

Phytoestrogen Content of Beverages, Nuts, Seeds, and Oils

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Phytoestrogens are secondary plant metabolites that have received increasing attention for their bioactivity, in particular due to their structural and functional similarity to 17β -estradiol. Although urinary and plasma phytoestrogens can be used as biomarkers for dietary intake, this is often not possible in large epidemiological studies or in the assessment of general exposure in free-living individuals. Accurate information about dietary phytoestrogens is therefore important, but there are very limited data concerning food contents. In this study was analyzed a comprehensive selection of tea, coffee, alcoholic beverages, nuts, seeds, and oils for their phytoestrogen content using a newly developed sensitive method based on LC-MS incorporating $^{13}\text{C}_3$ -labeled standards. Phytoestrogens were detected in all foods analyzed, although the contents in gin and bitter (beer) were below the limit of quantification ($1.5 \mu\text{g}/100 \text{g}$). Lignans were the main type of phytoestrogens detected. Tea and coffee contained up to $20 \mu\text{g}/100 \text{g}$ phytoestrogens and beer (except bitter) contained up to $71 \mu\text{g}/100 \text{g}$, mainly lignans. As these beverages are commonly consumed, they are a main source of dietary lignans. The results published here will contribute to databases of dietary phytoestrogen content and allow a more accurate determination of phytoestrogen exposure in free-living individuals.

KEYWORDS: Phytoestrogens; tea; coffee; beer; nuts; seeds; oils; lignans; isoflavones; LC/MS

INTRODUCTION

Dietary phytoestrogens have received increasing attention for their effect on human health due to their structural and functional similarity to 17β -estradiol (1) and, therefore, their ability to affect endocrine pathways. Several studies reported a beneficial effect on human health, for example, for cancer (2–5), cardiovascular disease (4, 6), osteoporosis (4, 7), menopausal symptoms (4, 8), male infertility (9), and obesity and type 2 diabetes (10). However, in livestock, diets rich in phytoestrogen—such as clover—can cause infertility and other symptoms of hyperestrogenization (11). Also, in humans, increased levels of endogenous sex hormones are generally associated with an increased risk of breast cancer in women (12), and recent studies have shown an increased risk for breast cancer associated with a high exposure to phytoestrogens (13, 14). It has also been shown that there are strong gene–nutrient interactions between phytoestrogens and polymorphisms of the estrogen receptor (ESR1 and NR1I2) (15, 16), the sex-hormone binding globulin (SHBG) (17), and probably aromatase (CYP19) (18). There are also concerns that phytoestrogens increase the risk of recurrence and stimulate tumor growth—and as these compounds can act

as either estrogens or antiestrogens, there are concerns surrounding the use of phytoestrogen supplements in breast cancer patients (19, 20). Accurate information on dietary phytoestrogens is crucial to determine exposure and investigate health effects further. However, despite several studies analyzing phytoestrogens in food, there is still insufficient information, and exposure is likely to be underestimated (21), in particular, because many studies focused only on a few compounds.

Previously, we have developed sensitive LC-MS/MS methods to determine urinary and plasma phytoestrogen concentrations as biomarkers of intake to assess cancer risks and interaction with gene variants (22). Adapting this method for the analysis of food samples, we have investigated the isoflavone (biochanin A, daidzein, formononetin, genistein, and glycitein), lignan (matairesinol and secoisolariciresinol), and coumestrol content in 38 beverages, nuts, seeds, and oils. These data will allow a more accurate determination of dietary phytoestrogen exposure and investigation of their effects in vivo.

EXPERIMENTAL PROCEDURES

Chemicals. Biochanin A, daidzein, genistein, glycitein, formononetin, secoisolariciresinol, matairesinol, and coumestrol were purchased from Plantech (Reading, Berkshire, U.K.). $^{13}\text{C}_3$ -Biochanin A $^{13}\text{C}_3$ -daidzein, $^{13}\text{C}_3$ -genistein, $^{13}\text{C}_3$ -glycitein, $^{13}\text{C}_3$ -formononetin, $^{13}\text{C}_3$ -matairesinol, $^{13}\text{C}_3$ -secoisolariciresinol, and $^{13}\text{C}_3$ -enterolactone were

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Table 1. Phytoestrogen Content in Beverages, Nuts, Seeds, and Oils^a

food	phytoestrogens	isoflavones							lignans		
		isoflavones	lignans	daidzein	genistein	glycitein	biochanin A	formononetin	secoisolariciresinol	metairesinol	coumestrol
Coffee											
coffee, instant powder	1833	913	920	153	594	162	—	4	862	58	—
coffee, instant, decaffeinated powder	647	5	641	—	4	—	—	1	610	32	—
coffee, infusion, average	17	<1	16	—	<1	—	<1	<1	16	<1	<1
coffee, infusion, decaffeinated	11	<1	10	—	<1	—	<1	<1	10	<1	<1
Tea											
tea, strong (15 g/L), from tea leaves	12	<1	11	<1	<1	—	<1	<1	9	2	<1
tea, weak (10 g/L), from tea leaves	8	<1	8	<1	<1	—	<1	<1	6	2	<1
tea, standard, from tea bag (4 bags/L)	7	<1	7	<1	<1	<1	<1	<1	4	2	<1
tea, decaffeinated, from tea bag	10	<1	9	<1	<1	<1	<1	<1	8	2	<1
tea, green	20	<1	20	<1	<1	<1	<1	<1	14	5	<1
tea, chamomile	8	6	2	<1	3	<1	3	<1	1	<1	—
Alcoholic Beverages											
lager, canned	68	13	54	<1	2	4	6	2	54	—	<1
beer, brown ale	71	8	63	<1	1	<1	4	2	63	—	<1
stout, 4–5%	45	9	35	<1	2	3	2	1	35	—	1
beer, bitter, best/premium	1	<1	1	<1	<1	<1	<1	<1	<1	<1	—
wine, red	76	<1	75	<1	<1	<1	—	<1	55	20	—
wine, white, dry	14	<1	14	<1	<1	<1	<1	<1	9	5	<1
cider, dry	55	6	48	<1	—	4	1	<1	48	—	<1
sherry, dry	41	3	38	—	—	<1	1	2	38	—	<1
sherry, cream	31	<1	31	<1	<1	<1	<1	<1	16	15	<1
gin	<1	<1	<1	<1	<1	<1	—	<1	<1	<1	—
whiskey	5	<1	4	<1	<1	<1	—	<1	4	<1	<1
Nuts and Seeds											
almonds, kernel only	112	27	84	<1	1	<1	25	<1	84	—	<1
Brazil nuts	887	105	781	6	85	<1	13	<1	770	12	<1
cashews, plain	182	12	170	—	2	1	7	2	165	5	—
coconut, desiccated	26	3	23	<1	2	<1	—	<1	19	4	—
coconut, fresh	42	10	32	—	—	4	6	—	30	2	<1
hazelnuts	80	22	57	<1	9	<1	12	<1	53	4	<1
peanuts, fresh	173	70	101	<1	48	10	8	4	97	5	2
peanuts, dry roasted	173	94	78	<1	58	12	22	1	71	7	1
peanuts, roasted, salted	427	154	273	4	70	56	21	3	256	17	—
peanut butter, smooth	140	61	79	<1	36	21	1	3	69	10	—
pecans	51	34	17	—	2	—	30	2	13	4	—
pine nuts	103	32	70	<1	4	<1	27	<1	46	25	<1
pistachios, roasted and salted	62	33	29	—	2	2	27	2	22	7	<1
pumpkin seeds	539	18	520	<1	5	2	7	3	510	11	<1
sunflower seeds	111	2	109	<1	1	<1	—	—	106	3	<1
walnuts	175	31	144	1	11	<1	17	<1	140	5	<1
Oils											
flaxseed	23	10	13	—	—	2	6	1	13	—	<1
roasted pumpkinseed	56	6	50	<1	<1	3	<1	1	50	—	<1
rapeseed	61	11	49	<1	2	3	4	3	49	—	—

^a Data are given as micrograms per 100 g of wet weight. Isoflavones are the sum of biochanin A, daidzein, formononetin, genistein, and glycitein; lignans are the sum of secoisolariciresinol and metairesinol. The data are the average of three different samples analyzed in duplicate. (A dash indicates the compound was not detected.)

obtained from Dr. Nigel Botting (University of St. Andrews, Fife, U.K.) (23–26). β -Glucuronidase (from *Helix pomatia*), β -glucosidase (from almonds), and cellulase (from *Trichoderma reesei*) were purchased from Sigma (Poole, Dorset, U.K.). Water, methanol, acetic acid, and ammonia were purchased from Sigma and Fisher Scientific (Loughborough, Leicestershire, U.K.). To inhibit losses of target compounds by adsorption to glassware, only silanized glassware was used.

Sampling. Samples of each food were purchased from at least five different food outlets (when possible) in Cambridgeshire, U.K. If possible, the foods bought at each outlet were from different manufacturers, varieties, countries of origin, and/or batch numbers. Each sample was weighed, and a representative portion (approximately 35 g of dry weight except for tea and coffee, for which approximately 700 g of prepared beverage, resulting in approximately 4–5 g of freeze-dried residue, was used) was taken from each of the five samples (except for alcoholic beverages and oils, which were analyzed directly by taking a 100 mg sample). The samples were frozen (–20 °C), freeze-dried (except nuts, seeds, oils, and alcoholic beverages) (BOC Edwards,

Crawley, Sussex, U.K.) and stored at –20 °C until analysis. For analysis, samples of each food were pooled (equal amounts), weighed, and processed as described above.

Analysis. Samples were analyzed as described previously (27). Briefly, approximately 100 mg of freeze-dried food was extracted three times with 2.0 mL of 10% methanol in sodium acetate (0.1%, pH 5) and deconjugated with a hydrolysis reagent consisting of purified *H. 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, U.K.), dried, reconstituted in 40% aqueous methanol, and analyzed using LC-MS/MS. Analysis was performed on a LC-MS/MS system consisting of a Jasco HPLC system (Jasco, Great Dunmow, U.K.) using a diphenyl column (Varian Pursuit, 3 μ m, 150 \times 2 mm, Varian, Oxford, Oxfordshire, U.K.) and a Waters Quattro Ultima triple-quadrupole MS instrument (Waters, Manchester, U.K.) 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, U.K.) and an ABI

Table 2. Comparison of Phytoestrogen Content Determined in This Study with Data by Liggins et al. (30), Milder et al. (28), Thompson et al. (31), and Smeds et al. (38)^a

food	phytoestrogens		isoflavones			lignans			
	this study	Thompson	this study	Thompson	Liggins	this study	Milder*	Thompson	Smeds
Coffee									
coffee, instant powder	1833		913			920			
coffee, instant, decaffeinated powder	647		5			641			
coffee, infusion, average	17	6.3	<1			16			
coffee, infusion, decaffeinated	11	5.5	<1			10			
Tea									
tea, strong	12		<1			11			
tea, weak	8		<1			8			
tea, standard, tea bag	7	8.9	<1	1		7	71	8	
tea, decaffeinated, tea bag	10		<1			9			
tea, green	20	13	<1	1		20	39	12	
tea, chamomile	8		6			2			
Alcoholic Beverages									
lager, canned	68		13			54	27		
beer, brown ale	71		8			63			
stout, 4–5%	45		9			35			
beer, bitter, best/premium	1		<1			1			
wine, red	76	53.9	<1	17		75	80	37	
wine, white, dry	14	12.7	<1	5		14	22	8	
cider, dry	55		6			48			
sherry, dry	41		3			38			
sherry, cream	31		<1			31			
gin	<1		<1			<1			
whiskey	5		<1			4			
Nuts and Seeds									
almonds, kernel only	112	131	27	18	nd	84		112	183
Brazil nuts	887		105		1	781			
cashews	182	122	12	22		170		99	371
hazelnuts	80	107.5	22	30	24	57		77	
peanut, fresh	173	34.5	70	27	24	101		7	135
peanut, dry roasted	173		94		21	78			
peanuts, roasted, salted	427		154			273			
peanut butter	140	80.1	61	42	10	79		38	
pecans	51	28.8	34	4		17		25	
pine nuts	103		32			70			
pistachios	62	382.5	33	177		29		199	
pumpkin seeds	539		18			520			
sunflower seeds	111	216	2	6	nd	109		210	
walnuts	175	139.5	31	55	nd	144		86	159

^aData are given as micrograms per 100 g of wet weight except for those marked with an asterisk, which are in micrograms per 100 mL. Lignans are the sum of secoisolariciresinol and matairesinol; isoflavones are the sum of biochanin A, daidzein, formononetin, genistein, and glycitein. nd, not detected.

4000 QTRAP mass spectrometer (Applied Biosystems, Warrington, Cheshire, U.K.) fitted with an electrospray ion source in negative ion mode. Compounds were quantified using ¹³C₃-labeled internal standards.

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

Data Analysis. Each sample was prepared in triplicate and each preparation analyzed twice. Data are presented as the average of these analyses in micrograms per 100 g of wet weight. Statistical analyses were performed using GraphPad Prism 4 for Macintosh, GraphPad Software, San Diego CA, www.graphpad.com.

RESULTS

All foods analyzed contained phytoestrogens, although the amounts present in gin and bitter beer were below the limit of quantification (**Table 1**). In all beverages analyzed, only small amounts of isoflavones were detected, with the notable exception of instant coffee powder, in which significant amounts of daidzein, genistein, and glycitein were found. In coffee and tea—but not chamomile tea—lignans were the main class of

phytoestrogens with an average content of 12 µg/100 g. The phytoestrogen content in alcoholic beverages (except for gin and bitter) was significantly higher (45 µg/100 g) with a notable difference between red (76 µg/100 g) and white wine (14 µg/100 g) and different types of beer (brown ale, 71 µg/100 g; lager, 68 µg/100 g; stout, 45 µg/100 g; bitter, 1 µg/100 g).

The phytoestrogen—and in particular lignan—content was much higher in nuts and seeds, in particular in Brazil nuts (887 µg/100 g) and pumpkin seeds (539 µg/100 g); the main lignan detected was secoisolariciresinol. In most samples, daidzein and formononetin were either absent or present in very low concentrations; the main isoflavones detected were biochanin A, genistein, and glycitein. In peanuts, roasting resulted in an increase in isoflavones detected, whereas peanut butter contained less phytoestrogens than fresh peanuts.

DISCUSSION

Previous analyses of beverages, nuts, seeds, and oils are limited to a few foods and focused mainly on their lignan content (28, 29). There is little information available about isoflavone content (30–32). **Table 2** shows a comparison of data published previously (28, 30, 31). For the limited com-

parison possible, levels were similar to those found elsewhere, with no significant difference between the data shown here and data published previously using a two-sided paired *t* test; there is no significant difference between our data and data published previously. In beverages, we found more lignans than Thompson et al. (31) and Milder et al. (28), but Thompson et al. found significantly more isoflavones in both red and white wines. The phytoestrogen content in soybeans is known to vary more than 4-fold (33, 34) depending on variety, harvesting, and processing, and in a recent study we showed a similar variability in a selection of other foods analyzed, including lignan-rich foods such as carrots and cabbage (35). For most foods analyzed, the variation in total phytoestrogen content is less than 3-fold when compared with the data of Thompson et al. (31). A notable exception are pistachio nuts, in which Thompson et al. found a higher amount of isoflavones (5-fold) and lignans (7-fold); however, we found a variability of up to 20-fold in foods from different sources of origin (35), and normal variability of phytoestrogen content occurring during sampling is a likely explanation. Another explanation for the observed differences from results published previously are differences in the analytical method, for example, different hydrolysis methods such as alkaline hydrolysis used by Milder et al. (28), which can result in higher yields for some compounds but may affect the stability of others.

In most foods analyzed, the lignan content is much higher than the isoflavone content, and lignans are the main contributor to total phytoestrogens. Although the phytoestrogen contents in coffee and tea are low (7–20 µg/100 g) compared with other plant-based foods, they are consumed regularly, and the average daily intake of coffee and tea as consumed by adults in the United Kingdom is >500 g (36). Average lignan intake from this source could therefore be as high as 80 µg/day. Similarly, the 600 g on average of beer and lager consumed by the adult British male beer consumer would result in a daily consumption of phytoestrogens of up to 378 µg/day. There are few data available about lignan consumption in the United Kingdom, but in a Swedish cohort an average total daily consumption of approximately 0.6–1 mg was found (37), suggesting that lignans from coffee, tea, and alcoholic beverages are an important contributor to daily phytoestrogen intake.

In summary, using a newly developed comprehensive analytical technique, all but 1 of the 38 foods analyzed here contained phytoestrogens—mainly lignans. 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.

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