source of daidzein and genistein (Reinli & Block, 1996), containing milligram quantities per g food. The isoflavones occur naturally in food as a variety of carbohydrate conjugates; three different glycosides have been identified in soyabean, all linked to C-7 of the isoflavone (Reinli & Block, 1996). It is possible that many other different carbohydrate conjugates of isoflavones exist, but these have yet to be identified. Both daidzein and genistein have hydroxyl groups other than that on C-7 to which the known monosaccharides or other saccharides

Abbreviations: E_2, 17β-oestradiol; ER, oestrogen receptor.

* **Corresponding author:** Dr Sheila Bingham, tel +44 1223 252760, fax +44 1223 252765, email Sheila.Bingham@mrc-dunn.cam.ac.uk

could glycosidically bond. An analogous compound that could be considered is quercetin, a flavone found in tea, onions and other plants. A wide variety of glycosides of this compound have been identified, including a tetraglycoside (Manguro *et al.* 1996). Phyto-oestrogens such as daidzein and genistein are formed only after removal of their conjugated carbohydrate by bacteria in the gut (Chang & Nair, 1995). Quantification of all the possible glycosides of these compounds would be time-consuming, even if authentic reference standards were available for comparison. The quantitative analysis is simplified by hydrolytically removing the carbohydrate and assaying only the aglycone compounds. A method of analysis using hydrolytic enzymes (cellulase) from *Aspergillus niger* with quantification via GC–MS was developed in this laboratory (Liggins *et al.* 1998). The concentration of daidzein and genistein in foodstuffs reported in the present paper can be used in future studies to assess the dietary intake of these compounds and their impact on health in epidemiological studies.

Materials and methods

Collection and preparation of food samples

Representative examples of each food were obtained by purchasing five samples of each food from different sources in the Cambridge area, typically two market stalls and three supermarkets. The food was weighed and any inedible matter was removed, weighed and discarded. If the food was normally eaten raw, each sample was placed in separate sealed plastic bags and frozen at −20°C on the day of purchase for later freeze-drying. Freeze-drying typically took 1 week or more; thereafter the samples were weighed, milled with a kitchen grinder (model BL350; Kenwood Ltd, Havant, Hants., UK) and stored in separate airtight jars. The samples were desiccated further before assay. If the food was normally eaten cooked, each prepared sample was divided into half and half was prepared as described for the raw food samples. The remaining half of each sample was finely chopped and pooled with the other four samples. The foods were boiled for a defined time, drained, frozen and freeze-dried in the same manner as the raw foods. After freeze-drying the food samples were stored in the dark in airtight glass jars and assayed for isoflavones within 4 months.

Quantification of daidzein and genistein in food

The protocol for the extraction of daidzein and genistein from food and their subsequent quantification has been published elsewhere (Liggins *et al.* 1998). The present paper contains only a brief description of the assay method, including any slight modifications used for the assay of the foods.

All enzymes, reagents and chemicals were purchased from Sigma/Aldrich, Poole, Dorset, UK, unless otherwise stated. In order to inhibit losses of target compounds by adsorption, all glassware was silanised in a solution of dimethyldichlorosilane in heptane (1:20 ratio, v/v), followed by deactivation of excess reagent in methylated spirits and oven drying (120°C).

A pooled example of each raw food type was prepared for assay by weighing 0·5 g of each of the five freeze-dried samples into a single 20 ml screw-cap test-tube (2·5 g in total). As the cooked foods had been pooled at an earlier stage, 1 g of the freeze-dried product was weighed into a similar tube. Five replicates of each of these pooled foods were prepared, one of which was assayed in advance of the other samples without internal standards to assess the approximate daidzein and genistein content. As reported elsewhere (Liggins *et al.* 1998), the synthetic glucosides daidzin (7-O-glucosyl-4′-hydroxyisoflavone) and genistin (7-O-glucosyl-4′5-dihydroxyisoflavone), both purchased from Plantech, Reading, Berks., UK, were used as the internal standards added to two of the replicates of each food type. The level of the standard was calculated to deliver the same concentration of daidzein and genistein as was already in the food. The two remaining replicate samples, containing no internal standard, were assayed as sample blanks. The difference in the average isoflavone concentration of the two samples containing standards and the sample blanks was used to calculate the recovery of the internal standards.

The isoflavone glycosides present in both the food and the internal standard were dissolved in at least 10 ml aqueous methanol (4:1 ratio, v/v), using sonication for 15 min to break up cellular material, followed by overnight soaking in the solvent. Insoluble material was filtered off through a double layer of filter paper (Whatman no. 4 on top of Whatman no. 1), and any adsorbed isoflavones were washed through with fresh aqueous methanol (4:1, v/v; >5 ml). The alcohol in the filtrate was removed by evaporation under N_2 to leave an aqueous extract, to which was added 5 ml 0·1 M-acetate buffer, pH 5, containing 100 units cellulase (*Aspergillus niger*; units as defined by Sigma, Poole, Dorset, UK). The mixture was incubated overnight at 37°C to remove the carbohydrate component of the isoflavone glycosides hydrolytically. The aglycone isoflavones were extracted from the aqueous hydrolysis solution by partitioning into ethyl acetate; three 2 ml washes of ethyl acetate were combined. 2 ml of the total was placed in a separate vial, and the solvent was evaporated under N_2.

The dried extracts were derivatised by adding 0·6 ml pyridine followed by 0·4 ml N-(*tert*-butyldimethylsilyl)-N-methyltrifluoro-acetamide containing 1 % (v/v) N-(*tert*-butyldimethylsilyl)-chloride. After 1 h at room temperature, 3 μl of the sample was injected onto the capillary column of the GC–MS (MD800; Thermoquest Ltd, Hemel Hempstead, Herts., UK). The GC–MS conditions used are described elsewhere (Liggins *et al.* 1998).

Calculation of isoflavone quantities in food

The daidzein and genistein concentration of each extracted sample was determined by comparison with pure authentic reference standards (Apin Chemicals Ltd, Abingdon, Oxon., UK), run simultaneously on the GC–MS. The recovery of the internal standards was used both to assess

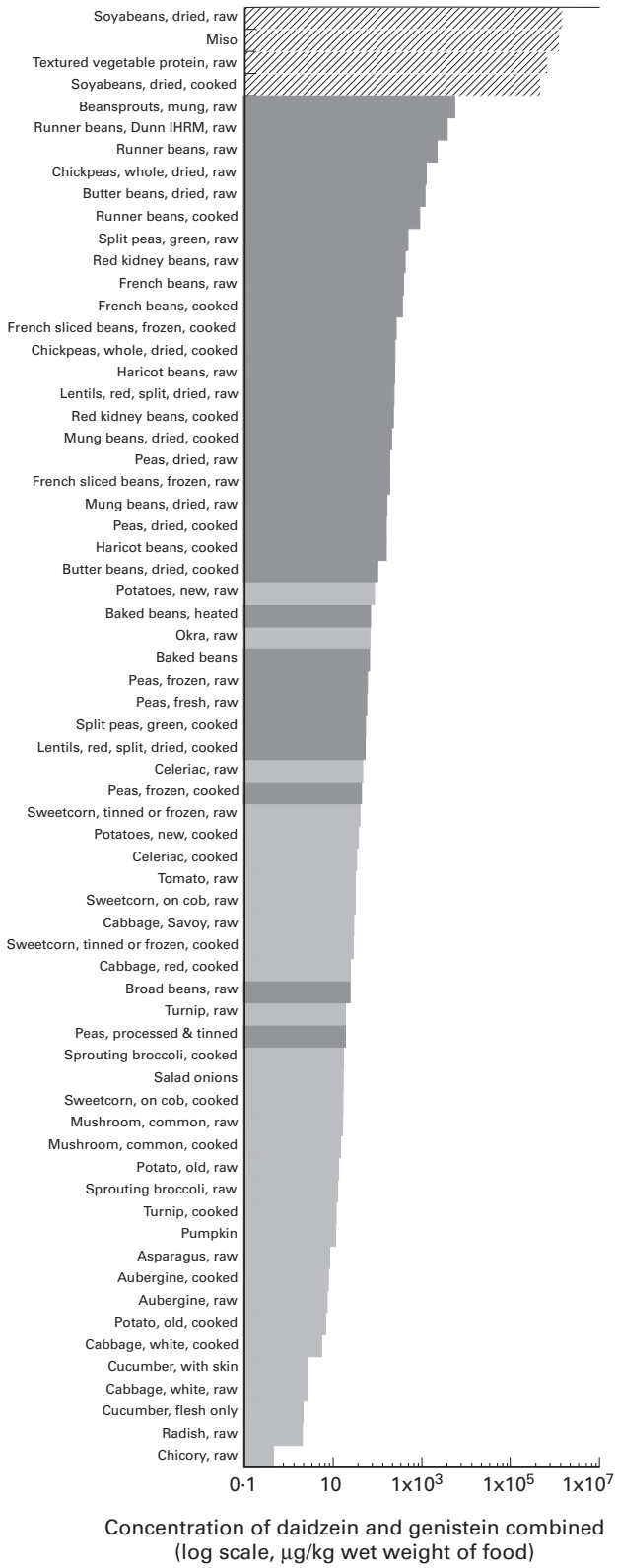

Fig. 1. Comparison of the combined concentrations of daidzein and genistein in different foods vegetables and vegetable products commonly used in Europe. (▨), Soyabean products; (■) legumes; (▩), other vegetables. For details of procedures for sampling and analysis, see p. 718.

the completeness of the extraction protocol and to adjust the concentration of isoflavones found to be in the food to account for any procedural losses. If the recovery of the internal standards fell below 70 % the assay procedure was repeated. The standard error of the mean was calculated from the assay initially employed to determine the concentration of internal standards, and the average of the two subsequent assays of samples which contained no internal standard performed on another day. Wet-weight concentrations of daidzein and genistein were calculated from the DM content of the food and the assayed dry-weight concentration.

Results

Of the 114 assayed foods reported in the present paper, sixty-six contained measurable quantities of daidzein or genistein. Table 1 illustrates the daidzein and genistein content of vegetables and vegetable products (on both a dry weight and wet weight basis). The individual foods are listed in the order of their codes in the supplements to *McCance and Widdowson's Composition of Foods* (Holland *et al.* 1991). Table 1 includes the percentage recovery of the internal standards assayed with the samples; recoveries ranged from 70 to 109 %.

The results indicate that daidzein and genistein were found in a broad concentration range across a wide variety of vegetables. Fig. 1 compares the combined concentration of daidzein and genistein in the foods that contained these isoflavones. Three groups were evident. Foods derived from soyabean contained by far the highest concentration, 5×10^5–14×10^5 μg daidzein and genistein/kg wet weight of food. The concentration observed in cooked soyabeans was two orders of magnitude higher than that in the next-richest food (raw mung beansprouts; 6×10^3 μg/kg). Mung beansprouts form part of the legume group highlighted in Fig. 1, which contained 2–6×10^3 μg daidzein and genistein/kg wet weight of food. The legume group, overall, contained more daidzein and genistein than the third group highlighted in Fig. 1 (other vegetables), but their concentration ranges overlapped, with the latter group containing up to 8 μg/kg.

Cooking by boiling in water generally caused a decrease in the combined daidzein and genistein concentration (on a wet weight basis); however, in four foods, cooking increased these levels. The increase in these four foods is, however, relatively small and could be accounted for by experimental error. Of the twenty-four foods in which cooking decreased the daidzein and genistein concentration, nine lost at least 75 % of the raw-weight value. Five contained very low amounts in the raw state and no isoflavone was detected after cooking. The remaining four foods that lost more than 75 % of their daidzein and genistein on cooking were all dried legumes, soaked overnight in water before cooking. However, the cooking-loss factors appear to be variable, as four other dried legumes, soaked overnight in water before cooking, retained more than 25 % of the isoflavones (soyabeans 33 %, red kidney beans 55 %, haricot beans 65 % and dried peas 84 %).

Table 2 compares the concentration of daidzein and

Table 1. Daidzein (Da) and genistein (Ge) contents of vegetables and vegetable products commonly eaten in Europe*
(Mean values with their interassay standard errors)

M&W code†	Vegetable	Date of assay	Recovery of internal standards (%)		Concentration (μg/kg dry weight)‡ (Da)		Concentration (μg/kg dry weight)‡ (Ge)		DM	Mean concentration (μg/kg wet weight)‡		
			Da	Ge	Mean	SE	Mean	SE		Da	Ge	Sum of Da and Ge
13-001	Potatoes, new, raw	Dec 97	72	75	132	8	304	47	0·190	25·1	57·9	83
13-003	Potatoes, new, cooked	Dec 97	75	78	55	12	147	13	0·186	10·2	27·3	37·5
13-009	Potatoes, old, raw	Dec 97	79	83	28	5	47	12	1·186	5·2	8·7	13·9
13-014	Potatoes, old, cooked	Dec 97	72	79	5		37	0	0·175	0·9	6·5	7·4
nc	Potatoes, red, cooked	Jun 98			nd		nd			nd	nd	
nc	Potatoes, red, raw	Mar 98			nd		nd			nd	nd	
13-043	Baked beans	Jul 97		102	nd		228	64	0·278	nd	63·5	63·5
13-044	Baked beans, heated	Jun 97	93	76	51	11	201	94	0·276	14·1	55·3	69·4
13-052	Beansprouts, mung, raw	Feb 98	88	77	39×10^3	329	68×10^3	3134	0·054	2066	3670	5736
13-064	Broad beans, raw	Oct 97	72	96	74	8	59	12	0·183	13·6	10·8	24·4
13-066	Broad beans, cooked	Oct 97			nd		nd			nd	nd	
13-070	Butter beans, dried, raw	Mar 98	82	73	305	16	847	25	1·000	305	847	1152
13-071	Butter beans, dried, cooked	Mar 98	76	78	185	6	65	6	0·375	69·4	24·4	93·8
13-074	Chickpeas, whole, dried, raw	Mar 98	71	87	475	19	766	21	1·000	475	766	1241
13-075	Chickpeas, whole, dried, cooked	Mar 98		83	nd		578	66	0·418	nd	241·4	241·4
13-081	French beans, raw	Feb 98	96	87	1198	26	3372	67	0·084	100·8	283·6	384·4
13-083	French beans, cooked	Dec 97	78	77	1151	189	3075	124	0·083	95·4	254·9	350·3
13-085	French sliced beans, frozen, cooked	Mar 98	92	81	686	42	1938		0·097	66·5	188	254·5
nc	French sliced beans, frozen, raw	Mar 98	85	88	479	5	1362		0·097	46·5	132·1	178·6
13-086	Haricot beans, raw	Mar 98	71	73	131	13	105	30	1·000	131	105	236
13-087	Haricot beans, cooked	Apr 98	93	91	186	17	173	7	0·425	79	73·5	152·5
13-091	Lentils, red, split, dried, raw	Mar 98	72	87	139	32	84	27	1·000	139	84	223
13-092	Lentils, red, split, dried, cooked	Mar 98	83	99	50	2	93	29	0·357	17·8	33·2	51
13-096	Mung beans, dried, raw	Apr 98	70	86	50	1	106	7	1·000	50	106	156
13-099	Mung beans, dried, cooked	Apr 98	72	80	154	43	399	36	0·373	57·4	148·8	206·2
13-109	Red kidney beans, raw	Mar 98	104	109	191	116	209	9	1·000	191	209	400
13-110	Red kidney beans, cooked	Mar 98	73	89	311	38	221	8	0·419	130·2	92·5	222·7
13-112	Runner beans, Dunn IHRM, raw	Mean§	96	95	23×10^3	532	31×10^3	2994	0·073	1672	2260	3932
13-112	Runner beans, raw	Jan 98	76	77	13×10^3	396	15×10^3	508	0·081	1036	1183	2219
13-114	Runner beans, cooked	Jan 98	72	78	7080	281	8860	616	0·056	396	495·9	891·9
13-115	Soyabeans, dried, raw	Apr 98	101	101	583×10^3	62×10^3	838×10^3	71×10^3	1·000	583×10^3	838×10^3	1421×10^3
13-116	Soyabeans, dried, cooked	Apr 98	79	74	411×10^3	36×10^3	839×10^3	98×10^3	0·375	154×10^3	315×10^3	469×10^3
nc	Miso	Aug 97	88	79	594×10^3	5×10^3	673×10^3	9×10^3	0·998	593×10^3	672×10^3	1265×10^3
17-081	Textured vegetable protein, raw	Oct 97	96	103	248×10^3	23×10^3	438×10^3	77×10^3	1·000	248×10^3	438×10^3	686×10^3
13-127	Peas, fresh, raw	Nov 97		94	nd		232	47	0·233	nd	54·1	54·1
13-129	Peas, fresh, cooked	Nov 97			nd		nd			nd	nd	
13-130	Peas, dried, raw	Apr 98	89	74	41	9	144	16	1·000	41·0	144	185
13-131	Peas, dried, cooked	Mar 98	91	74	32	10	381	9	0·375	12·0	142·9	154·9
13-132	Peas, frozen, raw	Apr 98	84	96	nd		268	2	0·208	nd	55·7	55·7
13-134	Peas, frozen, cooked	Jul 97	101	83	nd		215	40	0·194	nd	41·7	41·7
nc	Peas processed, tinned	Oct 97		89	nd		70		0·271	nd	19·0	19·0
13-141	Split peas, green, raw	Mar 98	76	70	130	4	347	16	1·000	130	347	477
13-142	Split peas, green, cooked	Mar 98		70	nd		128	10	0·408	nd	52·3	52·3
13-157	Asparagus, raw	Oct 97	96	93	36	15	79	1	0·079	2·8	6·2	9·0
13-158	Asparagus, cooked	Oct 97			nd		nd			nd	nd	0
13-161	Aubergine, raw	Oct 97	88	75	8	23	99	14	0·074	0·6	7·3	7·9
nc	Aubergine, cooked	Sep 97	105	90	50	37	105	27	0·054	2·7	5·7	8·4

Table 1. *continued*

M&W code†	Vegetable	Date of assay	Recovery of internal standards (%)		Concentration (μg/kg dry weight)‡ (Da)		Concentration (μg/kg dry weight)‡ (Ge)		DM	Mean concentration (μg/kg wet weight)‡		
			Da	Ge	Mean	SE	Mean	SE		Da	Ge	Sum of Da and Ge
13-166	Beetroot, precooked	Nov 97			nd		nd			nd	nd	
13-170	Calabrese, raw	Aug 97			nd		nd			nd	nd	
13-172	Calabrese, cooked	Aug 97			nd		nd			nd	nd	
13-174	Sprouting broccoli, raw	Jun 98	101	85	69	10	29	1	0·140	9·6	4·1	13·7
13-176	Sprouting broccoli, cooked	Jun 98	73	73	72	50	94	6	0·109	7·8	10·2	18·0
13-177	Brussels sprouts, raw	Jul 97			nd		nd			nd	nd	
13-179	Brussels sprouts, cooked	Jul 97			nd		nd			nd	nd	
13-183	Cabbage, green, raw	Dec 97			tr		tr			tr	tr	
13-185	Cabbage, green, cooked	Dec 97			tr		tr			tr	tr	
13-190	Cabbage, red, raw	Jul 97			nd		tr			nd	tr	
13-191	Cabbage, red, cooked	Sep 97		76	nd		276	14	0·088	nd	24·4	24·4
13-192	Cabbage, Savoy, raw	Jul 97		105	nd		289	68	0·100	nd	28·8	28·8
13-193	Cabbage, Savoy, cooked	Jul 97			nd		nd			nd	nd	
13-196	Cabbage, white, raw	Mar 98		88	nd		32	0	0·092	nd	2·9	2·9
13-197	Cabbage, white, cooked	Mar 98		78	nd		84	2	0·073	nd	6·1	6·1
13-200	Carrots, old, raw	Aug 97			nd		nd			nd	nd	
13-202	Carrots, old, cooked	Aug 97			nd		nd			nd	nd	
nc	Carrots, tinned	Feb-98			nd		nd			nd	nd	
13-215	Cauliflower, raw	Aug 97			nd		nd			nd	nd	
13-217	Cauliflower, cooked	Aug 97			nd		nd			nd	nd	
13-219	Celeriac, raw	Jul 97		106	nd		442	81	0·101	nd	44·7	44·7
13-220	Celeriac, cooked	Oct 97		73	nd		420	76	0·081	nd	33·9	33·9
13-221	Celery, raw	Jul 97			nd		nd			nd	nd	
13-222	Celery, cooked	Jul 97			nd		nd			nd	nd	
13-225	Chicory, raw	Jan 98	89		9		nd		0·053	0·5	nd	0·5
13-230	Courgette, raw	Aug 97			nd		nd			nd	nd	
13-231	Courgette, cooked	Aug 97			nd		nd			nd	nd	
13-233	Cucumber, with skin	Oct 97		71	nd		77	14	0·037	nd	2·9	2·9
nc	Cucumber, flesh only	Oct 97		81	nd		62	10	0·038	nd	2·4	2·4
13-241	Fennel, raw	Aug 97			nd		nd			nd	nd	
13-242	Fennel, cooked	Aug 97			nd		nd			nd	nd	
13-263	Leeks, raw	Sep 97			nd		nd			nd	nd	
13-265	Leeks, cooked	Sep 97			nd		nd			nd	nd	
13-266	Lettuce, round	Dec 97			nd		nd			nd	nd	
13-269	Lettuce, Iceberg	Dec 97			nd		nd			nd	nd	
13-274	Marrow, raw	Dec 97			nd		nd			nd	nd	
13-276	Marrow, cooked	Dec 97			nd		nd			nd	nd	
13-284	Mushroom, common, raw	Jul 97	83	82	12	6	209	21	0·077	0·9	16·0	16·9
13-285	Mushroom, common, cooked	Jun 97	71	99	nd		182	54	0·085	nd	15·4	15·4
13-300	Okra, raw	Jun 98	74	72	253	13	112	3	0·188	47·6	21·1	68·7
13-304	Onion, raw	Jan 98			nd		nd			nd	nd	
13-306	Onion, cooked	Jan 98			nd		nd			nd	nd	
13-312	Parsnip, raw	Jul 97			nd		nd			nd	nd	
13-313	Parsnip, cooked	Jul 97			nd		nd			nd	nd	
13-318	Pepper, green	Feb 98			nd		nd			nd	nd	
13-323	Plaintain, raw	Oct 97			nd		nd			nd	nd	
13-324	Plaintain, cooked	Oct 97			nd		nd			nd	nd	
13-326	Pumpkin	May 98	74		262	79	nd		0·047	12·2	nd	12·2

Table 1. *continued*

M&W code†	Vegetable	Date of assay	Recovery of internal standards (%)		Concentration (μg/kg dry weight)‡ (Da)		Concentration (μg/kg dry weight)‡ (Ge)		DM	Mean concentration (μg/kg wet weight)‡		
			Da	Ge	Mean	SE	Mean	SE		Da	Ge	Sum of Da and Ge
13-330	Radish, raw	Nov 97	80	88	nd		45	4	0·051	nd	2·3	2·3
13-343	Spinach, raw	Nov 97			nd		nd			nd	nd	
13-345	Spinach, cooked	Nov 97			nd		nd			nd	nd	
13-348	Spring greens, raw	Jul 97			nd		nd			nd	nd	
13-350	Spring greens, cooked	Jul 97			nd		nd			nd	nd	
13-352	Salad onions	Jul 97		100	nd		187	20	0·094	nd	17·6	17·6
13-359	Swede, raw	Aug 97			nd		nd			nd	nd	
13-361	Swede, cooked	Aug 97			nd		nd			nd	nd	
13-362	Sweet potato, raw	Aug 97			nd		nd			nd	nd	
13-364	Sweet potato, cooked	Aug 97			nd		nd			nd	nd	
13-368	Sweetcorn, on cob, raw	Dec 97	93	73	nd		134	2	0·235	nd	31·5	31·5
13-370	Sweetcorn, on cob, cooked	Jun 98	75	86	29	10	45	8	0·235	6·8	10·6	17·4
nc	Sweetcorn, tinned or frozen, cooked	Jun 98	93	77	40	5	66	2	0·262	10·5	17·3	27·8
nc	Sweetcorn, tinned or frozen, raw	Dec 97	79	82	74	25	59	7	0·308	22·8	18·2	41·0
13-384	Tomato, raw	Aug 97	72	80	nd		480	31	0·068	nd	32·5	32·5
13-389	Turnip, raw	Mar 98	91	79	165	15	47	3	0·091	15·0	4·3	19·3
13-391	Turnip, cooked	Mar 98	77	87	94	9	72	78	0·077	7·3	5·6	12·9
13-396	Salad cress	Oct 97			nd		nd			nd	nd	
13-396	Watercress	Nov 97			nd		nd			nd	nd	

M&W code, food coding as in Holland *et al.* (1991); nc, not coded in Holland *et al.* (1991); nd, not detected; tr, trace.
* For details of procedures for sampling and analysis, see p. 718.
† Items are listed in the order of their appearance in Holland *et al.* (1991).
‡ Concentrations adjusted to correct for any losses of the internal standards; for details, see p. 724.
§ This food was separately analysed twenty-four times over a period of 2 years and the values shown are an average of those assays.

Table 2. Daidzein and genistein concentrations (μg/kg) for vegetables and vegetable products determined in the present study compared with those previously reported*

Food	Present study		Other studies	
	Daidzein	Genistein	Daidzein	Genistein
Soyabeans, raw	58×10^4	84×10^4	33×10^4†	48×10^4†
Soyabeans, raw (ww)	58×10^4	84×10^4	54×10^4‡	84×10^4‡
Soyabeans, boiled (ww)	15×10^4	32×10^4	20×10^4‡	31×10^4‡
Miso	59×10^4	67×10^4	14×10^4§	15×10^4§
Miso (ww)	59×10^4	67×10^4	19×10^4§	31×10^4§
Beansprouts (mung)	39×10^3	68×10^4	8×10^3§	19×10^3§
			nd‖	nd‖
Broad bean	70	60	240†	tr†
Broccoli, calabrese	nd	nd	50§	70§
Carrot	nd	nd	20§	20§
Cranberry	50	210	0§	0§
Chickpeas	480	770	110§	760§
Green split pea	130	350	80†	tr†
			73×10^3‖	nd‖
Haricot beans	130	110	140†	4×10^3†
Oatmeal	nd	nd	0§	0§
Mung beans	50	110	100§	4×10^3§
			nd‖	nd‖
Mushroom	10	210	200§	1170§
Red kidney beans	190	210	80†	70†

nd, not detected; tr, isoflavone were identified but could not be quantified; ww, wet weight.
* Concentrations expressed on a dry weight basis, unless otherwise indicated.
† Mazur *et al.* (1998).
‡ Reinli & Block (1996).
§ Adlercreutz & Mazur (1997).
‖ Franke *et al.* (1994).

genistein reported here with concentrations for the same foods published by other authors. The results in Table 2 largely concur with published values; however, those for mung beansprouts and green split peas differ from those of Franke *et al.* (1994), but concur with those reported by other researchers (Adlercreutz & Mazur 1997; Mazur *et al.* 1998).

Discussion

The foods selected for the present study were chosen because they are staple ingredients of the UK diet. To date, the study of the dietary intakes of daidzein and genistein has been restricted mainly to soyabean products and other legumes. These foods, however, form only a small part of the 'Western diet', and in order to assess fully the dietary impact of phyto-oestrogens on the health of a population it is necessary to know their concentrations in other foods. The data reported here are not intended to provide a definitive phyto-oestrogen concentration for a given food, since natural variation could be considerable, as in soyabean (Wang & Murphy, 1994; Reinli & Block, 1996; Gang *et al.* 1997; Lers *et al.* 1998; Mazur *et al.* 1998). The concentrations reported here are derived from pooled samples of each food. No attempt has been made to measure the variation in concentration within the pooled samples, due to the extensive work necessary to obtain such data.

The analytical method employed for the quantification of daidzein and genistein in food was developed using a varied mixture of bleached white wheat flour and soyabean flour of known isoflavone content (Liggins *et al.* 1998). The soyabean flour had been analysed independently without hydrolysis, in such a way that the percentages of the three main known glycosides and the aglycone in soyabean flour were known (glucose 45, malonyl-glucose 23, acetyl-glucose 30, aglycone 2). The same soyabean flour was used as a reference material to ensure the quality of the assay procedure over time, using the recommendations made by Thompson & Wood (1995). To ensure that the assayed concentration reflected the actual content of the food, the completeness of hydrolysis and yield of aglycone isoflavones from each food was checked by the inclusion of glycosidic internal standards, at an aglycone concentration equal to that already present in the food. The recovery of the internal standards was used to normalise the results for different losses of the target compounds between assays, provided that the recovery of the internal standards was greater than 70 %; if the recovery was less than this amount, the results were rejected and the assay repeated.

The assay procedure employed for the present study could have been improved if other synthetic glycosidic conjugates of daidzein and genistein had been included with each food, together with the daidzin and genistin. Inclusion of such a variety of carbohydrate conjugates would give greater insight into the completeness of hydrolysis of each glycoside by cellulase. The assay used here relies on the assumption that all glycosides of daidzein and genistein are hydrolysed as completely as the synthetic daidzin and genistin added to each food. For the known carbohydrate conjugates present in soyabean (glucose, malonyl-glucose and acetyl-glucose) the assumption is valid. The hydrolytic assay procedure used here returned a total concentration of daidzein and genistein in soyabean equivalent to the sum of the three glucosides assayed independently (Liggins *et al.* 1998).

The foods collected for the present study could have been assayed using a non-glycolytic preparative procedure and quantification of the three known glycosides of daidzein and genistein. Such an approach would have been appropriate had the foods been of soyabean origin, where those glycosides were probably the predominant forms. However, the foods used here were not largely of soyabean origin, and the nature of the carbohydrate conjugated to the isoflavones was unknown. In foods where the predominant carbohydrate conjugate was not one of the three found in soyabean, quantification of just the three known glycosides would have underestimated their total daidzein and genistein content. It was, therefore, logical to employ an assay with a glycolytic step, to liberate the aglycone daidzein and genistein from all glycosides present in a food and to report the total content of each food. However, if some of these glycosides were not hydrolysed as completely as the synthetic daidzein and genistein that were added to each food, then the total concentrations may be underestimates. At present, however, lack of understanding of the nature of the glycosides and of the effect of the matrix on hydrolysis prevents further resolution of this problem.

The analytical data presented in Table 1 indicate that there are a number of possible sources of dietary daidzein and genistein, and that many and varied components of the

Table 3. Published concentrations (μg/24 h) of daidzein and genistein excreted by human subjects consuming Western-style diets of their own choice

Diet	Sex of participants	Sample	Daidzein	Genistein	Reference
Omnivorous	Male	Urine	48	50	Adlercreutz *et al.* (1991)
Omnivorous	Female	Urine	33	22	Cassidy *et al.* (1991)
Omnivorous	Female	Faeces	17	3	Adlercreutz *et al.* (1995)
Lactovegetarian	Female	Faeces	160	51	Adlercreutz *et al.* (1995)
Omnivorous	Male	Urine	144	42	Hutchins *et al.* (1995)
Omnivorous	Male and female	Urine	1065	297	Kelly *et al.* (1995)
Omnivorous	Female	Urine	203	65	Horn-Ross *et al.* (1997)
Omnivorous	Male	Urine	266	61	Karr *et al.* (1997)

human diet contain phyto-oestrogens. The richest sources are of soyabean origin, with concentrations two orders of magnitude higher than those in the next-richest grouping, the legumes. The legumes contain a wide concentration range of the isoflavones, overlapping that of other vegetables. The findings of the present study thus support those of previous studies illustrating the daidzein and genistein content of leguminous samples (Mazur *et al.* 1998).

The concentrations of daidzein and genistein in a limited number of other foods are compared with other published values in Table 2. Slight differences in concentration are probably due to natural variation, possibly compounded by differences in the techniques used for quantification. However, for the majority of the foods indicated in Table 2, the results presented here compare well with those published elsewhere. Two exceptions are mung beansprouts and green split peas: the results we report for green split peas agree with those of Adlercreutz & Mazur (1997) and of Mazur *et al.* (1998), who (like ourselves) used a GC–MS-based method of quantification; however, our results differ from those reported by Franke *et al.* (1994), who used HPLC combined with spectrophotometry. In the absence of a common reference material it is not known whether the high concentration of daidzein found in green split peas by Franke *et al.* (1994) is due to a genuine difference in the material tested. The difference in isoflavone concentration between mung beans and mung beansprouts in Table 1 seems to indicate that major changes may occur during sprouting. This factor might explain the difference between laboratories in concentrations of the isoflavones (Table 2).

A number of studies have been undertaken to investigate the concentration of daidzein and genistein in biological samples. Table 3 illustrates urine and faecal excretion of isoflavones in five studies of free-living individuals and in two studies where the diet was controlled. In the study by Karr *et al.* (1997), the subjects were supplied for 9 d with a soyabean-free diet; in the investigation by Kelly *et al.* (1995), individuals were asked to avoid legumes for 1 week before the study. The results of the studies illustrated in Table 3 support the findings, outlined in Table 1, that dietary sources of daidzein and genistein, other than soyabean, exist. The presence of daidzein and genistein in the plasma and prostatic fluid of free-living individuals from Australia (Morton *et al.* 1994), Portugal and the UK (Morton *et al.* 1997*a*,*b*) would also support such findings. The plasma or urine concentrations of daidzein and genistein populations regularly consuming soyabean as part of their normal diet are, however, considerably greater (Dalais *et al.* 1998; Maskarinec *et al.* 1998; Seow *et al.* 1998).

The present paper has concentrated on quantifying two phyto-oestrogens in a selection of foods eaten in Europe, for inclusion in databases of food composition. In this respect, identification of foods that contain no daidzein and genistein is equally important. Future research should include quantification of other phyto-oestrogens, in the foods listed here and also in other foods.

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References

Adlercreutz H, Fotsis T, Bannwart C, Wahala K, Brunow G & Hase T (1991) Isotope-dilution gas-chromatographic mass-spectrometric method for the determination of lignans and isoflavonoids in human urine, including identification of genistein. *Clinica Chimica Acta* **199**, 263–278.

Adlercreutz H, Fotsis T, Kurzer MS, Wahala K, Makela T & Hase T (1995) Isotope-dilution gas-chromatographic mass-spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. *Analytical Biochemistry* **225**, 101–108.

Adlercreutz H & Mazur W (1997) Phyto-oestrogens and Western diseases. *Annals of Medicine* **29**, 95–120.

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M & Fukami Y (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. *Journal of Biological Chemistry* **262**, 5592–5595.

Arts J, Kuiper G, Janssen J, Gustafsson JA, Lowik C, Pols HAP & VanLeeuwen J (1997) Differential expression of estrogen receptors alpha and beta mRNA during differentiation of human osteoblast SV-HFO cells. *Endocrinology* **138**, 5067–5070.

Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson JA & Nilsson S (1998) Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. *Molecular Pharmacology* **54**, 105–112.

Bingham SA, Atkinson C, Liggins J, Bluck L & Coward A (1998) Plant oestrogens: Where are we now? *British Journal of Nutrition* **79**, 393–406.

Brzezinski A, Adlercreutz H, Shaoul R, Rosler A, Shmueli A, Tanos V & Schenker JG (1997) Short-term effects of phytoestrogen-rich diet on postmenopausal women. *Menopause* **4**, 89–94.

Cassidy A, Bingham S, Setchel K & Watson D (1991) Urinary plant oestrogen excretion in post menopausal women. *Proceedings of the Nutrition Society* **50**, 105A.

Chang YC & Nair MG (1995) Metabolism of daidzein and genistein by intestinal bacteria. *Journal of Natural Products* **58**, 1892–1896.

Dalais FS, Rice GE, Wahlqvist ML, HsuHage BHH & Wattanapenpaiboon N (1998) Urinary excretion of isoflavonoid phytoestrogens in Chinese and Anglo-Celtic populations in Australia. *Nutrition Research* **18**, 1703–1709.

Enmark E, PeltoHuikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjold M & Gustafsson JA (1997) Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. *Journal of Clinical Endocrinology and Metabolism* **82**, 4258–4265.

Franke AA, Custer LJ, Cerna CM & Narala KK (1994) Quantitation of phytoestrogens in legumes by HPLC. *Journal of Agricultural and Food Chemistry* **42**, 1905–1913.

Gang DR, Dinkova-Kostova AT, Davin LB & Lewis NG (1997) Phylogenetic links in plant defense systems: lignans, isoflavonoids, and their reductases. *ACS Symposium Series* **658**, 58–89.

Holland B, Unwin ID & Buss DH (1991) *Vegetables and Spices: Fifth Supplement to McCance and Widdowson's The Composition of Foods*, 4th ed. Cambridge: Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Horn-Ross PL, Barnes S, Kirk M, Coward L, Parsonnet J & Hiatt RA (1997) Urinary phytoestrogen levels in young women from a multiethnic population. *Cancer Epidemiology, Biomarkers and Prevention* **6**, 339–345.

Hutchins AM, Slavin JL & Lampe JW (1995) Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *Journal of the American Dietetic Association* **95**, 545–551.

Karr SC, Lampe JW, Hutchins AM & Slavin JL (1997) Urinary isoflavonoid excretion in humans is dose dependent at low to moderate levels of soy-protein consumption. *American Journal of Clinical Nutrition* **66**, 46–51.

Kelly GE, Joannou GE, Reeder AY, Nelson C & Waring MA (1995) The variable metabolic response to dietary isoflavones in humans. *Proceedings of the Society for Experimental Biology and Medicine* **208**, 40–43.

Kuiper G & Gustafsson JA (1997) The novel estrogen receptor-beta subtype: potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. *FEBS Letters* **410**, 87–90.

Kuiper G, Lemmen JG, Carlsson B, Corton JC, Safe SH, VanderSaag PT, VanderBurg P & Gustafsson JA (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139**, 4252–4263.

Kurzer MS & Xu X (1997) Dietary phytoestrogens. *Annual Review of Nutrition* **17**, 353–381.

Lers A, Burd S, Lomaniec E, Droby S & Chalutz E (1998) The expression of a grapefruit gene encoding an isoflavone reductase-like protein is induced in response to UV irradiation. *Plant Molecular Biology* **36**, 847–856.

Liggins J, Bluck LJC, Coward WA & Bingham SA (1998) Extraction and quantification of daidzein and genistein in food. *Analytical Biochemistry* **264**, 1–7.

Manguro LOA, Midiwo JO & Kraus W (1996) A new flavonol tetraglycoside from Myrsine africana leaves. *Natural Product Letters* **9**, 121–126.

Maskarinec G, Singh S, Meng LX & Franke AA (1998) Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. *Cancer Epidemiology, Biomarkers and Prevention* **7**, 613–619.

Mazur WM, Duke JA, Wahala K, Rasku S & Adlercreutz H (1998) Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. *Journal of Nutritional Biochemistry* **9**, 193–200.

Messina MJ, Persky V, Setchell KDR & Barnes S (1994) Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutrition and Cancer* **21**, 113–131.

Morton MS, Chan PSF, Cheng C, Blacklock N, Matos-Ferreira A, Abranches-Monteiro L, Correia R, Lloyd S & Griffiths K (1997*a*) Lignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom. *Prostate* **32**, 122–128.

Morton MS, Matos-Ferreira A, Abranches-Monteiro L, Correia R, Blacklock N, Chan PSF, Cheng C, Lloyd C, Chiehping W & Griffiths K (1997*b*) Measurement and metabolism of isolflavonoids and lignans in the human male. *Cancer Letters* **114**, 145–151.

Morton MS, Wilcox G, Wahlqvist ML & Griffiths K (1994) Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. *Journal of Endocrinology* **142**, 251–259.

Murkies AL, Wilcox G & Davis SR (1998) Phytoestrogens. *Journal of Clinical Endocrinology and Metabolism* **83**, 297–303.

Reinli K & Block G (1996) Phytoestrogen content of foods – a compendium. *Nutrition and Cancer* **26**, 123–148.

Ruiz Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP & Rice Evans CA (1997) Antioxidant activity of phytoestrogenic isoflavones. *Free Radical Research* **26**, 63–70.

Seow A, Shi CY, Franke AA, Hankin JH, Lee HP & Yu MC (1998) Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle-aged and older Chinese in Singapore. *Cancer Epidemiology, Biomarkers and Prevention* **7**, 135–140.

Thompson M & Wood R (1995) Harmonised guidelines for internal quality control in analytical chemistry laboratories. *Pure and Applied Chemistry* **67**, 649–666.

Wang H & Murphy PA (1994) Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *Journal of Agricultural and Food Chemistry* **42**, 1674–1677.

Wei H, Bowen R, Cai Q, Barnes S & Wang Y (1995) Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proceedings of the Society for Experimental Biology and Medicine* **208**, 124–130.