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ORIGINAL COMMUNICATION

Daidzein and genistein content of cereals

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Objective: To analyse 75 cereals and three soy flours commonly eaten in Europe for the phytoestrogens daidzein and genistein.
Design: The phytoestrogens daidzein and genistein were extracted from dried foods, and the two isoflavones quantified 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.
Setting: Foods from the Cambridge UK area were purchased, prepared for eating, which included cooking if necessary, and freeze dried. Three stock soy flours were also analysed.
Results: Eighteen of the foods assayed contained trace or no detectable daidzein or genistein. The soy flours were rich sources, containing 1639–2117 mg/kg. The concentration of the two isoflavones in the remaining foods ranged from 33 to 11 873 μg/kg.
Conclusion: These analyses will supply useful information to investigators determining the intake of phytoestrogens in cereal products in order to relate intakes to potential biological activities.
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Introduction

Phytoestrogens are a diverse group of naturally derived compounds that bear a structural resemblance to 17β-oestradiol (E_2; Setchell & Adlercreutz, 1988). These 'phytoestrogens' include the lignans and coumestans in addition to daidzein, and genistein which have been shown to bind to oestrogen receptors, albeit at comparatively low levels (Shutt & Cox 1972). Sheep grazing on Australian pastures containing a particular type of clover rich in formononetin, which is converted to daidzein in the rumen during fermentation, developed widespread infertility in the 1940s. This problem was traced to these non-steroidal weak oestrogens (Shutt, 1976). Soya is rich in daidzein and genistein and use of soy in captive cheetah in a Cinncinnati zoo was also shown to be responsible for an infertility syndrome, reversed by its removal from the feed (Setchell *et al*, 1987).

In common with many other weak oestrogens, in model systems the isoflavones have been shown to compete for oestradiol at the receptor complex, yet fail to stimulate a full oestrogenic response after binding to the nucleus (Tang & Adams, 1980). In particular they have been shown to bind to the β estrogen receptor (Kuiper *et al*, 1998). The significance of the structural similarity of the isoflavones to mammalian oestrogens and possible effects on cancer prevention were first promulgated in the early 1980s in publications of Setchell and Adlercreutz (1988). Since that time the literature on the possible health benefits of the isoflavones found predominantly in soy beans has expanded exponentially. Now there are several research fronts covering most of the major public health problems of Western societies. In addition to hormone-related and bowel cancers, phytoestrogens are under active investigation for their protective effects in other hormone related conditions, such as menopausal symptoms, osteoporosis and heart disease (Bingham *et al*, 1998).

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Table 1 Daidzein (Da) and genistein (Ge) content of cereals and cereal products commonly consumed in the UK

		Concentration (μg/kg dry wt)					Mean concentration (μg/kg wet weight)		
			Daidzein		Genistein					
M&W code[a]	Food	Number of samples	Mean	s.e.	Mean	s.e.	DM	Da	Ge	Sum of Da and Ge
11-001	Arrowroot	4	36	1	16		1.000	36	16	52
11-002	Pearl barley	4	nd		86	7	1.000	nd	86	86
11-005	Wheat bran	5	nd		38	1	1.000	nd	38	38
11-010	Cornlour	5	58	1	51	1	1.000	58	51	109
11-015	Maize meal	3	95	16	142	2	1.000	95	142	237
11-017	Oatmeal	5	nd		nd			nd	nd	
11-018	Oatmeal, quick cook; Quaker oats	1	nd		nd			nd	nd	
nc	Oats, rolled	5	nd		nd			nd	nd	
11-023	Sago	2	36	1	nd		1.000	36	nd	36
11-024	Semolina	5	55	37	181	1	1.000	55	181	236
11-025	Soy flour, CSL stock, assay no. 1		778×10^3	4×10^3	889×10^3	21×10^3	1.000	778×10^3	889×10^3	1667×10^3
11-025	Soy flour, CSL stock, assay no. 2		816×10^3	40×10^3	823×10^3	49×10^3	1.000	816×10^3	823×10^3	1639×10^3
11-025	Soy flour, Dunn IHRM 2y average to August 1998		1037×10^3	10×10^3	1080×10^3	12×10^3	1.000	1037×10^3	1080×10^3	2117×10^3
11-027	Tapioca	5	nd		nd			nd	nd	
nc	Flour, brown, breadmaking	3	82	22	255	5	1.000	82	255	337
nc	Flour, brown, self-raising	1	279	11	83	24	1.000	279	83	362
nc	Flour, granary	1	82	2	31	10	1.000	82	31	113
11-031	Flour, plain, whitewheat	5	nd		nd			nd	nd	
11-033	Flour, wholemeal	5	24		146	8	1.000	24	146	170
nc	Wheat flakes	2	33	1	tr		1.000	33	tr	33
11-035	Rice, brown, wholegrain, raw	5	613	24	713	8	1.000	613	713	1326
11-036	Rice, brown wholegrain, boiled	5	125	2	141	3	0.337	42	48	90
nc	Rice, long grain, white, raw	5	nd		nd			nd	nd	
11-043	Rice, long grain, white, boiled	5	79	1	119	6	0.325	26	39	64
nc	Pasta, lasagne, green, raw	2	24	3	81	5	1.000	24	81	105
11-051	Pasta, lasagne, white, raw	5	21	3	31	4	1.000	21	31	52
11-052	Pasta, lasagne, white, boiled	5	nd		nd			nd	nd	
nc	Pasta, lasagne, wholewheat, raw	3	123		95	1	1.000	123	95	218
11-054	Pasta, macaroni, white, boiled	5	nd		nd			nd	nd	
11-055	Noodles, egg, medium, raw	4	144	72	89	2	1.000	144	89	233
11-056	Noodles, egg, medium, boiled	4	110	11	64	5	0.323	36	21	56
nc	Noodles, instant, raw	4	nd		48	3	1.000	nd	48	48
11-061	Pasta, spaghetti, white, raw	5	63	4	75	1	1.000	63	75	138
11-062	Pasta, spaghetti, white, boiled	5	nd		146	12	0.362	nd	53	53
11-063	Pasta, spaghetti, wholewheat, raw	5	nd		116	14	1.000	nd	116	116
11-064	Pasta, spaghetti, wholewheat, boiled	5	nd		126	7	0.304	nd	38	38
nc	Pasta, wholewheat (variety), raw	3	127	5	94	5	1.000	127	94	221
11-071	Bread, brown, pre-sliced	4	5005	544	3770	360	0.598	2992	2254	5245
11-078	Bread, granary, pre-sliced	3	2295	123	4614	125	0.486	1116	2244	3360
11-080	Bread, Hovis wheatgerm, pre-sliced	1	4223	52	3886	120	0.602	2541	2339	4880
11-102	Bread, white, pre-sliced	5	2297	386	2663	574	0.590	1356	1572	2928
11-113	Bread, wholemeal, pre-sliced	5	6387	668	7818	738	0.584	3731	4567	8298
11-126	Breakfast cereal; Kelloggs All Bran	1	nd		nd			nd	nd	
11-128	Breakfast cereal; Kelloggs Bran Flakes	1	nd		nd			nd	nd	
11-129	Breakfast cereal; Kelloggs Coco-Pops	1	nd		53	9	1.000	nd	53	53
11-130	Breakfast cereal; Kelloggs Corn Flakes	1	nd		nd			nd	nd	
11-131	Breakfast cereal; Kelloggs Crunchy Nut Cornflakes	1	120	12	195	25	1.000	120	195	315
11-133	Breakfast cereal; Kelloggs Frosties	1	nd		nd			nd	nd	
11-134	Breakfast cereal; Kelloggs Fruit and Fibre	1	nd		37	1	0.999	nd	37	37
nc	Breakfast cereal; muesli style, Alpen	1	tr		nd		1.000	tr	nd	
nc	Breakfast cereal; muesli, Jordans natural country muesli	1	244	3	273	7	1.000	244	273	517
nc	Breakfast cereal; muesli, Jordans original crunchy, almonds and raisins	1	tr		nd		1.000	tr	nd	
nc	Breakfast cereal; muesli, Jordans special recipe muesli	1	87	10	88	3	0.999	87	88	175
nc	Breakfast cereal; porridge, Scots porridge oats, raw	1	nd		43	10	1.000	nd	43	43
11-144	Breakfast cereal; Quaker Sugar Puffs	1	nd		nd			nd	nd	

Table 1 continued

Table 1 Cont.

M&W code[a]	Food	Concentration (μg/kg dry wt)					Mean concentration (μg/kg wet weight)			
		Number of samples	Daidzein		Genistein		DM	Da	Ge	Sum of Da and Ge
			Mean	s.e.	Mean	s.e.				
11-145	Breakfast cereal; Ready Brek, raw	1	nd		nd			nd	nd	
11-146	Breakfast cereal; Kelloggs Rice Krispies	1	nd		nd			nd	nd	
11-147	Breakfast cereal; Kelloggs Ricicles	1	nd		41	4	1.000	nd	41	41
11-148	Breakfast cereal; Nestle Shredded wheat	1	372	26	760	30	1.000	372	760	1132
11-149	Breakfast cereal; Nestle Shreddies	1	nd		nd			nd	nd	
11-150	Breakfast cereal; Kelloggs Special K	1	nd		nd			nd	nd	
11-151	Breakfast cereal; Kelloggs Start	1	101	1	87		1.000	101	87	188
11-153	Breakfast cereal; Kelloggs Sultana Bran	1	tr		nd		0.999	tr	nd	
11-154	Breakfast cereal; Weetabix	1	nd		nd			nd	nd	
11-167	Biscuits; Jacobs Cream Crackers	1	146	6	300	21	1.000	146	300	446
nc	Crispbread, multigrain	4	6085	443	5788	14	1.000	6085	5788	11 873
11-168	Crispbread, rye	5	25	5	tr		1.000	25	tr	25
nc	Crispbread, wheat	2	66	9	179	9	1.000	66	179	245
nc	Crispbread, wholemeal wheat	4	92	22	130	27	1.000	92	130	222
11-169	Biscuits; McVities Chocolate Homewheat	1	165	0	60	9	1.000	165	60	225
nc	Biscuits; McVities Cheddars	1	5		76	3	1.000	5	76	81
nc	Biscuits; Jacobs Choice Grain crackers	1	nd		61		1.000	nd	61	61
11-170	Biscuits; McVities Digestives	1	259	62	288	9	1.000	259	288	547
11-172	Biscuits; McVities Ginger Nuts	1	88	4	41	5	0.999	88	41	129
nc	Biscuits; Jacobs Fig Rolls	1	140	7	167	62	0.995	139	166	306
11-178	Biscuits; Rakusen's Matzos	1	4	6	75	12	1.000	4	75	79
11-182	Biscuits; Custard Creams, Golden Biscuit Co.	1	290	44	113	14	1.000	290	113	403
11-183	Biscuits; McVities Rich Tea Biscuits	1	132	3	149	7	1.000	132	149	281

[a]M&W code, food coding taken from Holland *et al* (1988). DM, dry matter; DA, daidzein; GE, genistein. nc, not coded in Holland *et al* (1988); nd, not detected in this food; tr, compound detected in unquantifiable trace concentration.

Effects unrelated to their agonist effects in estrogen receptors have also been established (Akiyama *et al*, 1987).

The variety of possible beneficial effects of phytoestrogens has stimulated much interest in the investigation of food intake in relation to risk of these diseases in epidemiological studies. However, little data exists on levels in foods, apart from soya for inclusion in food composition databases. We have previously published data on levels of isoflavones present in vegetables (Liggins *et al*, 2000b) and in fruit and nuts (Liggins *et al*, 2000a). Although lignans are present in cereals, no or only trace amounts of isoflavones have hitherto been reported in cereals (Than *et al*, 1998; Mazur & Adlercreutz, 1998). In order to investigate this further, we have analysed levels of daidzein and genistein in a variety of cereals, cereal products and three stock soy flours.

Methods

Collection and preparation of food samples

Representative examples for most cereals were obtained by purchasing five samples of each from different sources in the Cambridge area, typically two market stalls and three supermarkets. The food was weighed, 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 a week or more. Thereafter the samples were weighed, milled with a kitchen grinder (model BL350, Kenwood Ltd, Havant, Hampshire, UK) and stored in separate air-tight jars. Further desiccation was performed before the samples were assayed. If the food was normally eaten cooked, each prepared sample was split equally into two, and half was prepared as for the raw food samples. The remaining half of each sample were 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.

The breakfast cereals and biscuits were from single packets of brand-named goods purchased in a supermarket. If the food was a brand good made by only one manufacturer then only one sample would be purchased. Where the food was more generic then five distinct samples were purchased. Exceptions to the latter were where the food list specified branded goods because they were the market leader at the time the list was made, eg Kellogg's Cornflakes.

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). This article 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 silanized in a solution of dimethyldichlorosilane in heptane (1:20 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). Since the cooked foods were 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 others without internal standards to approximate the 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 (UK), Reading University, UK) were used as the internal standards spiked into two of the replicates of each food type. The spike was calculated to deliver the same concentration of daidzein and genistein as was already in the food. The two remaining replicate samples were unspiked and assayed as sample blanks. The difference in the average isoflavone concentration of the two spiked samples and the sample blanks was used to calculate the recovery of the internal standards.

The isoflavone glycosides present in both the food and the spike were dissolved into at least 10 ml aqueous methanol (4:1 v/v) using 15 min of sonication 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 no. 1), and any adsorbed isoflavones washed through with fresh aqueous methanol (4:1 v/v, > 5 ml). The alcohol in the filtrate was evaporated off, under nitrogen, to leave an aqueous extract, to which was added 5 ml of 0.1 M acetate buffer, pH 5, containing 100 Fishman units of cellulase (*Aspergillus niger*). The mixture was incubated overnight at 37°C to hydrolytically remove the carbohydrate component of the hydrolysis solution by partitioning into ethyl acetate; three 2 ml washes of ethyl acetate were combined, 2 ml of the total was aliquoted into a separate vial and the solvent evaporated under nitrogen.

The dried extracts were derivatized by adding 0.6 ml pyridine followed by 0.4 ml *N*-(tert-butyldimethylsilyl)-*N*-methyltrifluoro-acetamide (TBDMS) containing 1% TBDMS-chloride catalyst. After 1 h at room temperature, 3 µl of the sample were injected on to the capillary column of the gas chromatograph mass spectrometer (GC-MS, MD800, Fisons, UK). The GC-MS conditions used were as 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 the completeness of the extraction protocol and adjust the concentration of isoflavones determined to be in the food to account for any procedural losses. If the recovery of the internal standards fell below 70% due to losses in the extraction procedure, results were not reported and the analysis was repeated. The standard error from the mean (reported in the results) was calculated from the assay initially employed to determine the concentration of internal standards, and the average of the two subsequent unspiked assays, performed on another day.

Wet weight concentrations of daidzein and genistein were calculated from the percentage dry matter in the food and the assayed dry weight concentration.

Results

Of the 78 assayed foods reported in this article, 57 contained measurable quantities of daidzein and/or genistein. Three additional foods contained trace quantities. Table 1 illustrates the daidzein and genistein content of food (by both dry and wet weight). The foods are listed in the order of their codes in the supplement to the McCance and Widdowson's *Composition of Foods* (Holland *et al*, 1988). A number of the foods assayed have not yet been assigned codes but are listed in the results in an appropriate position.

Within the group of 57 foods that contain daidzein and genistein, the soy flours contained the most, 1.6–2.1 mg/g of the two isoflavones combined. The five breads and crispbread contained more than 1 µg/g, but less than 0.1 mg/g of daidzein and genistein combined. These foods are likely to contain soy flour as a minor ingredient. Wholegrain brown rice and shredded wheat also contained more than 1 µg/g food. Foods containing more than 100 ng/g but less than 1 µg/g varied and comprised raw pasta, brown, granary and wholemeal flours, some breakfast cereals, biscuits and crispbreads, and cereal grains such as semolina cornflour and maize meal. The lowest range of concentrations, less than 100 ng/g, comprised a varied selection of 20 foods, including cooked pasta and rice, other cereal grains, biscuits and breakfast cereals.

Discussion

The cereal foods, collected and assayed during the course of this work, were selected because they are commonly eaten in Europe. In order to minimize sampling error a pooled sample of each food was assayed and the results reported in this article are the average concentration of five samples of each non-branded food.

Table 2 Comparison of daidzein and genistein concentration (μg/100 g) in cereals with results published elsewhere

	This publication		*Other publications*		
Food	*Daidzein*	*Genistein*	*Daidzein*	*Genistein*	*Reference*
Bread, white	135.6	157.2	606.0	830.8	Horn-Ross *et al* (2000)
Bread, whole grain	373.1	456.7	155.8	141.8	Horn-Ross *et al* (2000)
Oatmeal	nd	nd	0/tr	0/tr	Horn-Ross *et al* (2000)
			0	0	Adlercreutz & Mazur (1997)
Wheat bran	nd	3.8	3.5	6.0	Adlercreutz & Mazur (1997)
Whole wheat flour	2.4	14.6	0	0	Adlercreutz & Mazur (1997)
Rice, brown, boiled	4.2	4.8	0/tr	0/tr	Horn-Ross *et al* (2000)
Rice, white, boiled	2.6	3.9	0/tr	0/tr	Adlercreutz & Mazur (1997)

The analytical data presented in Table 1 indicate that there are a number of possible sources of dietary daidzein and genistein. The richest sources are of soy origin, containing 1.6–2.1 mg/g as shown elsewhere (Reinli & Block 1996; Murphy *et al*, 1999; Franke *et al*, 1999; Wakai *et al*, 1999) Five types of bread contained between 1 μg and 0.1 mg of daidzein and genistein combined per gram wet weight of food. This is probably because soy flour is included as an ingredient of bread, since the daidzein and genistein content of flours only ranged from not detected to 362 ng/g. Raw whole grain brown rice and shredded wheat also had concentrations in this range. Whole grain brown rice would have had no added soy, thus it is in itself a source of daidzein and genistein.

These results were obtained without the use of labelled conjugated standards, since these are currently not available. In the absence of these to determine possible losses during the hydrolysis and extraction procedure, unlabelled conjugated pure compounds were added in known amounts in the second analyses (by the standard addition method) and the expected amount recovered compared with that observed. Although only results from analyses where more than 70% of the addition was recovered were obtained, this is not an entirely satisfactory way of determining accuracy since losses incurred will depend largely on the intact food matrix and the position and link type of the glycoside residue. Results published here were compared with results published elsewhere in Table 2. There was good agreement between analytical results for all foods except bread. Levels were higher in white bread and lower in wholemeal bread analysed in the US than in the UK. This probably results from different usage of soy in bread in these two countries. Nevertheless, good agreement with results published elsewhere, although reassuring, is not absolute proof of accuracy and future developments may result in improvements to the analytical method used.

The study reported in this article has concentrated on quantifying two phytoestrogens in cereals, in order that they can be included in databases of food composition. In this respect identification of foods that contain no daidzein and genistein is as important as knowledge of their concentrations in foods that do contain these phytoestrogens. Obviously other phytoestrogens and foods are consumed by the UK and world populations. The results presented in this article represent initial research aimed at elucidating the health implications of daidzein and genistein and will be of use in epidemiological and prospective studies. Future research should include quantification of other phytoestrogens both in the foods analysed in this article and others.

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