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Daidzein and genistein content of fruits and nuts

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Dietary phytoestrogens such as the isoflavones daidzein and genistein are thought to protect against chronic diseases that are common in Western societies, such as cancer, osteoporosis, and ischemic heart disease. In addition, there are concerns regarding the deleterious effects of hormone-like compounds, especially with respect to the development of infants. However, there is little information regarding the phytoestrogen content of foods, and therefore epidemiologic investigations of phytoestrogens are limited. As part of a study quantifying the consumption of phytoestrogens, the objective of this work was to assess the daidzein and genistein content of fruits and nuts commonly eaten in Europe. Eighty different fruits and nuts were sampled, prepared for eating, and freeze-dried. Daidzein and genistein were extracted from the dried foods, and the two isoflavones were 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. Of the 80 foods assayed, 43 contained no detectable daidzein or genistein, at a limit of quantification of 1 μg/kg dry weight of food. Nine foods contained more than 100 μg of the two isoflavones combined per kilogram wet weight, and 28 contained less than this amount. Currants and raisins were the richest sources of the isoflavones, containing 2,250 μg and 1,840 μg of the two isoflavones combined per kilogram of wet weight of food. Although fruits and nuts are not as rich in isoflavone phytoestrogens as are soy and other legumes, this is the first documentation of levels of daidzein and genistein occurring in these foods. (J. Nutr. Biochem. 11:326–331, 2000)

Keywords: phytoestrogens; isoflavones; daidzein; genistein

Introduction

Daidzein and genistein are isoflavones that form part of a diverse group of natural constituents of foods, which, because of their estrogenic activity in humans, are known as phytoestrogens. Phytoestrogens bear a structural resemblance to 17β-estradiol, and in vivo can interfere with mechanisms controlled by the hormone through competition for its receptors. In addition, phytoestrogens can have nonestrogen receptor mediated effects. Phytoestrogens may be important antioxidants.[1,2] They perturb the action of DNA topoisomerase II and ribosomal S6 kinase, which could explain their observed effects on cell cycle, differentiation, proliferation, and apoptosis. In addition, genistein is a potent tyrosine kinase inhibitor.[3,4] It has been proposed that through such mechanisms phytoestrogens protect against a wide range of ailments including breast, prostate, bowel, and other cancers, cardiovascular disease, osteoporosis, and menopausal symptoms.[3,5–9] However, apart from soy and a few other legumes, little is known of the phytoestrogen content of foods.[7,10] As part of our work to determine the levels of phytoestrogens in foods, we report here the concentrations of daidzein and genistein in a variety of fruits and nuts commonly eaten in Europe.

The method used to assay fruits and nuts for daidzein and genistein has been published previously.[11] It was developed using mixtures of bleached white wheat flour and soy flour with a known isoflavone composition. Daidzein and

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genistein are present in soy mainly as three types of glycosidic conjugate, linked either to glucose, malonyl, or acetyl derivatives of glucose. However, it is likely that other carbohydrate residues are linked in substitution of the glucose and that positions other than the carbon seven hydroxyl group of the isoflavone are used for a glycosidic bond. The carbohydrate component of the isoflavones is removed by the action of bacteria in the gut, producing the aglycones and other metabolites. Therefore, measurement of the total daidzin and genistin (the glucose derivatives) content of a food may underestimate the total daidzein and genistein content of a food after digestion and bacterial action. The assay procedure used in this article employs enzymes from *Aspergillus niger*, collectively known as cellulase, to hydrolyze the glycosidic bond and yield the aglycones daidzein and genistein. We have previously shown that this enzyme preparation works quantitatively with the various glycosides present in soya, and in the absence of knowledge of the identity and possession of reference standards of other glycosides, we have assumed that it hydrolyzes these with equal efficiency.[11] The data on the concentration of daidzein and genistein in foodstuffs reported in this article can be used in epidemiologic studies to assess the dietary intake of these compounds.

Materials and methods

Collection and preparation of food samples

Representative samples of each food 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, 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, Hampshire, UK), and stored in separate airtight jars. Further desiccation was performed before the samples were assayed. Two samples (dried prunes and chestnuts) were additionally assayed after boiling in water; the prepared raw samples were split into two parts, one of which was assayed as for the raw foods. The remaining portion was chopped into small pieces, pooled with the other four samples of that food type, and boiled with water (10 min for the prunes, 3 min for the chestnuts). After cooking the foods were drained, frozen, and freeze-dried in the same manner as the raw foods.

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.[11] This article contains only a brief description of the assay method including any slight modifications used for the assay of these foods.

All enzymes, reagents, and chemicals were purchased from Sigma/Aldrich (Poole, Dorset, UK) unless otherwise stated. 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 in a single 20-mL screw cap test tube (2.5 g in total). Because the cooked foods were pooled at an earlier stage, 1 g of the freeze-dried product was weighed in 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, the synthetic glucosides daidzin (7-O-glucosyl-4′-hydroxyisoflavone) and genistin (7-O-glucosyl-4′5-dihydroxyisoflavone; both purchased from Plantech, Reading, UK) were used as the internal standards spiked into two of the replicates of each food type.[11] 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 in 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 units of cellulase (*A. niger*, units as defined by Sigma). The mixture was incubated overnight at 37°C to hydrolytically remove the carbohydrate component of the isoflavone glycosides. 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 aliquoted into a separate vial, and the solvent was evaporated under nitrogen.

The dried extracts were derivatized by adding 0.6 mL pyridine followed by 0.4 mL N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (TBDMS) containing 1% TBDMS-chloride catalyst. After 1 hr at room temperature, 3 μL of the sample was injected into the capillary column of the gas chromatograph mass spectrometer (GC-MS; MD800, Fisons, UK). The GC-MS conditions used are as described elsewhere.[11]

Calculation of isoflavone quantities in food

The daidzein and genistein concentrations 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 to assess the completeness of the extraction protocol. In addition the recovery was used to adjust the concentration of isoflavones determined to be in the food to compensate for procedural losses. The results show the percentage recovery of the internal standards for each food, though if the recovery fell below 70% for either compound, the results were discarded and the assay repeated. The standard error of 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 was performed on another day. Wet weight concentrations of daidzein and genistein were calculated from the percentage of dry matter in the food and the assayed dry weight concentration.

Results

Eighty examples of fruits and nuts were analyzed for their daidzein and genistein content, of which 36 contained measurable quantities. In addition, raspberry tinned in syrup contained trace quantities of the isoflavones, but due to interference by other compounds these could not be mea-

Table 1 Daidzein and genistein content of fruits and nuts

Food (M&W code[1])		Assayed	Recovery of internal standards (%)		Concentration per dry weight (μg/kg)				Dry matter	Mean concentration per wet weight (μg/kg)		
					Daidzein		Genistein					
			Da	Ge	Mean	SE	Mean	SE		Da	Ge	Sum of Da and Ge
14-002	Apples, cooking, raw	Jan-98			nd		nd			nd	nd	
14-005	Apples, cooking, cooked	Jan-98			nd		nd			nd	nd	
14-017	Apple, Cox with skin	Jul-97			nd		nd			nd	nd	
nc	Apple, Cox no skin	Jul-97			nd		nd			nd	nd	
14-019	Apple, golden delicious with skin	Jan-98			nd		nd			nd	nd	
nc	Apple, golden delicious without skin	Jan-98			nd		nd			nd	nd	
14-021	Apple, Granny smith with skin, raw	Jul-97			nd		nd			nd	nd	
nc	Apple, Granny smith without skin	Jul-97			nd		nd			nd	nd	
14-023	Apple, red with skin	Jan-98			nd		nd			nd	nd	
nc	Apple, red without skin	Jan-98			nd		nd			nd	nd	
14-025	Apricots, raw	Jan-98			nd		nd			nd	nd	
14-031	Apricots, dried	Jun-98	82		50	3	nd		0.855	42.7	nd	42.7
14-034	Apricots, tinned	Feb-98			nd		nd			nd	nd	
14-037	Avocado	Jul-98			nd		nd			nd	nd	
14-045	Banana	Jul-98			nd		nd			nd	nd	
14-061	Cherries, raw	Jan-98			nd		nd			nd	nd	
14-071	Clementines	Mar-98	96	104	27	2	270	119	0.107	2.9	29	31.9
14-073	Cranberries	Aug-97	75	89	51	12	213	14	0.144	7.3	30.6	37.9
14-074	Currants	May-98	96	77	560	8	2167	2	0.823	461	1783.8	2244.8
14-085	Dates, dried	Jun-98	103	100	18	1	54	6	0.956	17.2	51.6	68.8
14-091	Figs, raw	Mar-98	87	91	28	20	77	3	0.180	5	13.9	18.9
14-092	Figs, dried	Apr-98	84	86	19	5	45		0.933	17.7	42	59.7
14-097	Fruit cocktail in syrup	Mar-98	103	80	nd		17		0.184	nd	3.1	3.1
14-100	Gooseberries, raw	Jan-98			nd		nd			nd	nd	
14-107	Grapefruit, tinned in natural juice	Feb-98			nd		nd			nd	nd	
14-109	Grapes, black	Oct-97			nd		nd			nd	nd	
14-109	Grapes, white	Oct-97			nd		nd			nd	nd	
14-111	Greengage, raw	Jan-98			nd		nd			nd	nd	
14-123	Kiwi fruit	Feb-98			nd		nd			nd	nd	
14-144	Lyches, tinned in syrup	Feb-98			nd		nd			nd	nd	
14-147	Oranges, mandarin, tinned	Feb-98			nd		nd			nd	nd	
14-148	Mango, raw	Oct-97	89	92	251	41	212	52	0.153	38.3	32.3	70.6
14-150	Mango tinned in syrup	Mar-98	109	75	39	13	54		0.235	9.2	12.7	21.9
14-156	Melon, canteloupe	Mar-98		107	nd		42		0.103	nd	4.3	4.3
14-159	Melon, galia	Jun-98	81	90	10	3	46	5	0.169	1.7	7.8	9.5
14-162	Melon, yellow honeydew	Dec-97	81	98	151	17	117	77	0.097	14.6	11.3	25.9
14-165	Watermelon	Oct-97			nd		nd			nd	nd	
14-171	Nectarines	Jan-98			nd		nd			nd	nd	
14-173	Olives, tinned in brine	Apr-98			nd		nd			nd	nd	
14-175	Oranges	Jul-97			nd		nd			nd	nd	
14-178	Passion fruit	Feb-98	80	86	245	18	403	11	0.268	65.8	108.2	174
14-183	Peach, raw	Jan-98			nd		nd			nd	nd	
14-189	Peaches, tinned in syrup	Mar-98	76	88	32		59	1	0.169	5.4	10	15.4
14-198	Pears, tinned in light syrup	Apr-98	75	82	10	2	52	3	0.200	2	10.4	12.4
nc	Pears, comice without skin	Mar-98	71	76	3		54	14	0.136	0.4	7.3	7.7
14-201	Pears, conference with skin	Mar-98			nd		nd			nd	nd	
nc	Pears, conference without skin	Mar-98			nd		nd			nd	nd	
14-208	Pineapple	Feb-98			nd		nd			nd	nd	
14-211	Pineapple, tinned in own juice	Feb-98			nd		nd					
14-213	Plums, red, raw	Mar-98			nd		nd			nd	nd	
14-220	Plums, Victoria, raw	Mar-98	105	73	5		551	24	0.135	0.7	74.4	75.1

(continued on next page)

Table 1 *(Continued)*

Food (M&W code[1])		Assayed	Recovery of internal standards (%)		Concentration per dry weight (μg/kg)				Dry matter	Mean concentration per wet weight (μg/kg)		
			Da	Ge	Daidzein Mean	Daidzein SE	Genistein Mean	Genistein SE		Da	Ge	Sum of Da and Ge
14-226	Pomegranates	Mar-98			nd		nd			nd	nd	
14-239	Prunes, dried, raw	Apr-98	74	93	52	33	104	35	0.820	42.6	85.3	127.9
nc	Prunes, dried, cooked	Apr-98	73	84	153	22	663	115	0.201	30.8	133.3	164.1
14-242	Raisins, California	May-98	80	98	690	24	1458	40	0.855	589.9	1246.5	1836.4
14-244	Raspberry, raw	Jan-98			nd		nd			nd	nd	
14-246	Rhubarb, cooked	Jul-97			nd		nd			nd	nd	
14-248	Raspberry, tinned in syrup	Jul-98			tr		tr			tr	tr	
14-252	Rhubarb, raw	Jul-97			nd		nd			nd	nd	
14-257	Satsumas	Mar-98	89		80	8	nd		0.102	8.2	nd	8.2
14-260	Strawberry, raw	Oct-97	79	102	45	30	457	108	0.101	4.5	46.1	50.6
14-262	Strawberries, tinned in syrup	Jul-98		88	nd		223	101	0.179	nd	40	40
14-277	Lemon juice, fresh	Jul-97			nd		nd			nd	nd	
14-283	Orange juice	Mar-98			nd		nd			nd	nd	
14-801	Almonds	Feb-98			nd		nd			nd	nd	
14-808	Brazil nut	Apr-98	83		12	2	nd		0.987	11.8	nd	11.8
14-813	Chestnuts, raw	Jun-98	72	96	79	17	59	5	0.498	39.3	29.4	68.7
nc	Chestnuts, cooked	Apr-98		77	nd		7		0.587	nd	4.1	4.1
14-816	Coconut, fresh	Jun-98	109	70	128	1	185	4	0.594	76	109.9	185.9
14-818	Coconut, desiccated	Mar-98			nd		nd			nd	nd	
14-821	Hazelnuts	Apr-98	93	70	58	2	194	28	0.952	55.2	184.7	239.9
14-829	Peanut butter	Apr-98		72	nd		98	1	1.000	nd	98	98
14-831	Peanuts, fresh	Apr-98	80	84	77	4	158	2	1.000	77	158	235
14-833	Peanuts, dry roasted	Apr-98	82	75	37	3	172	3	1.000	37	172	209
14-844	Sesame seeds	Apr-98	101	82	37	6	17	4	1.000	37	17	54
14-845	Sunflower seeds	Apr-98			nd		nd			nd	nd	
14-850	Walnuts	Apr-98			nd		nd			nd	nd	
nc	Fruit pie filling; bramley apple and raspberry, tinned	Jul-98	84		13	2	nd		0.212	2.8	nd	2.8
nc	Fruit pie filling; black cherry, tinned	Jul-98	73	77	10	3	182	3	0.255	2.6	46.4	49
nc	Fruit pie filling; black currant, tinned	Jul-98	70		6	1	nd		0.265	1.6	nd	1.6
nc	Fruit pie filling; red cherry, tinned	Jul-98			nd		nd			nd	nd	

The foods are listed in their order of appearance in Holland *et al.* (i.e., M & W code).[12] The month and year in which they were assayed is stated. The percentage recovery of the internal standards is indicated, but the analytical results presented have been corrected for losses of the internal standards. Da–daidzein. Ge–genistein. nc–not coded in Holland *et al.*[12] nd–not detected in this food. tr–compound detected in unquantifiable trace concentration.

sured. *Table 1* shows the daidzein and genistein content of all foods (by both dry and wet weight) corrected for losses of the internal standards. The foods are listed in the order of the codes given in Holland et al.[12] A number of the foods assayed have not yet been assigned codes but are listed in the results in an appropriate position. *Table 1* also includes the percentage recovery of the internal standards assayed with the foods; losses ranged from 70% to 109%.

The results indicate that daidzein and genistein are found in a variety of fruits and nuts at concentrations ranging from 1 to 2,250 μg of daidzein and genistein combined per kilogram wet weight of food. In comparison, soya beans contain approximately 2 g/kg wet weight.[13] Of the 36 samples of fruits and nuts that contained daidzein or genistein, currants and raisins were the richest, containing 2,250 and 1,840 μg of the analytes per kilogram of wet weight of the food, respectively. The other 34 foods contained between 250 and 1 μg/kg wet weight of food. *Figure 1* compares the combined concentration of daidzein and genistein in the 36 foods that contained these isoflavones. Three examples of peanut were analyzed and all had daidzein and genistein concentrations at the high end of the range illustrated in *Figure 1*. However, other non-leguminous nuts contained various concentrations and in three neither of the isoflavones was detected.

Discussion

The foods selected for this study were chosen because they are common items in the European diet. There are no other

published values for the amounts of daidzein and genistein in fruits and nuts. Published analytical data relating to the concentration of phytoestrogens is in the most part restricted to a small number of foods, most of which are relatively rich sources. Sources of low concentrations of phytoestrogens may be more common in particular dietary combinations, and cumulatively the low concentrations may have significant effects on health. The concentrations reported in this article are an average from five pooled samples of each food. The variation in concentration between these samples was not determined in this work due to the extensive work necessary to obtain such data. However, natural variation could be considerable, as is known to occur in soya.[13–17]

The carbohydrate component of glycosides of daidzein and genistein is currently considered to be glucose and its malonyl and acetyl derivatives. However, it is likely that the isoflavones mimic the carbohydrate conjugation of the flavones, each flavone existing naturally, across the plant kingdom, as a multiplicity of different glycosides. Assays restricted to the quantification of known glycosides would underestimate the quantity of isoflavones in food where the predominant form was an unknown glycoside. For this reason the study reported herein employed a hydrolytic step in the preparative procedure. This removed the carbohydrate component, enabling the assay of the liberated aglycone. The validation of the assay indicated that all of the known glycosides were completely hydrolyzed by the cellulase.[11] However, incomplete hydrolysis of glycosides (known or unknown) for which there are no reference standards cannot be definitively assessed. The concentrations of daidzein and genistein reported here may thus underestimate the actual content of the food.

The analytical data presented in *Table 1* indicate that there are a number of possible sources of dietary daidzein and genistein. The graphical comparison of daidzein and genistein content of fruits and nuts (*Figure 1*) illustrates that the three examples of peanut product investigated in this study appear to be among the richest 10 foods. The actual concentration range exhibited by peanuts (100–240 μg/kg wet weight) is, however, within the concentration range found in this laboratory (4 and 6,000 μg/kg wet weight) for other legumes.[18]

A number of the foods collected for the work described herein were not pure single ingredients, but had been processed in some way before purchase. It is therefore possible that during processing, the foods may have come into contact with other foodstuffs that contain daidzein and genistein. Currants and raisins, for example, may be good sources of the isoflavones naturally. However, the actual source of the daidzein and genistein in currants and raisins may have been vegetable oil, such as soy oil, or soy emulsifiers to which these foods might have been exposed to during processing. No detectable daidzein or genistein was apparent in grapes (*Table 1*), but this could be due to differences in cultivar between the fruits used for eating fresh or after drying.

The study reported in this article was designed to determine the the daidzein and genistein content of food. Analytical data for food types other than fruits will be published in due course.[18] Of the fruits and nuts that contained daidzein and genistein, the concentration was

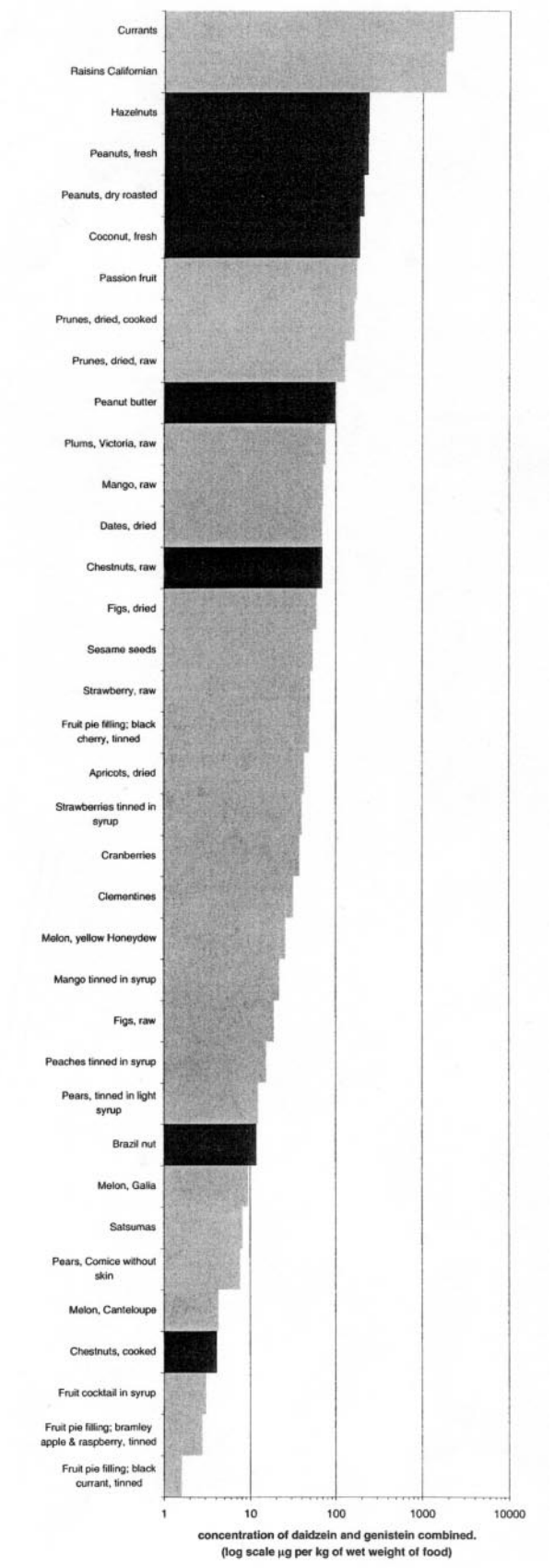

Figure 1 Comparison of the combined concentration of daidzein and genistein in different fruit (grey) and nuts (black).

largely similar to that of the vegetables, but a larger proportion of the vegetables contained the isoflavones. However, soy is a very rich source of daidzein and genistein, up to 3.8×10^6 μg/kg wet weight of soybean.[13] Clearly the inclusion of even a small portion of a soy product in a diet will expose the consumer to very significant concentrations of daidzein and genistein. Nevertheless, fruits, nuts, and vegetables contain a broad range of concentrations of these compounds and will contribute to the daily dietary intake. The results reported here will contribute to databases of the phytoestrogen content of diets applied to the dietary intake of foods of free living individuals and is the sole source of such information in the literature at the present time.

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