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Isoflavonoids and lignans in legumes: Nutritional and health aspects in humans

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Dietary factors are considered important environmental risk determinants for Western diseases. Studies have revealed beneficial or protective effects of the consumption of legumes with regard to hypercholesterolaemia and coronary heart disease, obesity, diabetes mellitus, and menopause. During the last decade attention has been focused on soy and soybean products. Several constituents have been isolated: isoflavones, phytosterols, protease inhibitors, inositol hexaphosphate, and saponins. Our interest concentrates on hormone-like bisphenolic phytoestrogens of dietary origin, the lignans and isoflavonoids. Their glycosides, converted by gut bacteria to mammalian derivatives with weak estrogenic and antioxidative activity, originate in leguminous seeds. We developed an isotope dilution gas chromatography-mass spectrometry method for quantitative determination of the isoflavones, formononetin, biochanin A, daidzein, genistein and coumestrol, and the lignans secoisolariciresinol and matairesinol, in food samples. We measured the four isoflavonoids and coumestrol, and, for the first time, the two lignans in 52 leguminous seeds and found high concentrations of isoflavonoids (0–1853.35 mg/kg; 0–7.3 mmol/kg dw) but lower amount of lignans (0–15.85 mg/kg; 0.05 mmol/kg dw). The highest plasma levels of their metabolites are found in individuals living in countries or regions with low cancer and cardiovascular disease incidence and these are probably sufficient to influence intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation, and angiogenesis. Leguminous seeds, therefore, in respect to their abundant concentrations of phytoestrogens, are strong candidates for a role as natural cancer-protective food. (J. Nutr. Biochem. 9:193–200, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Over 60 years ago veterinary field scientists reported the presence in plants of estrogen compounds that induce estrus in immature animals or interfere with normal reproductive processes.[1–3] To this day more than 300 plants have been found to possess compounds with estrogenic activity.[4,5] These compounds have been defined by the general name phytoestrogens. A few classes have now been identified, established, and studied: the hormone-like bisphenolic phytoestrogens, the isoflavonoids daidzein and genistein, the coumestans (coumestrol), and the lignans secoisolariciresinol (SECO) and matairesinol (MAT) are of great interest because of their estrogenic, antiestrogenic, anticarcinogenic, antiviral, antifungal, and antioxidant activities.[6–10]

The plant family most abundant in phytoestrogens is the Leguminosae. The legume family (Fabaceae) is generally characterized by seeds, often edible (though sometimes quite poisonous), borne in pods that often open along two seams, by pea-shaped flowers, and by compound stipulate leaves. Dried seeds of edible legumes are often called

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"pulses." Of the 13,000 species of legumes, only about 20 are commonly consumed by humans. Of all beans of the Leguminosae family the soybean and its products have attracted most attention. Several constituents of medical interest have been isolated. These include isoflavones, phytosterols, protease inhibitors, inositol hexaphosphate, and saponins.[11] Recently many other legumes have been reported to contain these same compounds as well. (For a listing of dozens of biologically active compounds in various legumes, including soy and nearly fifty other edible legumes, see: http://sun.ars-grin.gov/~ngrlsb/.) A great number of studies have been undertaken in a search for links between soy phytoestrogens and sex hormone metabolism and biological activity, intracellular enzymes, protein synthesis, growth-factor action, malignant-cell proliferation, and angiogenesis on the molecular as well as on tissue and organism levels.[10,12–24]

Nutritional properties of the Leguminosae have been investigated for a long time and the legumes have been shown to exert many physiologically beneficial effects in human and animal organisms. Dry beans supply protein, complex carbohydrates, soluble fiber, and essential vitamins and minerals to the diet, yet are low in fat and sodium and cholesterol-free. Although there is evidence that all the three major constituents of legumes (i.e., protein, starch, and dietary fiber) could be involved in lowering serum cholesterol and triglyceride levels in humans,[25–31] the mechanism(s) by which such effects are induced is (are), however, largely unknown. Human studies have also revealed the beneficial effects and health implications of the consumption of beans with regard to diabetes mellitus,[32,33] obesity,[34] and cancer.[35,36] Nevertheless, it remains to be seen whether their hypocholesterolemic, cardioprotective, and normoglycemic properties are related to some unique structural and/or compositional characteristics of legume seeds.

Precise quantitative data on isoflavone and lignan content in leguminous plants are lacking. Indeed, no food composition data for MAT and SECO are available. Although many methods for the separation and quantitation of phytoestrogens in plant (food) extracts by high performance-liquid chromatography (HPLC) have been described,[37–40] to the best of our knowledge only one study has analyzed the isoflavonoid content in various foods other than soy[41] and two other studies involved various legumes;[42,43] no one has yet performed any measurements of lignans in the Leguminosae. In the survey by Jones et al. no detectable isoflavonoid levels were reported for the 107 food items examined. The HPLC instrumentation applied in the other studies seems not to be sufficiently sensitive for those legumes with low concentrations of the compounds. Therefore, we have applied an isotope dilution gas chromatography–mass spectrometry in the selected monitoring mode (ID/GC/MS/SIM) method[44] for the identification and quantitative determination of these two lignans (SECO and MAT) and these four isoflavonoids (biochanin A, formononetin, daidzein, genistein)(structures in *Figure 1*) as well as coumestrol in 52 soybean and other leguminous seeds. We show for the first time the presence of the lignan SECO in soy beans and the lignans SECO and MAT in other legumes. The data we provide may be of use in future epidemiological and metabolic studies.

Methods and materials

Sources of legume samples

Legume seeds were purchased from commercial sources. Most of the legume seeds were germinated, planted and grown in the U.S. A few of the samples were kindly provided by Prof. McMichael (London School of Hygiene and Tropical Medicine, University of London). Before analysis the dry beans were ground three times with an electric-coffee grinder (Krups, Germany) and dry weight was determinated with a Moisture Analyser 40 (Sartorius AG, Göttingen, Germany).

Chemicals and standards

All chemicals and details of the synthesis of standards are presented in a publication describing ID/GC/MS/SIM method for the determination of lignans and isoflavonoids in food samples[44] as well as in two earlier publications on GC/MS methods for lignans and for the estrogen profile in human urine.[45,46] All glassware including Pasteur pipettes used for ion exchange chromatography and the glass liner of the injection system of the GC/MS instruments (HP 5995 quadrupole mass spectrometer with Autoinjector 7673A and data system HP 59970C MS Chem Station) was deactivated by silanization with 1% dimethylchlorosilane in toluene or Siliconimprägnierer (Carl Roth GMbH, Karlsruhe, Germany).

Analytical methods

Powdered legume samples were hydrolyzed, extracted with diethyl ether, and subsequently purified and separated on DEAE- and QAE-Sephadex columns. Isoflavonoids formononetin, daidzein, biochanin A, genistein, and coumestrol, and lignans SECO and MAT were silylated and analyzed by the ID/GC/MS/SIM method[44] (*Figure 2*) using synthesized deuterated internal standards for the correction of losses during the procedure. All assays were performed in duplicates or quadruplicates. In some samples we have found extremely high amounts of isoflavonoids, far exceeding the highest point on the GC/MS standard curve. In such cases we used ordinary gas chromatography with cholestane as the internal standard and a shortened procedure to avoid losses.[44,45]

The coefficient of variation of the method used was found to vary between 3.1% and 9.6% (concentration range, 0.05 to 0.13 mg/kg) for the seven compounds. The mean recovery for all the analyzed phytoestrogens was 99.5%, and the sensitivity limit was approximately 0.02 to 0.03 mg/kg (standard deviation of the assays at low levels multiplied by 2–3).

Results

The quantitative results for 48 cultivars of 16 food legume species, and for 4 forage legume samples, are listed in *Tables 1* and *2*.

The highest total concentration of isoflavones, with regard to edible seeds, was found in kudzu root (over 200,000 μg/100g; 8.14 mmol/kg), followed by soybeans [ranged from 37,300 μg/100g (1.4 mmol/kg) to 140,300 μg/100g (5.3 mmol/kg)] and chickpea [1,150 to 3,600 μg/100g (44.5 to 128.7 μmol/kg)]. The same relation in concentrations held also for daidzein and its precursor formononetin. The pea "Green split" was found to be the poorest source of all these phytoestrogens. All soybeans analyzed proved to be the richest source of genistein, reported to be the most biologically active phytoestrogen. However, significant amounts of this compound were,

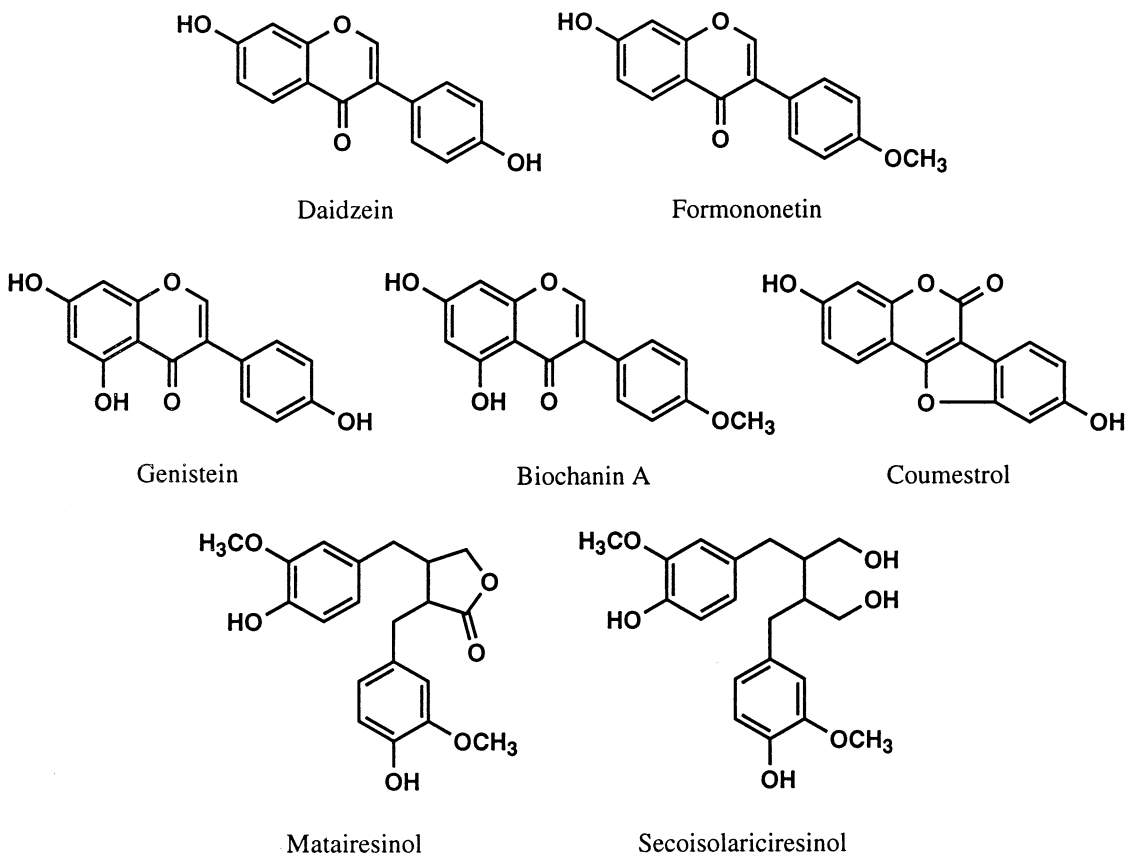

Figure 1 The isolated phytoestrogens from the Leguminosae.

besides kudzu, measured in pigeon pea, groundnuts, pinto, and haricot beans. The highest concentrations of its precursor, biochanin A, were found in *Cicer arietinum*, another commonly consumed legume species. Its cultivar, Garbanzo bean, had the third highest total isoflavonoid content after kudzu and soybeans. All the legumes, except the "Green split" pea, comprised daidzein and genistein, and, with a few exception, the amounts of genistein exceeded daidzein concentrations. Coumestrol was detected in most of the samples at very low concentrations (up to 10 μg/100g; 0.4 μmol/kg); the soybean "Santa rosa," kudzu leaf and red clover contained more (105.0 to 1,570 μg/100g; 3.9 to 58.6 μmol/kg). SECO at the concentration 1,590 μg/100g (44.9 μmol/kg) was detected in *Sophora japonica*. However, of the most commonly known and consumed legume species analyzed, the soybean and the peanut (*Arachis hypogaea*) contained the highest level of SECO [13.3–273 μg/100g (0.3 to 7.7 μmol/kg) and 333.0 μg/100g (9.3 μmol/kg), respectively] but no or minor amounts of MAT could be detected. Generally, all the other legume items analyzed contained SECO [from 2.8 to 475.8 μg/100g (0.08 to 13.5 μmol/kg)]. MAT seems to be the rarest phytoestrogen in the Leguminosae plant family; although considerable amounts were found in black gram samples. Red clover, a perpetrator of "clover disease" in sheeps in Australia, was found to contain the highest concentrations of formononetin and biochanin A. These phytoestrogens were not found in alfalfa, another common forage legume, at all. The content of lignans in the forage legumes was lower and ranged from trace to 19.4 μg/100g (0.5 μmol/kg).

Discussion

Our ID/GC/MS/SIM method applied to the analysis of phytoestrogen composition in the Leguminosae is a major methodological advance compared to the HPLC methodologies developed for quantitation of isoflavones in soybean and soyfoods. The majority of these methods have used gradient elution system and have been used for the analysis of phytoestrogens in samples with very high concentrations of isoflavones or lignans. For foods with exceptionally high amounts of lignans or isoflavones the HPLC (and gas chromatographic) techniques are more convenient than GC/MS methods. However, the method used in this investigation is more sensitive and specific than the HPLC methods, although more complicated. At low levels, HPLC, being a blind detection system, may easily produce nonspecific results. This seems to be particularly true for coumestrol, which in our experience occurs in very low amounts in food (legume) samples.

Franke et al.[42] have reported a concentration of coumestrol in a few samples ranging from 1,480 μg/100g (55.2 μmol/kg) in large lima beans to 561,140 μg/100g (20.9 mmol/kg) in clover sprouts (means of repeated analyses from dry or freeze-dried item). In the present survey we did determine this coumestan in a large part of the legumes

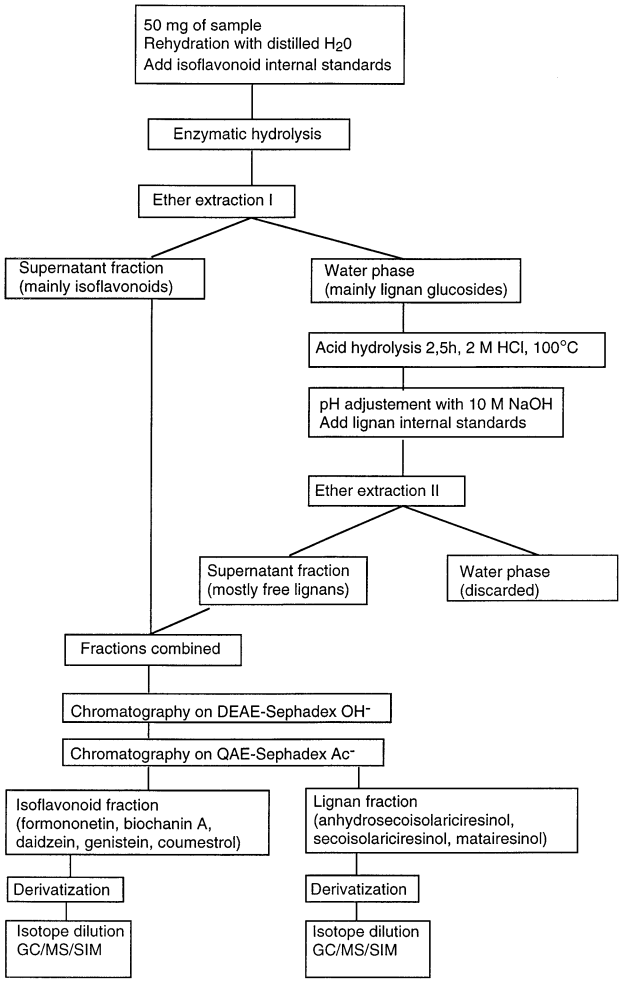

Figure 2 Flow-diagram of the method for the determination of lignans and isoflavonoids in legume samples.

analyzed; though the concentrations measured by us are many times lower. This finding correlates well with our studies on phytoestrogens in human urine; only once we have observed minimal amounts of coumestrol in urine in a vegetarian subject. In the same study by Franke all four isoflavonoids and coumestrol could not be detected in every legume item. This may result from differences in sensitivity. HPLC method detection limits, obtained from authentic standards, are from two to ten times higher than those determined by ID/GC/MS/SIM. However, at high concentrations of compounds, when we compared our results of daidzein and genistein in soybeans with other reports,[38,42,47] the levels are similar. In our study we have noticed a variation in the phytoestrogen content of various legume species. A significant amount of variability in values from one variety of a bean to another could be explained by differences in genetics, origin and environmental conditions (crop year and growth locations).[48]

A comparative survey of leguminous plants as sources of isoflavonoids by Kaufman et al.[43] reported HPLC analytical data being at wide variance with ours. Significant differences also remained when daidzein and genistein concentrations were compared with the isoflavonoid levels obtained by Franke et al.[42] The reason for these variances is unknown; however, differences in specificity of the analytical methods are probably the main cause.

On the other hand, it should be kept in mind that any comparisons between results and techniques applied do not hold true without analysis of the same samples. And, in general, there is a lack of procedures that allow to standardize methodologies and point out the reference methods.

A number of studies on hypercholesterolemia, diabetes mellitus, and obesity, have attributed a beneficial action of legumes to dietary fiber, protein content, protease inhibitors, or saponins.[49–52] So far, the only polyphenols in pulses that received more attention, the tannins, lignins, and phytic acid, have been regarded as antinutritional factors[53] and they have been eliminated from legumes by several processing methods and chemical treatments. It has been known for a very long time[1] that soybean contains isoflavones but only recently it has been suggested that the isoflavones may prevent chronic diseases including hormone-dependent cancers, coronary heart disease and atherosclerosis.[14,54–56]

From 1979 through 1980 the cyclical occurrence in the urine of the female vervet monkey and women of two unknown compounds was reported.[57–59] Afterward, these compounds were identified separately and independently by two groups[6,58] and given the names enterolactone (ENL) and enterodiol (END). These mammalian lignans are formed by the action of intestinal microflora from plant precursors, the lignans SECO (immediate precursor of the END) and MAT (immediate precursor of the ENL).[7,60] SECO and MAT have been identified and showed to occur mainly in the glycosidic form in the plants. These glycosides are readily hydrolyzed in the proximal colon to their aglycones and the aglycones subsequently absorbed from the gut.

Our early study[61] investigated urinary phytoestrogen excretion of lignans and isoflavonoids in relation to habitual diets of lactovegetarian, omnivoric and macrobiotic women living in Boston and Helsinki. Using GC/MS method the highest mean excretion of both ENL and END was found in Boston macrobiotics (23.940 nmol/24 hr, geometrical mean). This was 10 times higher than total lignan amounts excreted by omnivores in Boston (2.32 nmol/24 hr, geometrical mean) and in Helsinki (2.66 nmol/24 hr, geometrical mean). Lactovegetarians had values twice higher than the omnivoric groups. Isoflavonoid excretion showed the same pattern; but the range was not so high. Careful 3- or 5-day dietary records were made two to four times during 1 year and they revealed that the macrobiotic diet was mostly consisted of seeds, grains, leguminous beans, and legume sprouts. The amounts of phytoestrogens in legumes reported here, could explain such big differences among the groups.

These interesting findings induced another study on urinary phytoestrogen profile in Japanese women and men of rural origin consuming their habitual diets.[14] The urinary excretion of ENL was, with few exceptions, low. No gender differences in ENL and END excretion were asserted but mean urinary excretion of the isoflavonoids was high but slightly lower in Japanese men than in Japanese women and was strongly associated with the intake of various soy products. The authors found a weak correlation between

Table 1 Phytoestrogen content of food legumes

Botanical name Common name	Formo (μg/100 g)	Bioch. A (μg/100 g)	Da (μg/100 g)	Gen (μg/100 g)	Coumestrol (μg/100 g)	SECO tot[1] (μg/100 g)	Mat (μg/100 g)
Glycine max Soybean "Centennial"	119.4	14.7	25,200	34,300	10.1	273.0	tr
Glycine max Soybean INIAP Bolivia	18.1	nd	10,500	26,800	nd	111.0	nd
Glycine max Soybean "Santa rosa"	40.6	15.0	56,000	84,100	185.4	222.0	nd
Glycine max Soybean Chapman	121.0	tr	41,300	46,400	tr	13.3	0.00
Phaseolus vulgaris Kidney bean	4.4	2.6	28.2	158.0	2.4	56.0	nd
Phaseolus vulgaris K. b. Giant "Great Northern"	10.9	tr	7.1	52.3	0.00	113.0	tr
Phaseolus vulgaris K. b. "Blue Lake 274"	8.3	nd	19.0	68.3	tr	98.4	nd
Phaseolus vulgaris K. b. "Cajone Pinto"	5.0	tr	37.0	51.5	0.00	64.1	nd
Phaseolus vulgaris K. b. "Early Riser"	3.6	tr	19.7	13.8	2.2	153.0	tr
Phaseolus vulgaris K. b. "Kentucky Blue Pole"	6.6	nd	40.2	32.6	9.1	135.0	nd
Phaseolus vulgaris K. b. "Venture Bush"	4.7	2.5	9.4	30.9	tr	113.0	nd
Phaseolus vulgaris Red kidney beans	0.0	7.3	8.0	7.0	0.0	69.6	0.0
Phaseolus vulgaris Pinto beans	0.0	2.1	20.3	518.0	0.0	79.1	0.0
Phaseolus vulgaris Navy beans (Haricot)	0.0	4.4	13.7	408.0	0.0	85.8	0.0
Phaseolus vulgaris White kidney beans	0.0	11.7	11.4	18.2	0.0	123.0	0.0
Phaseolus lunatus Lima bean "Henderson's Bush"	11.3	2.3	12.2	12.2	0.0	184.0	tr
Phaseolus lunatus L. b. "Sieva" Pole	12.4	2.7	12.7	10.6	tr	185.0	tr
Phaseolus lunatus L. b. Seaside Large	9.0	nd	89.0	19.2	10.0	158.0	tr
Apios americana American "Groundnut" HA R19 PL5	0.0	3.6	3.9	216.0	0.00	58.1	2.2
Apios americana A. G. HA R19 PL12	3.6	4.6	0.0	341.0	0.0	23.1	3.1
Apios americana A. G. HA R19 PL44	0.0	7.6	0.0	560.0	nd	20.8	4.6
Apios americana A. G. HF R24 PL4	0.0	7.8	18.6	811.0	nd	54.1	3.3
Apios americana A. G. HF R24 PL5	0.0	7.8	0.0	108.0	tr	50.3	3.4
Cajanus cajan "Pigeon pea" (Red gram)	14.3	219.0	14.6	737.0	tr	50.3	0.0
Cajanus cajan P. p. GA-2	26.1	80.8	27.2	693.0	0.0	46.7	0.0
Cajanus cajan Pigeon pea (Tuwar dahl)	5.1	10.4	12.4	190.0	tr	18.7	0.0
Cicer arietinum Chickpea (Bengal gram) Goya "Garbanzo"	94.3	1,420	34.2	69.3	0.0	8.1	0.0
Cicer arietinum Chickpea (Garbanzo bean)	126.0	3,080	192.0	214.0	0.0	6.7	0.0
Cicer arietinum Chickpea	215.0	838.0	11.4	76.3	5.0	8.4	0.0
Pisum sativum Pea "Green Split"	4.9	3.2	11.3	tr	0.0	9.3	nd
Pisum sativum Pea "Yellow Split"	10.0	4.4	3.7	tr	0.0	8.2	tr
Pisum sativum Pea "Green Split"	0.0	3.0	5.1	0.0	0.0	2.8	0.0
Pisum sativum Split peas (Chann dhal)	4.7	5.6	7.9	22.8	tr	12.8	nd

(continued)

Table 1 (continued)

Botanical name Common name	Formo (μg/100 g)	Bioch. A (μg/100 g)	Da (μg/100 g)	Gen (μg/100 g)	Coumestrol (μg/100 g)	SECO tot[1] (μg/100 g)	Mat (μg/100 g)
Trigonella foenumgraecum "Fenugreek"	tr	tr	10.2	9.8	0.0	8.6	tr
Vicia faba Broad bean "Diana"	6.3	tr	31.8	tr	0.0	26.0	tr
Vicia faba Broad bean "Herz Freya"	39.0	tr	15.8	tr	0.0	31.8	131.0
Vigna mungo Black gram "Smile"	2.0	0.0	6.9	tr	0.0	45.7	262.0
Vigna mungo Black gram	0.0	11.4	35.9	16.2	tr	105.0	70.8
Vigna mungo Black gram (Urid dahl)	0.0	81.1	30.3	60.3	9.5	240.0	79.4
Vigna unguiculata Cowpea (Black-eyed pea)	0.0	0.0	30.3	55.7	tr	195.0	nd
Vigna unguiculata Blackeyed bean	5.5	7.7	20.5	11.4	7.7	196.0	nd
Vigna radiata Green gram (Mung bean)	7.5	14.1	9.7	365.0	tr	172.0	nd
Arachis hypogaea Groundnut (Peanut)	6.8	6.5	49.7	82.6	0.0	333.0	tr
Lens culinaris Lentil "Jack Rabbit"	10.7	tr	10.4	18.8	0.0	12.3	tr
Lens culinaris Lentil (Masoor dahl)	7.5	7.1	3.3	7.1	6.8	8.9	nd
Pueraria lobata Kudzu (leaf)	87.0	1,240	375.0	2,520	18.1	476.0	tr
Pueraria lobata Kudzu (root) Japanese Arrowroot	7,090	1,400	185,000	12,600	1,570	30.7	tr
Sophora japonica "Japanese Pagoda Tree"	322.0	830.0	319.0	265.0	9.9	1,590	3.8

[1]Total amount of anhydrosecoisolariciresinol and secoisolariciresinol.
nd, not determinated because of low concentrations.
tr, Present in trace amounts; over 50 to 60% of detection limit.

intake of soybeans (excluding soy sauce) and excretion of ENL and total lignans; the same was found for green and yellow vegetables. Daily dietary intake of soy products (sauce excluded) by the two groups studied amounted 54 g (women) and 39 g (men) on the average. Additionally every day some 100 to 120 g of legumes and green and yellow vegetables were consumed. The excretion of MAT was very low. The excretion of its mammalian derivative END, though low, was also observed to be associated with the intake of beans and soy products in general. It is likely that the majority of the lignans in the Japanese subjects is derived from non-grain plant products, mostly legumes.

Thus, it seems that the results of the present study are in good agreement with the earlier studies relating urinary phytoestrogen excretion to diet. The low excretion of ENL in the Japanese subjects compared, e.g., with Finnish women,[62] was partly attributed to low intake of grain (whole-grain) products such as bread. Using our ID/GC/MS/SIM technique we have recently analyzed the lignan content of whole-grain products and cereals.[56] These foods

Table 2 Phytoestrogen content of forage legumes

Botanical name Common name	Formo μg/100 g	Bioch. A μg/100 g	Da μg/100 g	Gen μg/100 g	Coumestrol μg/100 g	SECO tot[1] μg/100 g	Mat μg/100 g
Lupinus mutabilis Lupin "Tarvi"	23.1	0.0	tr	2,420	0.0	3.1	0.0
Medicago spp. Smile Sprouting Alfalfa	0.0	0.0	3.7	11.5	tr	19.4	0.7
Trifolium spp. Smile sprouting clover (seed)	1,270	381.0	178.0	323.0	5.4	13.2	tr
Trifolium pratense Red clover	22,300	20,400	12,200	4,010	105.0	tr	tr

[1]Total amount of anhydrosecoisolariciresinol and secoisolariciresinol.
tr, Present in trace amounts; over 50 to 60% of detection limit.

contain SECO and MAT. Generally the concentrations of e.g. MAT determinated in cereals and grains are much higher than those in the legumes, because MAT was found in substantial amounts only in a few cultivars of black gram and one broad bean sample.

In 1995 Hutchins et al.[63] compared the effect of basal, vegetable/fruit (low and high), and legume/allium diet on urinary isoflavonoid phytoestrogen and lignan excretion in seven men and three women. The urine samples were analyzed by ID/GC/MS/SIM according to the method developed.[46] The urinary excretion of neither END, ENL, and MAT nor the sum of lignans on the legume/allium diet differed from the basal diet. This could be partly explained by low plant lignans concentrations in the legume component of this diet: garbanzo bean (*Cicer arietinum*) contains only 6.65 μg/100 g of dry weight of SECO and no MAT. On the other hand, significantly greater quantities of genistein ($P = 0.0001$), *O*-desmethylangolensin ($P = 0.04$), and the sum of isoflavonoids ($P = 0.02$) were excreted on the legume/allium diet compared with the other experimental diets. These findings could be attributed to high concentrations of isoflavonoids in the garbanzo bean (the total isoflavonoid content over 3,500 μg/100 g).

The results of our study show that the members of the Leguminosae family contain, apart from the isoflavones, the precursors of mammalian lignans, SECO, and MAT. These plant lignans as well as their mammalian metabolites ENL and END, possessing a number of biological properties[64,65] and found in relatively high concentrations in human plasma, may be candidates for a role in the prevention of cancer and other chronic diseases. Our investigation provides quantitative data which are of value for evaluation of the potential health effects of these foods.

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