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Analytical Methods

Phytoestrogen content of fruits and vegetables commonly consumed in the UK based on LC–MS and ^{13}C-labelled standards

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ABSTRACT

Phytoestrogens are a group of non-steroidal secondary plant metabolites with structural and functional similarity to 17β-oestradiol. Urinary and plasma phytoestrogens have been used as biomarkers for dietary intake, however, this is often not possible in large epidemiological studies or to assess general exposure in free-living individuals. Accurate information about dietary phytoestrogens is therefore important but there is very limited data concerning food contents. In this study, we analysed the phytoestrogen (isoflavone, lignan and coumestrol) content in more than 240 different foods based on fresh and processed fruits and vegetables using a newly developed sensitive method based on LC–MS incorporating $^{13}C_3$-labelled standards. Phytoestrogens were detected in all foods analysed with a median content of 20 μg/100 g wet weight (isoflavones: 2 μg/100 g; lignans 12 μg/100 g). Most foods contained less than 100 μg/100 g, however, 5% of foods analysed contained more than 400 μg/100 g, in particular soya-based foods and other legumes. The results published here will contribute to databases of dietary phytoestrogen content and allow the more accurate determination of phytoestrogen exposure in free-living individuals.

1. Introduction

Phytoestrogens are a group of non-steroidal polyphenolic plant metabolites that induce biological responses and can mimic or modulate the action of endogenous oestrogens, often by binding to oestrogen receptors (Committee on Toxicity of Chemicals in Food, 2003). The bioactivity of these compounds is based on their structural similarity with 17β-oestradiol (Branham et al., 2002; Martin, Horwitz, Ryan, & McGuire, 1978; Setchell & Adlercreutz, 1988; Verdeal, Brown, Richardson, & Ryan, 1980) and their ability to bind to oestrogen receptors (Shutt & Cox, 1972). Apart from their effect on oestrogen receptors, phytoestrogens can also act as antioxidants (Wei, Bowen, Cai, Barnes, & Wang, 1995) and inhibitors of enzymes such as tyrosine kinase (Akiyama et al., 1987) and DNA topoisomerase (Markovits et al., 1989). As a result of their bioactivity, these compounds have received increasing attention for potentially beneficial effects for a wide range of human conditions such as cancer (Adlercreutz, 2002; Duffy, Perez, & Partridge, 2007; Peeters, Keinan-Boker, van der Schouw, & Grobbee, 2003; Stark & Madar, 2002), cardiovascular disease (Anthony, 2002; Stark & Madar, 2002), osteoporosis (Dang & Lowik, 2005; Stark & Madar, 2002) menopausal symptoms (Krebs, Ensrud, MacDonald, & Wilt, 2004; Stark & Madar, 2002), male infertility (Phillips & Tanphaichitr, 2008), obesity and type 2 diabetes (Bhathena & Velasquez, 2002). However, elevated endogenous sex hormone levels are generally associated with an increased risk of breast cancer in women (The Endogenous Hormones and Breast Cancer Collaborative, 2002) and not all studies have shown a beneficial effect on breast cancer risk associated with increased exposure to phytoestrogens in Western societies (Grace et al., 2004; Ward et al., 2008). There are also strong gene–nutrient interactions between phytoestrogens and oestrogen receptor polymorphisms (ESR1 and NR1I2) (Low et al., 2005b, 2007), polymorphisms in the gene for the sex-hormone binding globulin (SHBG) (Low et al., 2006) and probably polymorphisms in the gene encoding aromatase (CYP19) (Low et al., 2005a) which influence their bioactivity. Despite the large number of studies conducted, there is still no clear evidence whether phytoestrogen intake has a beneficial or detrimental effect on human health and the UK Committee on Toxicity (COT) has recommended further research (Committee on Toxicity of Chemicals in Food, 2003).

Exposure to phytoestrogens can be determined either directly by measuring diet or indirectly by using biomarkers in plasma or urine (Grace et al., 2004). Although biomarkers are often more reliable due to the limitations in dietary assessment (Day, McKeown,

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Table 1

Phytoestrogen content of fruits and vegetables analysed. The data is the average of three samples analysed in duplicate and given in μg/100 g wet weight. Isoflavones are the sum of daidzein, genistein, glycitein, biochanin A and formononetin, lignans the sum of secoisolariciresinol and matairesinol. Unless stated otherwise, food analysed was unprepared.

Food (taxonomic name)	Preparation	Variety	Family	Phytoestrogens	Isoflavones	Lignans	Daidzein	Genistein	Glycitein	Biochanin A	Formononetin	Secoisolariciresinol	Matairesinol	Coumestrol
Apple (*Malus domestica*)	Cored	Cox	Rosaceae	4	2	2	<1	<1	–	1	<1	2	<1	<1
Apple (*Malus domestica*)	Cored	Golden Delicious	Rosaceae	5	2	3	<1	<1	–	<1	<1	3	<1	<1
Apple (*Malus domestica*)	Cored	Granny Smith	Rosaceae	4	2	2	<1	<1	–	<1	1	2	<1	<1
Apple (*Malus domestica*)	Cored	Red dessert	Rosaceae	3	1	2	<1	<1	<1	<1	<1	2	–	–
Apple (*Malus domestica*)	Peeled, cored & cooked	Cooking apple	Rosaceae	9	7	2	2	<1	<1	4	<1	2	<1	–
Apple (*Malus domestica*)	Peeled & cored	Cooking apple	Rosaceae	5	3	2	1	<1	<1	1	<1	1	<1	–
Apple (*Malus domestica*)	Peeled & cored	Cox	Rosaceae	5	2	2	<1	<1	–	<1	<1	2	<1	<1
Apple (*Malus domestica*)	Peeled & cored	Golden Delicious	Rosaceae	5	3	2	<1	<1	<1	2	<1	2	<1	–
Apple (*Malus domestica*)	Peeled & cored	Granny Smith	Rosaceae	4	2	2	<1	<1	–	<1	<1	2	<1	<1
Apple (*Malus domestica*)	Peeled & cored	Red dessert	Rosaceae	2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Apricot (*Prunus armeniaca*)	Stoned		Rosaceae	53	1	52	<1	<1	–	<1	<1	51	<1	–
Apricot (*Prunus armeniaca*)	Dried		Rosaceae	443	12	431	–	–	8	4	–	430	<1	<1
Apricot (*Prunus armeniaca*)	Tinned in syrup, drained		Rosaceae	24	2	22	<1	<1	<1	1	<1	22	–	<1
Asparagus (*Asparagus officinalis*)	Cooked		Asparagaceae	154	2	152	–	<1	<1	2	<1	149	3	–
Aubergine (*Solanum melongena*)	Raw		Solanaceae	9	<1	8	<1	<1	<1	<1	<1	8	<1	<1
Aubergine (*Solanum melongena*)	Cooked		Solanaceae	8	<1	8	<1	<1	<1	<1	<1	8	<1	–
Avocado (*Persea americana*)	Peeled & stoned		Lauraceae	43	9	34	<1	<1	6	<1	<1	24	8	–
Banana (*Musa* sp.)	Peeled		Musaceae	3	2	1	<1	<1	<1	1	<1	<1	<1	–
Beans, baked (*Phaseolus vulgaris*)	Cold		Fabaceae	28	5	22	2	3	<1	<1	<1	22	<1	<1
Beans, baked (*Phaseolus vulgaris*)	Heated		Fabaceae	25	6	19	2	3	<1	<1	<1	19	<1	<1
Beans, Broad beans (*Vicia faba*)	Fresh, podded		Fabaceae	21	<1	21	<1	<1	<1	<1	<1	20	<1	–
Beans, Broad beans (*Vicia faba*)	Cooked		Fabaceae	22	<1	21	<1	<1	<1	<1	<1	21	<1	–
Beans, Butter beans (*Phaseolus limensis*)	Dried		Fabaceae	196	51	143	24	21	6	–	–	141	2	2
Beans, Butter beans (*Phaseolus limensis*)	Cooked from dried		Fabaceae	36	13	22	6	5	1	–	<1	22	<1	<1
Beans, French beans (*Phaseolus vulgaris*)			Fabaceae	147	50	94	12	35	2	1	<1	94	<1	3
Beans, French beans (*Phaseolus vulgaris*)	Cooked		Fabaceae	159	48	109	8	36	2	2	<1	108	<1	2
Beans, Haricot beans (*Phaseolus vulgaris*)	Dried		Fabaceae	132	21	106	6	14	<1	–	–	106	–	5

Beans, Haricot beans (*Phaseolus vulgaris*)	Cooked from dried	Fabaceae	29	6	22	2	4	<1	–	–	22	<1	<1
Beans, Kidney beans (*Phaseolus vulgaris*)	Dried	Fabaceae	172	73	89	15	26	32	–	–	88	<1	10
Beans, Kidney beans (*Phaseolus vulgaris*)	Cooked from dried	Fabaceae	41	14	26	2	6	5	<1	–	26	<1	<1
Beans, Runner beans (*Phaseolus coccineus*)	Trimmed & strung	Fabaceae	201	164	26	64	78	22	<1	<1	26	<1	11
Beans, Runner beans (*Phaseolus coccineus*)	Trimmed, strung & cooked	Fabaceae	156	132	18	45	70	16	<1	<1	17	–	7
Beansprouts (*Vigna radiata*)	Pre-washed	Fabaceae	798	351	86	110	225	16	–	<1	86	<1	361
Beetroot (*Beta vulgaris*)	Raw, peeled	Chenopodiaceae	8	1	7	–	<1	<1	1	–	6	1	–
Beetroot (*Beta vulgaris*)	Cooked	Chenopodiaceae	10	<1	10	<1	<1	<1	–	<1	10	<1	–
Beetroot (*Beta vulgaris*)	Pickled	Chenopodiaceae	5	1	4	–	<1	<1	<1	<1	4	<1	–
Beetroot (*Beta vulgaris*)	Precooked	Chenopodiaceae	8	1	7	–	<1	<1	1	<1	7	<1	–
Blackberries (*Rubus* sp.)	Fresh	Rosaceae	57	<1	56	<1	<1	<1	<1	<1	55	1	<1
Blackberries (*Rubus* sp.)	Stewed from fresh	Rosaceae	221	<1	220	–	<1	–	–	<1	220	–	<1
Blackcurrant (*Ribes nigrum*)	Fresh	Grossulariaceae	109	2	107	<1	–	<1	2	<1	105	2	<1
Blackcurrant (*Ribes nigrum*)	Tinned in juice and syrup, drained	Grossulariaceae	69	<1	69	–	<1	–	–	<1	65	4	<1
Broccoli (*Brassica oleracea*)	Fresh, thick stalks removed	Brassicaceae	71	<1	71	<1	<1	<1	<1	–	71	<1	–
Broccoli (*Brassica oleracea*)	Cooked, thick stalks removed	Brassicaceae	96	3	90	<1	<1	<1	3	<1	89	<1	3
Broccoli, sprouting (*Brassica oleracea*)	Tough stalks removed	Brassicaceae	68	1	66	–	<1	–	<1	<1	66	<1	<1
Broccoli, sprouting (*Brassica oleracea*)	Cooked from fresh	Brassicaceae	41	3	38	–	2	–	1	<1	37	<1	<1
Brussel sprouts (*Brassica oleracea*)		Brassicaceae	75	<1	74	<1	–	<1	<1	<1	74	<1	<1
Brussel sprouts (*Brassica oleracea*)	Cooked	Brassicaceae	59	<1	58	<1	<1	<1	<1	<1	58	<1	–
Cabbage, green (*Brassica oleracea*)		Brassicaceae	11	<1	10	–	<1	<1	<1	<1	10	<1	<1
Cabbage, green (*Brassica oleracea*)	Cooked	Brassicaceae	8	<1	7	–	<1	<1	<1	<1	7	<1	<1
Cabbage, January King (*Brassica oleracea*)		Brassicaceae	14	4	10	<1	3	<1	–	<1	10	<1	–
Cabbage, January King (*Brassica oleracea*)	Cooked	Brassicaceae	6	1	5	<1	<1	<1	–	<1	4	<1	–
Cabbage, red (*Brassica oleracea*)		Brassicaceae	7	<1	6	<1	<1	<1	<1	<1	5	<1	–
Cabbage, red (*Brassica oleracea*)	Cooked	Brassicaceae	4	<1	4	<1	<1	<1	<1	<1	4	<1	–
Cabbage, Savoy (*Brassica oleracea*)		Brassicaceae	30	4	26	<1	2	<1	1	<1	26	<1	–
Cabbage, Savoy (*Brassica oleracea*)	Cooked	Brassicaceae	15	3	12	<1	<1	<1	3	<1	12	<1	–
Cabbage, white (*Brassica oleracea*)		Brassicaceae	12	<1	11	<1	<1	<1	<1	<1	11	<1	<1
Cabbage, white (*Brassica oleracea*)	Cooked	Brassicaceae	8	<1	8	<1	<1	<1	<1	<1	8	<1	<1
Carrots (*Daucus carota*)		Apiaceae	125	4	121	2	1	<1	1	<1	114	7	<1
Carrots (*Daucus carota*)	Cooked	Apiaceae	114	3	111	1	2	<1	–	–	107	3	–
Carrots (*Daucus carota*)	Tinned	Apiaceae	49	<1	48	<1	<1	<1	<1	<1	47	1	<1

(*continued on next page*)

Table 1 (*continued*)

Food (taxonomic name)	Preparation	Variety	Family	Phytoestrogens	Isoflavones	Lignans	Daidzein	Genistein	Glycitein	Biochanin A	Formononetin	Secoisolariciresinol	Matairesinol	Coumestrol
Cauliflower (*Brassica oleracea*)			Brassicaceae	15	<1	14	<1	<1	<1	<1	<1	14	<1	–
Cauliflower (*Brassica oleracea*)	Cooked		Brassicaceae	12	<1	11	<1	<1	<1	<1	–	11	<1	–
Celeriac (*Apium graveolens*)	Peeled		Apiaceae	14	2	13	<1	1	<1	<1	<1	10	<1	–
Celeriac (*Apium graveolens*)	Peeled, cooked		Apiaceae	36	<1	35	<1	<1	<1	<1	<1	33	<1	–
Celery (*Apium graveolens*)			Apiaceae	7	<1	7	<1	<1	<1	–	–	6	<1	<1
Celery (*Apium graveolens*)	Cooked		Apiaceae	8	<1	8	<1	<1	<1	–	–	7	<1	<1
Cherries (*Prunus* sp.)	Stoned		Rosaceae	27	20	6	5	2	13	1	<1	6	–	–
Cherries (*Prunus* sp.)	Glace		Rosaceae	7	4	3	<1	<1	<1	3	<1	1	2	<1
Chestnuts (*Castanea sativa*)			Fagaceae	217	2	214	<1	<1	<1	2	<1	201	13	<1
Chestnuts (*Castanea sativa*)	Cooked		Fagaceae	283	2	280	<1	<1	<1	1	<1	265	15	<1
Chick peas (*Cicer arietinum*)	Dried		Fabaceae	609	607	2	16	79	9	441	62	<1	2	<1
Chick peas (*Cicer arietinum*)	Cooked from dried		Fabaceae	420	416	4	4	35	3	351	22	<1	3	<1
Chick peas, as Houmous				169	135	34	<1	4	<1	127	4	<1	34	<1
Chicory (*Cichorium intybus*)			Asteraceae	19	<1	19	<1	<1	<1	–	–	18	<1	<1
Chinese leaves (*Brassica rapa*)			Brassicaceae	12	1	11	<1	<1	<1	<1	<1	11	<1	–
Clementine (*Citrus reticulata*)	Pith and skin removed		Rutaceae	6	<1	5	<1	<1	–	–	<1	5	<1	<1
Coconut (*Cocos nucifera*)	Fresh		Arecaceae	42	10	32	–	–	4	6	–	30	2	<1
Coconut (*Cocos nucifera*)	Desiccated		Arecaceae	26	3	23	<1	2	<1	–	<1	19	4	–
Courgette (*Cucurbita pepo*)			Cucurbitaceae	35	<1	35	<1	<1	<1	<1	–	35	<1	–
Courgette (*Cucurbita pepo*)	Cooked		Cucurbitaceae	46	3	43	<1	<1	<1	2	<1	43	<1	<1
Cranberries (*Vaccinium* sp.)			Ericaceae	93	3	88	2	<1	1	–	<1	88	<1	<1
Cucumber (*Cucumis sativus*)			Cucurbitaceae	12	<1	12	<1	<1	<1	–	<1	12	<1	–
Cucumber (*Cucumis sativus*)	w/o Skin		Cucurbitaceae	13	<1	13	<1	<1	<1	<1	<1	13	<1	<1
Curly kale (*Brassica oleracea*)	Cooked		Brassicaceae	8	2	6	–	2	<1	<1	<1	5	1	<1
Dates (*Phoenix dactylifera*)	Boxed, stones removed		Arecaceae	168	4	163	<1	<1	1	2	–	161	3	<1
Dates (*Phoenix dactylifera*)	Dried		Arecaceae	599	14	584	–	–	7	7	<1	581	3	<1
Dates, medjool (*Phoenix dactylifera*)	Stones removed		Arecaceae	192	35	157	5	19	6	4	<1	156	2	<1
Fennel (*Foeniculum vulgare*)			Apiaceae	72	<1	72	<1	<1	<1	<1	–	59	12	–

Fennel (*Foeniculum vulgare*)	Cooked	Apiaceae	85	<1	85	<1	<1	<1	<1	<1	72	13	–
Fig. (*Ficus* sp.)		Moraceae	389	12	376	–	10	2	–	<1	372	3	<1
Fig. (*Ficus* sp.)	Dried	Moraceae	129	14	114	–	12	<1	1	<1	113	1	1
Garlic (*Allium sativum*)	Peeled	Alliaceae	99	2	97	–	–	<1	2	<1	93	4	<1
Gooseberries (*Ribes uva-crispa*)		Grossulariaceae	72	<1	71	<1	<1	<1	<1	<1	71	<1	<1
Gooseberries (*Ribes uva-crispa*)	Stewed with sugar	Grossulariaceae	121	<1	121	–	<1	<1	<1	–	119	2	<1
Grapefruit (Citrus x paradisi)	Peel, pith and pips removed	Rutaceae	39	17	21	3	2	–	11	<1	21	<1	<1
Grapefruit (Citrus x paradisi)	Tinned in juice	Rutaceae	21	13	4	5	–	–	8	<1	4	<1	4
Grapes, black (*Vitis* sp.)	Seeds removed	Vitaceae	19	5	14	1	4	<1	–	<1	7	7	–
Grapes, dried as Currants (*Vitis* sp.)	Dried	Vitaceae	87	17	70	–	–	7	10	–	36	34	<1
Grapes, dried as Raisins (*Vitis* sp.)	Dried	Vitaceae	88	7	81	–	<1	<1	5	1	50	31	<1
Grapes, red, seedless (*Vitis* sp.)		Vitaceae	21	6	15	<1	3	<1	2	–	7	8	–
Grapes, white, seedless (*Vitis* sp.)		Vitaceae	18	<1	17	<1	<1	<1	<1	<1	8	9	–
Green beans (*Phaseolus vulgaris*)	Frozen, sliced	Fabaceae	58	19	38	3	16	<1	<1	<1	38	–	<1
Green beans (*Phaseolus vulgaris*)	Frozen, sliced, cooked	Fabaceae	46	16	30	3	13	-	<1	<1	29	<1	<1
Greengage (*Prunus* sp.)	Stoned	Rosaceae	105	2	103	<1	<1	1	<1	<1	102	1	<1
Kiwi (*Actinidia delicosa*)	Skin removed	Actinidiaceae	111	<1	111	<1	<1	<1	–	–	107	4	–
Leek (*Allium ampeloprasum*)		Alliaceae	66	1	65	<1	<1	<1	1	<1	65	<1	–
Leek (*Allium ampeloprasum*)	Cooked	Alliaceae	61	9	52	<1	<1	<1	8	<1	52	<1	–
Lemon (Citrus x limon)	Freshly juiced	Rutaceae	4	1	2	<1	<1	<1	<1	<1	2	<1	1
Lemon (Citrus x limon)	Peeled	Rutaceae	29	4	25	–	3	<1	–	<1	25	–	<1
Lentils, red (Lens culinaris)	Dried	Fabaceae	54	51	3	26	19	6	–	–	2	1	<1
Lentils, red (Lens culinaris)	Cooked	Fabaceae	14	13	<1	6	5	2	–	<1	<1	<1	–
Lettuce, cos (*Lactuca sativa*)		Asteraceae	5	<1	4	<1	<1	<1	<1	<1	4	–	–
Lettuce, iceberg (*Lactuca sativa*)		Asteraceae	7	<1	7	<1	<1	<1	<1	–	7	<1	<1
Lettuce, little gem (*Lactuca sativa*)		Asteraceae	7	1	6	<1	<1	<1	<1	–	5	1	–
Lettuce, round (*Lactuca sativa*)		Asteraceae	8	<1	8	<1	<1	<1	<1	–	7	<1	<1
Lychees (*Litchi chinensis*)	Tinned in syrup	Sapindaceae	4	3	<1	<1	<1	<1	3	<1	<1	<1	<1
Mandarin (*Citrus reticulata*)	Tinned	Rutaceae	5	2	2	1	–	1	<1	<1	2	<1	1
Mangetout (*Pisum sativum*)	Cooked	Fabaceae	47	38	9	–	<1	37	–	<1	9	–	<1
Mango (*Magnifera* sp.)	Skinned & stoned	Anacardiaceae	20	1	19	<1	<1	<1	<1	<1	17	1	–
Mango (*Magnifera* sp.)	Tinned in syrup	Anacardiaceae	4	3	<1	–	<1	–	3	<1	–	<1	–
Marrow (*Cucurbita* sp.)		Cucurbitaceae	9	<1	9	<1	<1	<1	<1	<1	9	<1	–
Marrow (*Cucurbita* sp.)	Cooked	Cucurbitaceae	8	<1	8	–	–	–	–	<1	8	<1	<1
Melon, cantaloupe (*Cucumis melo* ssp.)	Skin & seeds removed	Cucurbitaceae	16	<1	16	<1	<1	<1	<1	<1	16	<1	–

(*continued on next page*)

Table 1 (*continued*)

Food (taxonomic name)	Preparation	Variety	Family	Phytoestrogens	Isoflavones	Lignans	Daidzein	Genistein	Glycitein	Biochanin A	Formononetin	Secoisolariciresinol	Matairesinol	Coumestrol
Melon, galia (*Cucumis melo* ssp.)	Skin & seeds removed		Cucurbitaceae	11	<1	11	<1	<1	<1	<1	<1	11	<1	<1
Melon, honeydew (*Cucumis melo* ssp.)	Skin & seeds removed		Cucurbitaceae	25	3	22	1	1	<1	<1	<1	21	<1	<1
Melon, water (*Citrullus lanatus*)	Skin & seeds removed		Cucurbitaceae	35	<1	34	<1	<1	<1	<1	<1	34	<1	<1
Mung beans (*Vigna radiata*)	Dried		Fabaceae	323	32	289	6	15	2	8	<1	289	–	2
Mung beans (*Vigna radiata*)	Cooked from dried		Fabaceae	50	8	42	<1	4	<1	3	<1	42	<1	<1
Mushrooms (*Agaricus bisporus*)	Wiped & trimmed		Agariaceae	n/a	n/a	<1	–	Not determined	52	<1	<1	<1	<1	–
Mushrooms (*Agaricus bisporus*)	Cooked		Agariaceae	2	2	<1	<1	<1	<1	1	<1	<1	<1	–
Mushrooms (*Agaricus bisporus*)	Microwaved		Agariaceae	<1	<1	<1	–	<1	<1	<1	<1	<1	–	<1
Nectarine (*Prunus persica*)	Stones removed		Rosaceae	25	1	24	<1	<1	<1	<1	<1	24	<1	–
Okra (*Abelmoschus esculentus*)	Topped & tailed		Malvaceae	86	2	84	–	<1	<1	1	<1	84	<1	<1
Olives, black, pitted (*Olea europaea*)	Tinned in brine, drained in jar of brine,		Oleaceae	16	2	14	<1	1	<1	<1	–	5	9	<1
Olives, green (*Olea europaea*)	stoned, drained		Oleaceae	33	1	32	–	–	<1	<1	–	25	6	<1
Onion rings (*Allium cepa*)	Breaded/ battered		Alliaceae	55	44	11	6	28	5	4	<1	8	3	<1
Onions (*Allium cepa*)			Alliaceae	31	<1	31	–	<1	<1	<1	<1	30	<1	–
Onions (*Allium cepa*)	Cooked		Alliaceae	21	<1	20	–	<1	<1	<1	<1	20	<1	<1
Orange (*Citrus sinensis*)	peel & pith removed		Rutaceae	36	12	21	4	3	–	5	<1	21	<1	2
Orange (*Citrus sinensis*)	Longlife juice		Rutaceae	9	<1	4	<1	<1	–	–	<1	4	<1	4
Papaya (*Carica papaya*)	Peel & seeds removed		Caricaceae	4	2	2	–	<1	–	2	–	<1	2	–
Parsley (*Petroselium crispum*)	Leaves		Apiaceae	197	59	137	-	57	<1	<1	<1	137	<1	<1
Parsnip (*Pastinaca sativa*)			Apiaceae	65	5	60	<1	<1	<1	5	–	36	21	–
Parsnip (*Pastinaca sativa*)	Cooked		Apiaceae	66	<1	65	–	<1	<1	<1	<1	49	17	–
Passion Fruit (*Passiflora edulis*)	Juice & seeds		Passifloraceae	71	43	26	42	<1	<1	<1	<1	26	<1	2
Peach (*Prunus perica*)	Stoned		Rosaceae	43	<1	42	<1	<1	<1	<1	<1	42	<1	–
Peach (*Prunus perica*)	Tinned in syrup, drained		Rosaceae	2	2	<1	–	<1	–	2	<1	–	<1	–
Pear (*Pyrus communis*)	w/o Skin	Comice	Rosaceae	19	2	17	–	<1	1	–	<1	17	<1	<1
Pear (*Pyrus communis*)	w/o Skin	Conference	Rosaceae	6	<1	5	–	<1	<1	–	–	5	<1	–
Pear (*Pyrus communis*)	w/o Skin	Williams	Rosaceae	6	6	<1	<1	<1	5	–	<1	<1	–	–
Pear (*Pyrus communis*)	Tinned, drained		Rosaceae	1	<1	<1	<1	<1	<1	–	<1	<1	<1	<1
Pear (*Pyrus communis*)	w/o skin	Comice	Rosaceae	8	<1	7	–	<1	<1	–	–	7	<1	<1
Pear (*Pyrus communis*)	w/o Skin	Conference	Rosaceae	3	<1	3	–	<1	<1	–	–	2	<1	–
Pear (*Pyrus communis*)	w/o Skin	Williams	Rosaceae	3	3	<1	–	<1	2	–	<1	<1	–	–
Peas (*Pisum sativum*)	Tinned, processed, drained		Fabaceae	2	2	<1	<1	<1	<1	–	<1	<1	<1	<1
Peas, fresh (*Pisum sativum*)			Fabaceae	3	2	1	<1	<1	1	–	<1	1	<1	–

Peas, fresh (*Pisum sativum*)	Cooked	Fabaceae	1	1	<1	<1	<1	<1	<1	<1	–	<1	<1
Peas, frozen (*Pisum sativum*)		Fabaceae	3	1	2	<1	<1	<1	<1	<1	1	<1	<1
Peas, frozen (*Pisum sativum*)	Cooked	Fabaceae	2	1	1	<1	<1	<1	<1	<1	<1	<1	–
Peas, garden (*Pisum sativum*)	Tinned, drained	Fabaceae	2	1	1	<1	<1	<1	<1	–	<1	<1	<1
Peas, marrowfat (*Pisum sativum*)	Tinned, drained	Fabaceae	51	50	<1	<1	3	46	<1	<1	<1	<1	–
Peas, mushy (*Pisum sativum*)	Tinned/frozen	Fabaceae	15	14	1	<1	3	10	<1	<1	1	–	–
Peas, petit pois (*Pisum sativum*)	Frozen	Fabaceae	8	6	2	–	2	1	2	<1	<1	2	–
Peas, split, dried (*Pisum sativum*)		Fabaceae	15	11	4	2	2	7	–	<1	<1	4	<1
Peas, split, dried (*Pisum sativum*)	Cooked	Fabaceae	13	12	<1	4	5	3	–	<1	<1	<1	–
Peas, sugar snap (Pisum sativum)	Cooked	Fabaceae	44	31	13	–	<1	29	<1	<1	13	–	<1
Peas, whole, dried (*Pisum sativum*)		Fabaceae	29	28	<1	7	9	9	–	3	–	<1	–
Peas, whole, dried (*Pisum sativum*)	Cooked	Fabaceae	10	9	<1	2	3	4	<1	<1	–	<1	–
Pepper, green (*Capsicum annuum*)		Solanaceae	11	<1	11	<1	<1	–	–	–	11	<1	–
Pepper, red (*Capsicum annuum*)		Solanaceae	16	5	11	–	<1	<1	4	<1	11	–	<1
Pepper, yellow (*Capsicum annuum*)		Solanaceae	11	6	5	–	–	–	6	<1	5	<1	<1
Pineapple (*Ananas comosus*)		Bromelidaceae	38	21	17	–	–	–	21	–	2	15	–
Pineapple (*Ananas comosus*)	Tinned in juice, drained	Bromelidaceae	14	2	12	<1	<1	<1	1	<1	<1	11	<1
Plum, red (*Prunus domestica*)		Rosaceae	8	2	6	<1	<1	1	<1	<1	6	<1	–
Plum, Victoria (*Prunus domestica*)		Rosaceae	26	2	24	<1	<1	<1	<1	<1	24	<1	<1
Plum, yellow (*Prunus domestica*)		Rosaceae	72	2	69	–	<1	1	–	<1	69	–	<1
Plum, yellow (*Prunus domestica*)	Cooked	Rosaceae	152	2	150	–	<1	<1	<1	<1	150	<1	<1
Pomegranate (*Punica granatum*)	Flesh & seeds	Lythraceae	304	<1	304	<1	<1	<1	<1	<1	294	9	<1
Potato, chips (*Solanum tuberosum*)	From chip–shop	Solanaceae	11	7	3	<1	2	<1	5	<1	<1	2	<1
Potato, chips (*Solanum tuberosum*)	Oven chips	Solanaceae	15	11	4	–	3	2	5	<1	3	1	–
Potato, crisps (*Solanum tuberosum*)		Solanaceae	22	5	17	–	3	<1	2	–	14	2	<1
Potato, for baking (*Solanum tuberosum*)	Boiled	Solanaceae	3	2	1	<1	<1	<1	<1	<1	<1	<1	<1
Potato, for baking (*Solanum tuberosum*)	w/o Skin, boiled	Solanaceae	2	1	<1	<1	<1	<1	<1	–	<1	<1	<1
Potato, mashed, instant (*Solanum tuberosum*)		Solanaceae	4	2	1	<1	2	<1	–	<1	1	<1	–

(*continued on next page*)

Table 1 (*continued*)

Food (taxonomic name)	Preparation	Variety	Family	Phytoestrogens	Isoflavones	Lignans	Daidzein	Genistein	Glycitein	Biochanin A	Formononetin	Secoisolariciresinol	Matairesinol	Coumestrol
Potato, new (*Solanum tuberosum*)			Solanaceae	18	16	2	<1	4	11	<1	–	1	<1	<1
Potato, new (*Solanum tuberosum*)	Boiled		Solanaceae	5	2	3	<1	<1	<1	<1	<1	2	<1	<1
Potato, new (*Solanum tuberosum*)	w/o Skin		Solanaceae	4	2	2	<1	–	<1	2	<1	1	<1	–
Potato, new (*Solanum tuberosum*)	w/o Skin, boiled		Solanaceae	3	3	<1	–	–	<1	3	<1	–	<1	–
Potato, old (*Solanum tuberosum*)			Solanaceae	10	8	2	<1	1	5	<1	<1	<1	2	–
Potato, old (Solanum tuberosum)	Baked		Solanaceae	3	2	1	–	<1	–	1	<1	–	1	<1
Potato, old (*Solanum tuberosum*)	Boiled		Solanaceae	1	<1	<1	–	<1	–	<1	<1	<1	<1	–
Potato, old (*Solanum tuberosum*)	w/o Skin, baked		Solanaceae	3	1	2	–	<1	<1	<1	<1	<1	1	<1
Potato, red (*Solanum tuberosum*)	w/o Skin		Solanaceae	10	1	8	<1	<1	<1	<1	<1	8	<1	–
Potato, red (*Solanum tuberosum*)	w/o Skin, boiled		Solanaceae	20	5	15	2	3	<1	<1	<1	14	<1	<1
Potato, waffle (*Solanum tuberosum*)	Cooked		Solanaceae	9	5	4	<1	4	<1	–	<1	2	1	<1
Prune (*Prunus domestica*)	Dried		Rosaceae	363	6	357	1	2	2	<1	<1	352	4	<1
Prune (*Prunus domestica*)	Cooked from dried		Rosaceae	108	2	106	<1	<1	<1	–	<1	105	<1	<1
Prune (*Prunus domestica*)	Semi–dried, ready to eat		Rosaceae	284	3	281	<1	1	<1	–	<1	278	3	<1
Prune (*Prunus domestica*)	Tinned in syrup & juice, not drained		Rosaceae	75	3	72	–	1	<1	<1	<1	68	4	<1
Pumpkin (*Cucurbita* sp.)			Cucurbitaceae	154	<1	154	<1	<1	<1	–	<1	153	<1	<1
Quince (*Cydonia oblonga*)	Stewed		Rosaceae	9	2	7	<1	<1	1	–	<1	6	<1	<1
Radish (*Raphanus sativus*)			Brassicaceae	3	<1	3	<1	<1	<1	<1	<1	3	<1	–
Raspberries (*Rubus idaeus*)			Rosaceae	26	2	24	<1	<1	<1	1	<1	24	<1	<1
Raspberries (*Rubus idaeus*)	Tinned in syrup, drained		Rosaceae	21	5	15	<1	<1	<1	5	<1	15	<1	<1
Redcurrants (*Ribes rubrum*)			Grossulariaceae	47	<1	46	<1	<1	<1	–	–	45	<1	<1
Rhubarb (*Rheum* sp.)			Polygonaceae	1	1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Rhubarb (*Rheum* sp.)	Cooked		Polygonaceae	3	2	<1	<1	<1	<1	<1	<1	<1	<1	<1
Salad cress (*Lepidium sativum*)			Brassicaceae	18	3	15	1	1	<1	–	<1	15	<1	–
Satsuma (*Citrus unshiu*)			Rutaceae	24	2	12	2	–	–	–	<1	12	<1	10
Sharon fruit (*Diospyros* sp.)			Ebenaceae	11	6	5	<1	1	<1	4	<1	4	<1	<1
Soya bean (Glycine max)	Cooked		Fabaceae	17556	17544	11	5730	10664	1144	1	4	11	<1	<1
Soya bean (*Glycine max*)	Frozen, cooked		Fabaceae	10687	10621	64	3864	5540	1210	<1	6	64	<1	2
Soya bean, Tofu (*Glycine max*)	Microwaved		Fabaceae	10619	10609	10	2528	7292	781	3	6	10	–	<1
Soya flour (*Glycine max*)			Fabaceae	124727	124381	345	54128	62125	8114	<1	14	337	9	<1
Soya mince granules (*Glycine max*)	Cooked		Fabaceae	20850	20745	101	5747	13770	1225	<1	3	99	2	4
Spinach (*Spinacia olearcea*)			Amaranthaceae	7	2	5	<1	–	<1	2	<1	5	<1	<1
Spinach (*Spinacia olearcea*)	Cooked		Amaranthaceae	4	<1	3	<1	<1	<1	<1	<1	3	<1	<1
Spring green (*Brassica oleracea*)			Brassicaceae	56	11	45	<1	<1	<1	10	<1	44	<1	–
Spring green (*Brassica oleracea*)	Cooked		Brassicaceae	43	13	30	<1	<1	<1	12	<1	30	<1	–
Spring onion (*Allium* sp.)			Alliaceae	62	9	53	–	<1	<1	8	<1	52	<1	–

Strawberries (Fragaria × ananassa)		Rosaceae	8	<1	7	<1	–	<1	–	<1	7	<1	<1
Strawberries (Fragaria ananassa)	Tinned in syrup, drained	Rosaceae	40	2	38	–	<1	–	2	<1	38	<1	–
Sultanas (*Vitis* sp.)		Vitaceae	54	11	44	–	<1	<1	8	1	13	31	<1
Swede (*Brassica napobrassica*)		Brassicaceae	6	1	5	<1	<1	<1	<1	<1	5	<1	–
Swede (*Brassica napobrassica*)	Cooked	Brassicaceae	2	<1	2	<1	<1	<1	<1	<1	2	<1	–
Sweet potato (*Ipomoea batatas*)		Convolvulaceae	259	1	258	<1	<1	<1	<1	–	136	122	<1
Sweet potato (*Ipomoea batatas*)	Cooked	Convolvulaceae	251	1	249	<1	<1	<1	<1	–	118	132	<1
Sweetcorn (Zea mays)	Boiled on the cob	Poaceae	9	2	7	<1	<1	<1	2	<1	5	2	<1
Sweetcorn (Zea mays)	Frozen, tinned, drained	Poaceae	<1	<1	<1	–	<1	<1	<1	–	<1	<1	<1
Sweetcorn (Zea mays)	Frozen, tinned, drained, heated	Poaceae	3	<1	2	–	<1	<1	<1	<1	1	<1	–
Sweetcorn (Zea mays)	Kernels from the cob	Poaceae	2	<1	1	<1	<1	<1	<1	<1	1	<1	<1
Sweetcorn, baby (Zea mays)		Poaceae	6	<1	5	<1	<1	<1	<1	<1	5	<1	<1
Tomato (*Solanum lycopersicum*)		Solanaceae	6	1	4	<1	<1	<1	<1	–	4	<1	–
Tomato (*Solanum lycopersicum*)	Grilled	Solanaceae	7	<1	6	–	<1	<1	–	<1	6	–	<1
Tomato (*Solanum lycopersicum*)	Pureed	Solanaceae	9	5	5	<1	4	<1	–	<1	4	<1	<1
Tomato (*Solanum lycopersicum*)	Tinned	Solanaceae	3	1	2	<1	<1	<1	–	–	2	<1	<1
Tomato ketchup			14	7	8	<1	2	<1	4	–	2	6	<1
Turnip (*Brassica rapa*)		Brassicaceae	12	<1	12	–	<1	<1	–	–	12	<1	–
Turnip (*Brassica rapa*)	Cooked	Brassicaceae	8	<1	8	<1	<1	<1	–	<1	8	<1	–
Watercress (*Nasturtium* sp.)		Brassicaceae	45	<1	45	<1	<1	<1	<1	<1	45	<1	–
Vegetable grills	Cooked		43	16	27	<1	15	1	–	–	23	3	<1
Brown sauce			214	46	168	14	25	4	2	–	162	7	–
Fruit cocktail	Tinned in syrup, drained		3	<1	3	<1	<1	<1	–	<1	2	<1	–
Mixed peel			38	32	6	5	19	3	5	<1	<1	5	<1
Pate vegetarian assorted			581	488	92	110	317	42	19	1	87	4	<1
Pate vegetarian chick pea based (*Cicer arietinum*)		Fabaceae	1494	1444	49	351	806	111	169	7	36	13	<1
Pate, mushroom (*Agaricus bisporus*)		Agariaceae	17	8	8	<1	5	<1	2	<1	4	3	<1

Wong, Welch, & Bingham, 2001; Kipnis et al., 2003), their use is often not feasible, particularly in larger studies, and intake has to be either calculated from dietary information provided by participants or determined by a combination of biomarkers and dietary information. Accurate information on the phytoestrogen content in foods is therefore crucial for the investigation of effects on health; and to determine population levels for surveillance purposes.

The main dietary sources of phytoestrogens are plant-based foods such as fruits and vegetables. In plants, where these compounds occur predominantly as glycosides, they act as antioxidants, screen against light and most importantly act as defensive agents against predators (Mazur & Adlercreutz, 1998a). The principal phytoestrogen-classes are isoflavones (found mainly in legumes, e.g. chickpeas and soybean), lignans (e.g. in cereals, linseed and other fruits and vegetables) and coumestans (e.g. in young sprouting legumes like clover or alfalfa sprouts) (Committee on Toxicity of Chemicals in Food, 2003).

Several detailed studies have been conducted to determine the phytoestrogen content of food previously, amongst others in the UK (Liggins, Bluck, Coward, & Bingham, 1998a, 1998b; Liggins et al., 2000; Liggins, Grimwood, & Bingham, 2000; Liggins, Mulligan, Runswick, & Bingham, 2002), Finland (Dwyer et al., 1994; Mazur, 1998; Mazur et al., 1996, 1998b; Valsta et al., 2003), and the US (US Department of Agriculture, 2002); however, these studies provide only data for approximately 12% of the UK diet (Mulligan, Welch, McTaggart, Bhaniani, & Bingham, 2007) and had methodological limitations (Adlercreutz et al., 1993; Wähälä, Hase, & Adlercreutz, 1995; Wähälä & Rasku, 1997). Previously, we have developed a sensitive LC/MS/MS method using $^{13}C_3$-labelled standards to analyse phytoestrogens in plasma and urine (Grace et al., 2003). We have adapted this method to be used for food samples and have measured the phytoestrogen content (isoflavones: biochanin A, daidzein, formononetin, genistein, glycitein; lignans: matairesinol, secoisolariciresinol; coumestrol) in more than 240 foods based on fruits and vegetables commonly consumed in the UK. This is one of the most comprehensive analysis of plant-based phytoestrogens in the UK and elsewhere.

2. Experimental

2.1. Chemicals

Biochanin A, daidzein, genistein, glycitein, formononetin, secoisolariciresinol, matairesinol and coumestrol were purchased from Plantech (Reading, Berkshire, UK). $^{13}C_3$-biochanin A $^{13}C_3$-daidzein, $^{13}C_3$-genistein, $^{13}C_3$-glycitein, $^{13}C_3$-formononetin, $^{13}C_3$-matairesinol, $^{13}C_3$-secosiolariciresinol and $^{13}C_3$-enterolactone were obtained from Dr. Nigel botting (University of St. Andrews, Fife, UK) (Fryatt & Botting, 2005; Haajanen & Botting, 2006; Whalley, Bond, & Botting, 1998; Whalley, Oldfield, & Botting, 2000). β-Glucuronidase (from *Helix pomatia*), β-glucosidase (from almonds) and cellulase (from *Trichoderma reesi*) were purchased from Sigma (Poole, Dorset, UK). Water, methanol, acetic acid and ammonia were purchased from Sigma (Poole, Dorset, UK) and Fisher Scientific (Loughborough, Leicestershire, UK). To inhibit losses of target compounds by adsorption to glassware, only silanised glassware was used.

2.2. Sampling

Samples of each food were purchased from at least five different food outlets (where possible) in Cambridgeshire, UK. If possible, the foods bought at each outlet were from different manufacturers, varieties, country of origin and/or batch numbers. Each sample was weighed, prepared and a representative portion (approximately 35 g dry weight) was taken from each of the five samples. Cooked food was boiled in water until tender and the water discarded; more details on preparation are given in Table 1. Tinned foods were drained unless indicated otherwise; outer leaves were removed from cabbages; lettuce was analysed as purchased. The samples were frozen (−20 °C), freeze-dried if necessary (BOC Edwards, Crawley, Sussex, UK) and stored at −20 °C until analysis. For analysis, samples of each food were pooled (equal amounts), weighed and processed as described below.

2.3. Analysis

Samples were analysed as described previously (Kuhnle, Dell'Aquila, Low, Kussmaul & Bingham, 2007). Briefly, approximately 100 mg freeze-dried food was extracted three times with 2.0 ml 10% methanol in sodium acetate (0.1%, pH 5) and deconjugated with a hydrolysis reagent consisting of purified *Helix pomatia* juice (β-glucuronidase), cellulase and β-glucosidase. Deconjugated samples were then extracted using Strata C-18E SPE cartridges (50 mg/ml; Phenomenex, Macclesfield, Cheshire, UK), dried, reconstituted in 40% aqueous methanol and analysed using LC/MS/MS. Analysis was performed on an LC/MS/MS system consisting of a Jasco HPLC system (Jasco, Great Dunmow, UK) using a diphenyl column (Varian Pursuit, 3 μm, 150 × 2 mm, Varian, Oxford, Oxfordshire, UK) and a Waters Quattro Ultima triple quadrupole MS instrument (Waters, Manchester, UK) fitted with an electrospray ion source in negative ion mode and a LC/MS/MS system consisting of an Agilent 1100 CapHPLC System (Agilent, Wokingham, Berkshire, UK) and an ABI 4000 QTRAP mass spectrometer (Applied Biosystems, Warrington, Cheshire, UK) fitted with an electrospray ion source in negative ion mode. Compounds were quantified using $^{13}C_3$-labelled internal standards; Compounds were quantified using $^{13}C_3$-labelled internal standards; coumestrol was quantified using $^{13}C_3$-enterolactone.

The method was validated on both LC/MS/MS systems. The intra-batch CV of this method is between 3% and 14% and the inter-batch between 1% and 6%. As quality control, a sample consisting of equal amounts of red cabbage, orange and celery was analysed with each batch. The limit of detection of this method is 1.5 μg/100 g dry weight.

2.4. Data analysis

Each sample was prepared in triplicate and analysed twice. Data are presented as the average of two analyses and is in μg/100 g wet weight. Data was analysed using SPSS 16 (SPSS Inc., Chicago, IL) for Mac OS X. The data was not normally distributed and therefore non-parametric tests were used. Differences between plant-families were analysed using the Kruskal–Wallis test, the effect of preparation was investigated using Wilcoxon signed rank test. $p < 0.05$ was considered to be statistically significant.

3. Results

In all foods analysed, with the exception of microwaved mushrooms and unheated tinned sweet-corn, phytoestrogens were detected (Table 1). In most foods, the phytoestrogen content was below 100 μg/100 g wet weight (median: 20 g/100 g; IQR (interquartile range): 7–66 μg/100 g) with less isoflavones (median: 2 μg/100 g; IQR: 1–8 μg/100 g) than lignans (median: 12 μg/100 g; IQR 3–47 μg/100 g) and a low amount of coumestrol (median: <1 μg/100 g). However, 5% of foods analysed contained more than 400 μg/100 g phytoestrogens (>134 μg/100 g isoflavones in top 5% of foods; >218 μg/100 g lignans in top 5% of foods), with the highest content in soya flour (125,000 μg/100 g) and cooked soya beans (18,000 μg/100 g).

Daidzein, genistein and glycitein were the main isoflavones in legumes, in particular in soya-based foods such as soya flour, soya mince granules or tofu. A notable exception was passion fruit which was the only non-legume with a daidzein content of more than 40 μg/100 g. Biochanin A was only found in some foods and in most foods the content was below 1 μg/100 g. High contents of biochanin A were found mainly in chick peas and chick-pea based foods such as vegetarian pâte and houmous. Similarly, formononetin was found only in a few foods with the highest content in chick peas and – to a lesser extent – soya. Most types of food analysed contained lignans, in particular secoisolariciresinol, which was only absent or in very low concentration in just a few foods, notably peas and potatoes. The highest amount of secoisolariciresinol was found in dried dates and apricots, but high amounts were also found in figs, prunes, soya flour and pomegranate. In contrast to secoisolariciresinol, matairesinol was either absent or present in very low concentrations in most foods. Cooked sweet potatoes contained the highest amount of matairesinol (132 μg/100 g in cooked sweet potatoes). High amounts were also found in dried grapes (currants, raisins and sultanas), parsnips and chestnuts. Most foods contained only small amounts of coumestrol with only 5% containing more than 2 μg/100 g. Coumestrol was mainly found in legumes and citrus fruits (Rutaceae) with the highest content in beansprouts (361 μg/100 g), raw runner (11 μg/100 g) and kidney (10 μg/100 g) beans.

The phytoestrogen content and composition (proportion of isoflavones on total phytoestrogens) varied significantly ($p < 0.05$, Kruskal–Wallis test) between foods from different plant families (Table 2). Legumes (Fabaceae) contained the highest amount of isoflavones whereas Alliaceae, such as garlic, leek and onion, and Apiaceae, such as carrots, fennel and parsnips, had the highest content of lignans. In most foods, lignans were the main class of phytoestrogens found, with the exception of legumes which contained mainly isoflavones.

Sixty-one types of food were analysed cooked and raw (Table 3). There was a significant difference in phytoestrogen, isoflavone and lignan content between raw and cooked foods (Wilcoxon signed rank test, $p < 0.05$) but no difference in phytoestrogen composition, suggesting that cooking affects isoflavones and lignans in a similar way. For most foods, the phytoestrogen content was higher in raw samples when compared with cooked samples, however, the phytoestrogen content increased in some foods, notably in red potatoes and celeriac. Peeling decreased the phytoestrogen content in most foods analysed (Wilcoxon signed rank test, $p < 0.05$); it also affected the phytoestrogen composition although not in a uniform manner. The effect of stewing and drying could only be investigated in a small number of foods but generally resulted in an increase in phytoestrogen content. Tinned food had a lower overall phytoestrogen content, but the difference was not statistically significant (Wilcoxon signed rank test, $p < 0.06$). Analysis of variance was conducted, but due to the small numbers of samples analysed, it was not to investigate the effect of plant family on phytoestrogen content and composition in more detail.

Table 3
Comparison of 61 raw and cooked foods. Phytoestrogen content is given in μg/100 g wet weight (median and inter-quartile range).

	Raw	Cooked
Total phytoestrogens	29 (9–72)*	18 (7–52)
Isoflavones	2 (1–11)*	2 (1–6)
Lignans	14 (5–66)*	12 (3–39)
%Isoflavones	11% (2–44%)	17% (3–37%)

* Indicates a significant difference between raw and cooked food (Wilcoxon signed rank test, $p < 0.05$).

4. Discussion

Phytoestrogens are formed as secondary metabolites by most plants and are therefore ubiquitous in plant products (Mazur & Adlercreutz, 1998a). In this study, we have analysed more than 240 foods based on fruit and vegetables for their phytoestrogen content to provide a comprehensive database for the assessment of dietary intake and exposure. The results are expressed per 100 g wet weight to facilitate the use in epidemiological studies and diet composition databases. Phytoestrogens were found in virtually all foods analysed although the content in most foods was well below 100 μg/100 g wet weight with the exception of legumes like soya and some other foods such as dried fruits, figs, pomegranate, chestnuts and sweet potatoes. Staple foods such as potatoes contained on average less than 10 μg/100 g phytoestrogens. Other fruits and vegetables commonly consumed in a large study of free-living adults in the UK (Day et al., 1999), such as bananas (3 μg/100 g), raw tomatoes (6 μg/100 g), apples (12 μg/100 g) and cucumbers (12 μg/100 g), also contained only small amounts of phytoestrogens.

In contrast to bioanalytical methods for the determination of compounds in a single matrix such as plasma or urine, the analysis of food stuff is made difficult by the large variety of different matrices and moreover the lack of true quality controls (Kuhnle, Dell'Aquila, Low, Kussmaul, & Bingham, 2007). Although most methods use "standard foods" as quality control to monitor method performance and precision, it is very difficult to monitor accuracy, in particular since the true content of each compound is not known. Fortifying samples with neat standards does not provide sufficient information on recovery and accuracy because most compounds are present as glycosides and are embedded in the cellular matrix. To assess relative quality and accuracy of data, they can be compared with data published elsewhere. However, data for comparison is only available for a limited number of foods and most studies focus on different types of phytoestrogens; for example Horn-Ross et al. (2001) do not include formononetin whereas

Table 2
Phytoestrogen content (in μg/100 g wet weight; median and inter-quartile range) and composition (% of total phytoestrogen content) in foods from different plant families. For this table, only unprocessed foods from families with at least five samples were compared. Percentage of the phytoestrogen content in Solanaceae is too small to provide reliable information of phytoestrogen composition.

Family	*n*	Phytoestrogens	Isoflavones	Lignans	Percentage of isoflavones (%)
Alliaceae	5	62 (31–83)	2 (0–26)	53 (11–81)	2 (2–47%)
Apiaceae	6	69 (7–143)	3 (0–18)	66 (7–125)	6 (2–16%)
Asteraceae	5	7 (5–13)	<1 (0–1)	7 (4–13)	2 (1–8%)
Brassicaceae	17	14 (5–51)	<1 (0–2)	12 (4–45)	7 (2–7%)
Cucurbitaceae	9	16 (9–35)	<1	16 (8–34)	2 (0–4%)
Fabaceae	23	51 (3–201)	28 (2–51)	13 (0–89)	70 (33–92%)
Rosaceae	28	8 (3–37)	2 (0–2)	6 (0–35)	14 (5–46%)
Rutaceae	7	24 (4–36)	2 (0–12)	12 (2–21)	14 (8–34%)
Solanaceae	13	9 (3–11)	2 (0–5)	4 (1–8)	–

Thompson, Boucher, Liu, Cotterchio, and Kreiger (2006) do not include biochanin A. Furthermore, the phytoestrogen content in foods depends on a large number of genetic and environmental factors such as variety, harvest and processing (Eldridge & Kwolek, 1983; Wang & Murphy, 1994), making a comparison difficult. For soya-based food, fourfold differences between growth location and varieties have been observed. Previously, we compared the effect of different sources or countries of origin in nine different foods and found an average variability of threefold with a coefficient of variation of more than 30%; however, for some foods the observed variability was much higher (Kuhnle, Dell'Aquila, Runswick & Bingham, 2009). A comparison of our data with Horn-Ross et al. (2000), Milder, Arts, Van De Putte, Venema, and Hollman (2005) and Thompson et al. (2006) using Wilcoxon's signed rank test showed no overall significant difference between the phytoestrogen and isoflavone content and phytoestrogen composition (as proportion of isoflavones on total phytoestrogens) found in this study and the average content found elsewhere. However, lignan contents were significantly different ($p < 0.01$) with most values found in this study being higher, suggesting a better extraction of these compounds from the sample matrix.

Only limited information is available about the effect of cooking on the phytoestrogen content of foods. Milder et al. (2005) and Thompson et al. (2006) investigated the effect for some types of food and found a decrease in phytoestrogen content. The protocol for this study was not designed to assess the effect of cooking on phytoestrogen levels and, although compared with previous studies, this study includes a larger variety of foods, it was not possible to control for the effect of family on the probability shown in Table 3 that there are losses during cooking. An explanation for this loss during cooking is the leaching of phytoestrogens into the water which is later discarded. Although prolonged heating could also result in the decomposition of phytoestrogen, this effect was not seen in stewed fruits and it is therefore likely that these compounds are stable during preparation, at least under acidic conditions.

In summary, this study provides so far the most comprehensive database of isoflavones, lignans and coumestrol in more than 240 foods based on fruits and vegetables commonly consumed in the UK. The selection of food was based on consumption data of the EPIC-Norfolk cohort (Day et al., 1999) and will allow the more accurate determination of phytoestrogen intake and exposure in this and other studies and free-living individuals.

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