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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