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Phytoestrogen Content of Foods of Animal Origin: Dairy Products, Eggs, Meat, Fish, and Seafood

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Dietary phytoestrogens may be involved in the occurrence of chronic diseases. Reliable information on the phytoestrogen content in foods is required to assess dietary exposure and disease risk in epidemiological studies. However, existing analyses have focused on only one class of these compounds in plant-based foods, and there is only little information on foods of animal origin, leading to an underestimation of intake. This is the first comprehensive study of phytoestrogen content in animal food. We have determined the phytoestrogen content (isoflavones: biochanin A, daidzein, formononetin, genistein, and glycitein; lignans: secoisolariciresinol and matairesinol; coumestrol; equol; enterolactone; and enterodiol) in 115 foods of animal origin (including milk and milk-products, eggs, meat, fish, and seafood) and vegetarian substitutes using liquid chromatography−mass spectrometry (LC−MS) with ^{13}C-labeled internal standards. Phytoestrogens were detected in all foods analyzed; the average content was 20 μg/100 g of wet weight (isoflavones, 6 μg/100 g; lignans, 6 μg/100 g; equol, 3 μg/100 g; and enterolignans, 6 μg/100 g). In infant soy formula, 19 221 μg/100 g phytoestrogens were detected (compared to 59 μg/100 g in non-soy formula). Our study shows that all foods analyzed contained phytoestrogens and most foods (except for fish, seafood, and butter) contained mammalian phytoestrogens (enterolignans and equol). This is the first comprehensive study of phytoestrogen content of foods of animal origin and will allow for a more accurate estimation of exposure to dietary phytoestrogens.

KEYWORDS: Phytoestrogens; enteroligans; dairy products; meat

1. INTRODUCTION

Phytoestrogens, secondary plant metabolites, which are structurally or functionally similar to 17β-estradiol (*1*), have received increasing attention for their potentially beneficial effects as estrogen agonists or anti-estrogens in health and disease. Their possible effects have been implicated in the etiology of hormone-dependent cancers (*2–5*), cardiovascular disease (*4, 6*), osteoporosis (*4, 7*) menopausal symptoms (*4, 8*), male infertility (*9*), obesity, and type-2 diabetes (*10*). Evidence that these compounds are biologically active even at low levels in humans comes from interactions between phytoestrogens and gene variants of the estrogen receptor (ESR1 and NR1I2) (*11, 12*), sex-hormone binding globulin (SHBG) (*13*), and probably aromatase (CYP19) (*14*). Recent studies have shown either increased or decreased breast cancer risk associated with phytoestrogens at low levels (*15–17*). However, these compounds can act as either estrogens or anti-estrogens, leading to concerns surrounding the use of phytoestrogen supplements in breast cancer patients (*18, 19*).

The major classes of phytoestrogens are isoflavones, lignans, and coumestans (*20*). In plants, they occur in general as glycosides, which are deconjugated by intestinal glucosidases to release the aglycones (*21*). The aglycones can then be further metabolized by the intestinal microflora into hormone-like compounds with weak estrogenic activity (*5*). Lignans can be converted into the mammalian lignans enterodiol and enterolactone, whereas the isoflavones daidzein can be converted into *O*-desmethylangolensin (*O*-DMA) and equol (*22*). Phytoestrogens are mainly excreted in urine, but they have also been found in human (*23*) and cow's milk (*24, 25*).

Using new highly sensitive mass spectrometry methods incorporating ^{13}C-labeled internal standards, we have used urinary and plasma phytoestrogen concentrations as biomarkers of intake to assess risks of cancer and interactions with gene variants in a number of studies (*11, 14, 15, 26, 27*). However, not all epidemiological and surveillance studies

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Table 1. Phytoestrogen Content in Foods of Animal Origin[a]

food	phytoestrogens	isoflavones	lignans	coumestrol	equol	enterolactone	enterodiol
Milk and Related Products							
coffee whitener, not reconstituted	22	6	14	1	1	2	—
cream double	8	4	3	<1	—	1	—
cream single	8	2	2	<1	1	3	—
cream substitute, double	13	6	4	2	—	2	—
cream, UHT whipping	10	7	1	<1	—	1	—
crème fraiche	9	2	5	<1	—	1	—
goat's milk, whole	5	1	1	<1	3	1	—
milk, skimmed, condensed	20	4	6	<1	2	8	—
milk, condensed, evaporated/unsweetened, carnation type	20	3	6	<1	3	8	—
milk, condensed, evaporated/unsweetened, low fat, carnation light type	17	1	3	<1	3	9	—
milk, whole, full fat, condensed	25	6	9	1	—	9	—
milk, semi-skimmed	8	4	1	<1	1	3	—
milk, skimmed	20	14	1	<1	1	3	—
milk, whole	12	6	1	<1	1	4	—
milk, skimmed, dried, w/o vegetable fat (powder)	58	5	8	1	8	37	—
milk, skimmed, dried, with vegetable fat (powder)	58	7	15	<1	7	28	—
soy milk, sweetened, fortified, unflavored	9313	9307	6	<1	—	—	—
soy milk, unsweetened	6028	6018	9	1	—	—	—
dessert topping, canned	16	5	5	—	1	5	—
UHT cream (for coffee	64	6	57	1	—	—	—
Baby Formula Powder							
baby formula	59	19	16	1	5	19	—
soy infant formula	19221	19211	10	<1	—	—	—
Cheese							
cheese, brie, French and English	30	10	2	<1	7	11	—
cheese, Camembert	29	3	7	<1	4	15	—
cheese, Cheddar vegetarian	27	9	2	<1	4	13	—
cheese, Cheddar, Canadian	36	10	2	1	14	10	—
cheese, Cheddar, English (nonvegetarian)	28	7	1	<1	6	13	—
cheese, Cheddar, reduced fat	62	14	17	2	8	22	—
cheese, Cheshire	62	17	18	<1	4	23	—
cheese, Danish blue	34	9	2	<1	11	11	—
cheese, double Gloucester	22	6	3	<1	4	9	—
cheese, Edam	20	7	3	<1	1	8	—
cheese, Feta (ewe's and goat's milk)	12	4	<1	<1	2	5	—
cheese, Gouda	24	10	3	<1	2	9	—
cheese, mozzarella (buffalo milk)	24	4	6	<1	6	6	—
cheese, Parmesan	27	6	5	1	4	11	—
cheese, red Leicester	22	7	3	<1	4	8	—
cheese, spread, low/reduced fat/light (soft processed cheese)	23	2	2	<1	6	12	—
cheese, spread, plain, triangles and tube	29	7	7	<1	4	10	—
cheese, Stilton	29	8	2	<1	7	12	—
cheese, Wensleydale	54	4	25	<1	10	15	—
cottage cheese	11	2	2	<1	1	7	—
cottage cheese, low fat	12	2	2	<1	1	7	—
fromage frais virtually fat free	11	2	1	<1	2	6	—
Mascarpone	13	2	4	1	3	3	—
processed cheese slices, plain	36	12	11	<1	4	9	—
quark	14	1	5		2	6	—
soft cheese, Philadelphia type, full fat	41	9	13	<1	13	6	—
soft cheese, Philadelphia type, medium fat	14	2	2		4	6	—
Butter							
butter, brand 1, salted	13	10	2	<1	—	—	—
butter, brand 1, unsalted	11	9	1	<1	—	—	—
butter, brand 2, slightly salted	17	12	5	<1	—	—	—
butter, brand 2, unsalted	15	9	5	<1	—	—	—
Ice Cream and Desserts							
ice cream in cone, luxury	28	21	4	<1	—	3	—
ice cream dessert	15	2	6	1	2	5	—
ice cream, ice cream roll	789	779	7	1	1	2	—
ice cream, choc ice	36	20	9	1	1	5	—
ice cream, dairy	16	7	3	<1	2	4	—
ice cream, low fat and cream	9	2	<1	<1	1	5	—
ice cream dessert, low fat	8	1	1	—	1	5	—
ice cream, nondairy	17	7	4	<1	1	5	—
ice cream, with cone	19	10	4	<1	1	3	—
mousse, chocolate, commercial	8	2	2	—	2	3	—
mousse, chocolate, commercial, not rich	7	2	1	—	1	4	—
trifle, commercial	19	2	15	—	—	2	—
soy ice cream	13494	13488	5	1	—	—	—

Table 1. Continued

food	phytoestrogens	isoflavones	lignans	coumestrol	equol	enterolactone	enterodiol
			Yogurt				
yogurt, authentic Greek (cow's, ewe's, and goat's milk)	8	1	2	<1	1	4	—
yogurt, bio, strawberry	39	4	27	<1	4	4	—
yogurt, Greekstyle natural	14	1	3	<1	5	5	—
yogurt, Greekstyle natural lowfat	12	3	2	<1	2	5	—
yogurt, Greekstyle natural probiotic	12	2	1	<1	4	4	—
yogurt, hazelnut	12	3	2	<1	2	4	—
yogurt, natural, low fat	19	12	1	—	2	5	<1
yogurt, natural, whole milk	20	10	2	—	2	5	<1
yogurt, soy	8286	8235	51	1	—	—	—
yogurt, strawberry light	15	2	8	<1	1	3	—
yogurt, strawberry lowfat	13	4	4	<1	2	4	—
yogurt, strawberry with cream	56	5	46	<1	1	4	—
			Egg and Egg Products				
egg noodles, cooked	2	2	<1	—	—	—	—
egg noodles, raw	12	9	3	—	—	—	—
egg, barn-kept hens, white, raw	5	2	2	<1	<1	<1	<1
egg, barn-kept hens, whole, raw	18	8	2	—	4	3	1
egg, barn-kept hens, yolk, raw	47	31	6	<1	8	2	<1
egg, caged hens, white, raw	5	2	2	<1	<1	—	—
egg, caged hens, whole, raw	12	6	3	<1	2	1	—
egg, caged hens, yolk, raw	31	12	10	<1	7	2	—
egg, free-range hens, white, raw	6	2	3	<1	<1	<1	—
egg, free-range hens, whole, raw	11	6	3	<1	1	1	—
egg, free-range hens, yolk, raw	27	11	9	1	5	2	—
			Meat				
beef, fat, roast	19	3	16	1	—	—	—
beef, roast, lean only	7	1	6	<1	—	—	—
chicken, breast, roast	6	4	2	<1	—	—	—
chicken, dark meat, roast	4	2	1	—	—	—	—
chicken skin, dry	12	9	2	—	—	—	—
chicken skin, moist	6	5	1	<1	—	—	—
corned beef	32	27	4	1	—	1	<1
lamb, fat, roast	10	5	4	1	—	—	—
lamb, liver, grilled	35	12	17	—	—	6	—
lamb, roast, lean only	5	1	4	<1	—	<1	—
pig, liver, grilled	12	5	5	—	1	1	—
pork crackling	20	8	9	—	—	2	—
pork, fat, roast	8	3	5	1	—	—	—
pork, roast, lean only	4	1	3	<1	—	—	—
			Meat Substitutes				
burger, soy based	4430	4410	19	1	—	—	—
burger, vegetarian	4449	4415	34	<1	—	—	—
mince, savory, soy or TVP based (high soy content)	28758	28618	139	1	—	—	—
mycoprotein pieces chicken style	10	6	3	<1	—	—	—
sausage, mycoprotein based	23	11	11	<1	—	1	—
sausage, TVP/soy	3994	3946	49	<1	—	—	—
sausage, vegetarian	21	7	12	<1	—	1	—
			Fish and Seafood				
salmon	4	3	1	—	—	—	—
cod, microwaved	2	1	1	—	—		—
mussels	9	7	2	—	—	—	—
prawns, frozen	8	3	4	1	—	—	—
tuna, canned, brine	6	5	<1	<1	—	—	—

[a] Data are given as μg/100 g of wet weight. Isoflavones are the sum of biochanin A, daidzein, formononetin, genistein, and glycitein, and lignans are the sum of secoisolariciresinol, matairesinol, and shonanin. More detailed data can be found in the Supporting Information. Unless indicated otherwise, dairy products are from cow's milk (— indicates not detected).

have biological samples available, and many studies have relied on reports of food intake and associated phytoestrogen databases to assess phytoestrogen exposure in relation to disease risk (*28–32*). Previous food analyses have mainly been limited to isoflavones in fruit, vegetables, nuts, and seeds, and there is little data available for lignans and animal foods (*33–36*) (*37*). This could lead to an underestimation and misclassification of dietary exposure, because phytoestrogens are found in milk and, possibly, other animal foods (*38*).

We have therefore modified our technique for blood and urine (*15, 39*) to analyze food samples (*40*). Using this technique, we analyzed the variability of phytoestrogen content in foods from different sources (e.g., manufacturer or country of origin) and could show that the variability (as a coefficient of variation) is 33% for lignans and 37% for isoflavones (*41*). We have now applied this technique to determine the phytoestrogen content (isoflavones: biochanin A, daidzein, formononetin, genistein, and glycitein; lignans: matairesinol and secoisolariciresinol; enterolignans: enterolignan and enterolactone; coumestrol; and equol) of 115 foods of animal origin and their corresponding vegetarian substitutes. Milks, cheese, butter, ice cream, yogurt, eggs, meat, and fish were investigated.

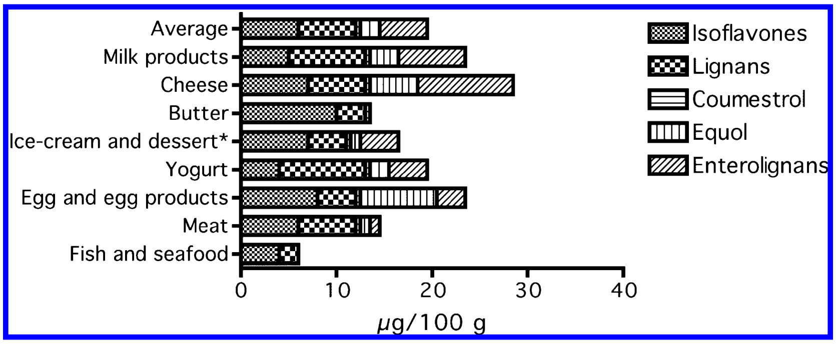

Figure 1. Summary of phytoestrogen content (in μg/100 g of wet weight) in different types of food of animal origin [excluding soy-, TVP-, or mycoprotein-based substitutes; (*) excluding ice-cream roll].

EXPERIMENTAL PROCEDURES

Chemicals. Biochanin A, daidzein, genistein, glycitein, formononetin, secoisolariciresinol, matairesinol, coumestrol, enterodiol, enterolactone, and equol were purchased from Plantech (Reading, Berkshire, U.K.). $^{13}C_3$-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, $^{13}C_3$-enterodiol, and $^{13}C_3$-enterolactone were obtained from Dr. Nigel Botting (University of St. Andrews, Fife, U.K.) (*42–45*). β-Glucuronidase (from *Helix pomatia*), β-glucosidase (from almonds), and cellulase (from *Trichoderma reesi*) were purchased from Sigma (Poole, Dorset, U.K.). Water, methanol, acetic acid, and ammonia were purchased from Sigma (Poole, Dorset, U.K.) 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 (where possible) in Cambridgeshire, U.K. Where possible, the foods bought at each outlet were from different manufacturers, varieties, countries of origin, and/or batch numbers. Each sample was weighed and, if necessary, cooked. A representative portion (approximately 35 g of dry weight) was taken from each of the five samples for each food. The samples were frozen (−20 °C), freeze-dried if necessary (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 (*40*). Briefly, approximately 100 mg of freeze-dried food was extracted 3 times with 2.0 mL of 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, 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 μm, 150 × 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 4000 QTRAP mass spectrometer (Applied Biosystems, Warrington, Cheshire, U.K.) fitted with an electrospray ion source in negative-ion mode. Compounds were quantified using $^{13}C_3$-labeled internal standards; for compounds where labeled internal standards were not available, $^{13}C_3$-enterolactone (for lignans) and $^{13}C_3$-glycitein (for isoflavones) were used.

The method was validated on both LC/MS/MS systems. The intrabatch CV of this method is between 3 and 14%, and the interbatch is between 1 and 6%. As quality control for the main plant phytoestrogens, 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 analyzed twice. Data given are the average of two analyses and in μg/100 g of wet weight. For brevity, results are presented as total food isoflavones, total food lignans, and metabolized equol, enterolactone, and enterodiol. Data on separate phytoestrogens analyzed are shown in the Supporting Information.

RESULTS

Phytoestrogens were detected in all food groups analyzed, although the amount in some foods was very low. **Table 1** shows the results for all foods analyzed (more detailed information can be found in the Supporting Information); enterolignans (mainly enterolactone) and equol were found in all foods of animal origin, except for butter, fish, and seafood.

Traces of coumestrol were detected in many foods but mainly below the limit of quantification (1.5 μg/100 g of dry weight). In cheese, the phytoestrogen content varied considerably, but there was no clear link between the type of cheese and phytoestrogen content. For most cheeses, enterolactone and equol were the main contributors to the total phytoestrogen content.

The phytoestrogen content in eggs was lower than in milk products, and most phytoestrogens (mainly isoflavones) were found in the egg yolk. In contrast to milk-based products, the amount of equol was higher than the amount of enterolignans present. Although the isoflavones content was highest in eggs from hens kept in barns, there was no clear difference between different types of chicken husbandry. The amount of equol and enterolignans in meat, fish, and seafood was very low, and the main phytoestrogens detected were isoflavones and lignans.

The phytoestrogen and, in particular, isoflavones content were considerably higher in soy-based foods. Soy milk and yogurt contained almost 500 times more phytoestrogens when compared to their milk-based counterpart. In contrast, mycoprotein-based meat substitutes contained only small amounts of phytoestrogens. The infant formula analyzed contained similar amounts of phytoestrogen compared to milk powder; however, it contained more isoflavones and lignans and less equol and enterolignans. In contrast, the amount of phytoestrogens in soy-based infant formula was more than 300 times higher than in normal infant formula.

Figure 1 gives a summary of phytoestrogens detected in different types of food. A major proportion of the phytoestrogens in eggs and dairy products, apart from butter, were derived from the gastrointestinal metabolism of plant precursors, whereas in meat, fish, and seafood the phytoestrogens were virtually all plant-derived.

DISCUSSION

Phytoestrogens are often associated with plant-based diets only, and despite knowledge of the presence of phytoestrogens in foods of animal origin, in particular, in milk (*24, 25*), for

Table 2. Average Proportion of Different Classes of Phytoestrogens in Foods Derived from Animals (Excluding Soy-, TVP-, or Mycoprotein-Based Substitutes)

food type	isoflavones (%)	lignans (%)	coumestrol (%)	equol (%)	enterolignans (%)
animal-based food	31	29	2	12	26
milk and related products	21	34	2	13	30
cheese	25	21	2	18	35
butter	74	22	4		
ice cream and desserts	42	24	3	6	24
yogurt	21	46	3	10	21
egg and egg products	34	17	2	34	13
meat	41	41	3	7	7
fish and seafood	67	33			

some time, very little data about the phytoestrogen content in foods of animal origin are available. Consequently, the contribution to total phytoestrogen exposure is often neglected (*32*). The data presented in this study are the first comprehensive investigation of phytoestrogens in animal products and will allow for a more accurate determination of exposure to dietary phytoestrogens from foods. Apart from some exceptions, we found that lignans were as important as isoflavones, contributing approximately equal proportions to total phytoestrogen levels, thus emphasizing the need for chromatographic analytical systems that incorporate a spectrum of compounds and use as many labeled standards as possible rather than, for example, single focus analytical methods based on immunofluorescence.

Our data show that most animal foods contained phytoestrogens, which would have been derived from animal feeds and pastures, especially those containing clover and other legumes. Furthermore, although the phytoestrogen content in animal products is low when compared to soy-based foods (e.g., soy milk), the range is similar to that of many commonly consumed vegetables. For example, staple foods, such as potatoes (total phytoestrogen content < 10 μg/100 g (*46*)), contain less phytoestrogens than most animal products. Eggs (particularly the yolk), cheese, and other milk-derived products contained a high proportion of mammalian phytoestrogens, whereas the phytoestrogens in meat and fish were almost entirely composed of the unmetabolised plant precursors. **Table 2** shows that the proportion of isoflavones and lignans varies considerably between different types of foods.

It is known that cow's milk contains phytoestrogens from limited analyses, and we show here that goat's milk also contains these compounds. Only a few studies have investigated phytoestrogen contents of cow's milk (*37, 46, 47*); however, these studies did not include plant lignans. The phytoestrogen content in milk depends upon a variety of factors, such as fodder and season, and the results obtained in this study are lower but still comparable to data obtained previously from Australia (5−29 μg/100 mL equol) (*47*), France (1−29 μg/100 mL equol and 1−9 μg/100 mL enterolactone) (*37*), and Finland (6 μg/100 mL equol) (*48*). Soy is used as a food additive, and some of the somewhat higher levels of isoflavones in processed foods, such as ice cream, are indicative of their presence. The health effects of very high levels of phytoestrogens present in soy infant formula, several orders of magnitude higher than in human breast milk and milk-based formula, have been a matter of concern for some time (*49*).

This study is the first comprehensive investigation of phytoestrogens in animal products, and this data will allow for a more accurate determination of exposure to dietary phytoestrogens.

Supporting Information Available: Phytoestrogen content in food of animal origin in μg/100 g of wet weight. This material is available free of charge via the Internet at http://pubs.acs.org.

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