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Extraction and Quantification of Lignan Phytoestrogens in Food and Human Samples

Jason Liggins, Rebecca Grimwood, and Sheila A. Bingham[1]
Dunn Human Nutrition Unit, Medical Research Council, Hills Road, Cambridge CB2 2XY, United Kingdom

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Dietary phytoestrogens have a number of biological effects, including endocrine disruption, antioxidant potential, and protein tyrosine kinase inhibition. Secoisolariciresinol, matairesinol, and shonanin are lignan phytoestrogens found in foodstuffs, especially flaxseed. Normally they are glycosidically linked to carbohydrates and in the large intestine are deconjugated from the carbohydrate portion by bacteria. The aglycone lignans can be further modified to form the mammalian phytoestrogens enterodiol, enterolactone, and enterofuran, which are absorbed into the body and excreted in urine. To assess the health implications of phytoestrogens in general populations, knowledge of the quantity in the foods eaten is necessary. This article describes a simple preparative procedure for the assay of secoisolariciresinol, matairesinol, and shonanin in foodstuffs after hydrolytic removal of any conjugated carbohydrate. The difficulties in the practical application of the assay procedure are illustrated and discussed. Analytical results indicating the concentration of secoisolariciresinol, matairesinol, and shonanin in a number of foodstuffs are presented. Also, the mass spectral data of a putative mammalian phytoestrogen, called enterofuran, identified in urine are presented. © 2000 Academic Press

Key Words: **lignan; phytoestrogen; food; urine; secoisolariciresinol; matairesinol; shonanin; enterofuran; enterodiol; enterolactone.**

Phytoestrogens are a wide variety of phenolic compounds derived from plant material which are stereochemically similar to the hormone 17-β-estradiol (E_2).[2] Phytoestrogens compete with E_2 for its receptors, but estrogen receptor β (ERβ) illustrates greater translational responses to particular phytoestrogens than ERα does to the same compounds (1–3). Phytoestrogens are thought to act both agonistically and antagonistically to the action of E_2, with possible beneficial effects in cancer and cardiovascular disease in adults, but uncertain effects in infants (4–12).

At least three types of compound are classed as phytoestrogens, including the flavonoids, coumestans, and lignans. Setchell *et al.* illustrated that the concentration of the lignans enterolactone and enterodiol in human urine was dependent on the amount of linseed consumed (13–15). Enterolactone (CAS 78473-71-9) and enterodiol (CAS 80226-00-2) (see Fig. 1, mammalian lignans) are gut bacterial metabolites, formed by modification of lignans from food after removal of carbohydrate conjugates. Because these two compounds are produced in animals as opposed to plants they are sometimes termed the mammalian lignans to distinguish them from lignans from plants. Two of the plant lignans known to form enterolactone and enterodiol upon bacterial fermentation are matairesinol (CAS 580-72-3) and secoisolariciresinol (CAS 29388-59-8), respectively (see Fig. 1), although enterodiol also rearranges to form enterolactone (16–18). All of the lignans, both mammalian and plant, occur naturally mostly as conjugates of hydrophilic compounds. Mammalian lignans as ethers of glucuronic and sulfuric acid and plant lignans glycosidically linked to a wide variety of different carbohydrates of differing chain length (17, 19–23).

In biological materials, quantitative assays of phytoestrogens are complicated by the conjugation to a variety of carbohydrates. It is unlikely that all of the possible glycosides of the target lignans are known, and, of the known examples, synthetic versions exist for only a very few. However, even if all were known and synthetic versions were available, it would be im-

[1] To whom correspondence should be addressed. Fax: +44 1223 252765. E-mail: sheila.bingham@mrc-dunn.cam.ac.uk.

[2] Abbreviations used: E_2, 17-β-estradiol; ERβ, estrogen receptor β; DHT, 5α-dihydrotestosterone; SHBG, sex hormone binding globulin; TMS, *N,O*-bis(trimethylsilyl)trifluoroacetamide; TBDMS, *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroactamide; CV, coefficient of variation.

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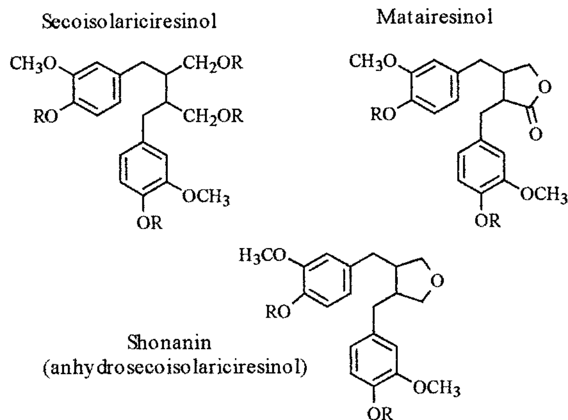
Plant lignans



Mammalian lignans

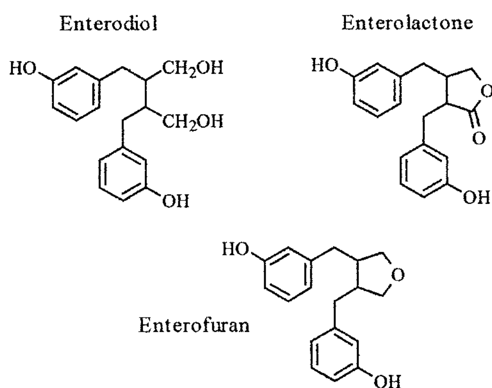


FIG. 1. Lignans of interest. The R group could be a hydrogen or a carbohydrate residue.

practical to quantify all them for more than a very few samples. A more practical approach is to remove the carbohydrate portion in the sample preparation and quantify the liberated aglycones, consolidating the analysis to a few compounds. Such an approach has been used for many years in hormone assays in blood and urine, using glucuronidase and sulfatase to remove glucuronic acid and sulfate from the hormone prior to assay. Indeed, the preparative steps of hormone assays have been adapted for the analysis of the mammalian lignans, since they likely are conjugated to glucuronic acid and sulfate in a similar manner (13–15).

The plant lignans present a greater analytical challenge than their mammalian counterparts; the variety of conjugated carbohydrates is much greater, both in terms of the monosaccharides forming the glycosidic bond and in the number and nature of further monosaccharides glycosidically linked to the first (19–23). Furthermore, both secoisolariciresinol and matairesinol have more than a single hydroxyl group, any one or more of which could be involved in glycosidic bonds to carbohydrates (see Fig. 1). This large variety of plant lignan carbohydrate conjugates reinforces the case for analysis of aglycone lignans after hydrolytic removal of the carbohydrate.

However, enzymatic approaches to the problem are complicated by the large variety of conjugates. The enzyme or collection of enzymes would have to break glycosidic bonds between the lignan and many different monosaccharides. Also the enzyme would have to overcome steric hindrance of the phenolic links on the neighboring methoxy groups and cope with glycosidic bonds to a variety of carbons on the monosaccharide ring (see Fig. 1).

Two approaches to the hydrolysis of glycosidic bonds between plant lignans and carbohydrate have been published in the literature. Both methods have been used to produce analytical results presenting the lignan concentration of a variety of foodstuffs (24, 25). The Thompson method employs anaerobic *in vitro* fermentation of food containing plant lignans with gut bacteria harvested from feces. This produces enterolactone and enterodiol from the plant lignans present in the food, the concentration of which was subsequently measured by gas chromatography and flame ion detection (24). The method is dependent on the survival of a wide variety of anaerobic bacteria from the gut, through passing of feces to culture *in vitro.* It relies on the collection of a comprehensive library of anaerobic flora, to ensure that the entire variety of the various plant lignans and their glycosidic conjugates are converted to their mammalian derivatives. This is a technically difficult task to set up initially and maintain with the same hydrolytic properties over a period of time and has not been widely utilized.

The method described by Mazur employs hot acid to break the glycosidic bonds (25). The method is a many-stepped procedure using both enzymes and hot acid to hydrolytically remove the carbohydrate component of lignan and isoflavone phytoestrogens in food (25). The steps for preparing the lignans and isoflavones for quantification by gas chromatography–mass spectrometry follow different paths. The food extract is first subjected to enzyme hydrolysis, liberating the isoflavones from their respective glycosides, but incompletely hydrolyzing the lignan glycosides, which were retained in the aqueous fraction after partitioning with organic solvent. The lignan glycosides were then hydrolyzed with hot acid and the aglycones are partitioned off with organic solvent. Both organic fractions of isoflavone and lignan aglycones are then combined, further purified, derivatized, and analyzed by gas chromatography–mass spectrometry. A problem with acid hydrolysis of natural products is their stability, Mazur *et al.* noted that one of the target plant lignans, sec-

oisolariciresinol, in acid dehydrated to a compound they called anhydrosecoisolariciresinol, which when quantified was taken to be solely derived from secoisolariciresinol (25). However, anhydrosecoisolariciresinol is chemically identical to another naturally occurring lignan called shonanin (3,4-divanillyltetrahydrofuran) (19–21, 23). Shonanin would be liberated from its glycosides alongside secoisolariciresinol upon acid hydrolysis; thus, the Mazur method actually quantifies both the naturally occurring Shonanin and what is produced from secoisolariciresinol as a result of the acid hydrolysis (25). Shonanin is, however, a significant phytochemical in its own right, competing with 5α-dihydrotestosterone (DHT) for sex hormone binding globulin (SHBG) which potentially could help prevent prostate cancer (26–28). Shonanin has the same arrangement of phenolic hydroxyl groups as secoisolariciresinol and matairesinol and as such is likely another phytoestrogen that should be quantified alongside the latter two. Furthermore, if the postingestion metabolism of shonanin is analogous to secoisolariciresinol and matairesinol, then it will likely form the compound enterofuran (see Fig. 1) which can be formed by dehydration of enterodiol; c.f. dehydration of secoisolariciresinol to shonanin (26). Enterofuran has the same arrangement of phenolic hydroxyl groups as enterolactone and enterodiol, making it a good candidate to be a mammalian phytoestrogen and should be quantified alongside the latter two compounds.

This article describes a modification of the assay developed by Mazur *et al.* simplified for the quantification of the lignans secoisolariciresinol, matairesinol, and shonanin in food after hydrolytic removal of any conjugated carbohydrate (25). The difficulties in the practical application of such an assay procedure are illustrated and discussed. Analytical results indicating the concentration of secoisolariciresinol, matairesinol, and shonanin in a number of foodstuffs are presented. Also the mass spectral data of a putative phytoestrogen, called enterofuran, identified in urine are presented.

MATERIALS AND METHODS

All enzymes, reagents, and chemicals were purchased from Sigma/Aldrich (Poole, Dorset, UK) unless otherwise stated. The food samples were purchased from local stores, freeze-dried, and milled to a fine flour. Secoisolariciresinol, secoisolariciresinol diglucoside, shonanin, matairesinol, and matairesinol glucoside were all purchased from Plantech (Reading, UK). To inhibit losses of target compounds by adsorption to glassware, all glassware was silanized in a 10% solution of dimethyldichlorosilane in heptane, followed by deactivation of excess reagent in methylated spirits and oven drying (120°C).

Food Assay Optimization

The assay procedure was optimized from pilot experiments using samples of milled flax seed (0.5 g). The concentration of hydrochloric acid, time of hydrolysis, and extraction solvent were determined by measuring maximum yield of lignans from the food. These optimal conditions were then applied to other foods.

Food Assay Procedure

The following procedure was performed in duplicate on separate occasions. To three 0.5-g samples of each freeze-dried food, 5 ml of 1.5 M hydrochloric acid was added in a screw-capped tube (15 ml) with a Teflon-coated stirring flea. The tubes were heated to 100°C and stirred on a dry block (Reacti-Therm III; Pierce & Warriner, Chester, UK) for 1, 2, and 3 h, respectively. Thereafter the samples were removed from the heat and allowed to cool for 30 min and 0.75 ml of 10 M sodium hydroxide was added to neutralize the acid. Aglycone lignans from each sample were separately partitioned from the aqueous hydrolysis mixture into 2 ml of an equal mixture of ethyl acetate and methyl *tert*-butyl ether (1:1, v/v). The organic phase was separated from the aqueous phase by centrifugation (3*g* for 3 min) followed by aspiration to a separate tube. The wash with organic solvent was repeated twice more and the three organic phases were combined in the separate tube. A total of 2 ml of the organic extract was removed from the combined 6 ml to a 1.5-ml autosampler vial in two 1-ml aliquots. The Reacti-Therm III (Pierce & Warriner) was used to evaporate the solvent from the vial, after each aliquot, by gentle heating ($<$40°C) and passing a stream of nitrogen (BOC, Guildford, UK) over the surface.

The dried samples were dissolved in a 1-ml mixture (4:1, v/v) of pyridine and *N,O*-bis(trimethylsilyl)trifllouroacetamide with 1% trimethylchlorosilane. Two micrograms of anthraflavic acid was included in the pyridine, to correct for differences in instrument response between injections. The vials were sealed and heated to 60°C for 1 h to facilitate the formation of trimethylsilyl derivatives of the lignans. Calibration standards of known quantities of secoisolariciresinol, shonanin and matairesinol were derivatized in an identical manner. One microliter of the derivatized samples or standards was injected into a gas chromatograph–mass spectrometer (GC–MS, MD800 or GCQ Thermoquest Ltd., Hemel Hempstead, UK). The GC–MS parameters (oven program, chromatographic retention times, and mass ions) were obtained using the pure synthetic lignan standards. The injection port was operated in the splitless mode at 280°C and lined with silanized glass. The capillary column (15 m long, 0.25 mm internal diameter) was lined with 1 μm of 100% polysiloxane (Hall Analytical Laboratories Ltd.,

Manchester, UK). The initial oven temperature was 180°C; after 5 min this was ramped at 15°C per minute to 320°C. The mass spectrometer was operated in its selected ion mode; the ions chosen were determined using pure samples of the lignans. The ions (mass to charge ratio) selected for each compound were as follows: anthraflavic acid, 369.2 and 384.3; secoisolariciresinol, 560.3 and 470.3; shonanin, 488.3 and 179.1; matairesinol, 502.1 and 487.0. The lignans in the samples were identified and quantified by comparison with known quantities of the reference standards analyzed in the same run. The authenticity of a lignan from a sample was determined by a combination of its chromatographic retention time and a ratio of its two mass ions, quantification was based on the area of one of the mass ions divided by that of anthraflavic acid calculated against a calibration curve formed from the results of the known quantities of reference standards. Gas chromatographic and mass spectral data were similar to those already published and are not replicated here (24, 25).

Enterofuran

Artificial enterofuran was formed according to the method of Schottner *et al.* by dehydration of enterodiol. Enterodiol was heated to 100°C for 30 min in 1.5 M HCl; c.f. dehydration of secoisolariciresinol to shonanin (26). Following dehydration the product was derivatized with either *N,O*-bis(trimethylsilyl)trifluoroacetamide (TMS) containing 1% TMS chloride catalyst or *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroactamide (TBDMS) containing 1% TBDMS chloride catalyst, in pyridine. The derivatized enterofuran was injected (1 μl) into the GCQ GC–MS, using the GC conditions described above. The mass spectrometer was operated in the full-scan mode. Urine was assayed for phytoestrogens using an adaptation of the method described previously (29). To 0.5 ml of urine was added 0.5 ml of 0.2 M acetate buffer (pH 5.0) containing approximately 140 units of β-glucuronidase (10 μl of *Helix pomatia* extract HP-2). After vortexing, hydrolysis of the glycosidic bonds was achieved by overnight incubation at 37°C in a shaking water bath. Aglycone phytoestrogens were extracted from the aqueous solution by partitioning with ethyl acetate (three 0.5 ml washes). The ethyl acetate fractions were combined, dried under a stream of nitrogen using a little heat (<40°C), ready for derivatization as described above. The GC–MS conditions used were the same as those for the synthetic enterofuran. The enterofuran in the urine sample was identified by comparison with the major compound produced by dehydration of enterodiol and the mass spectral information provided by Schottner *et al.* (26).

RESULTS

A range of test foods containing a variety of lignan concentrations was formed by blending bleached white wheat flour with milled flaxseed in different but known proportions. The concentration of lignans in the test foods was calculated from both the amount in the two pure foods and the proportion of each in the blend, but also assayed using the conditions optimized for pure milled flaxseed (3 h of acid hydrolysis). Figure 2 compares the assayed concentration of lignans in the test foods with the calculated as both regression and Bland and Altman plots (30). The high correlation coefficient relating the assayed results to the calculated indicates that the assay procedure yields a similar proportion of lignans from foods containing a wide concentration range of the target compounds. This observation is supported by the Bland and Altman analysis of the same results (Figs. 2C and 2D). The lack of a trend in the least-squares fit and the grouping of the data around the *y* axis zero indicate that any errors related to the magnitude of the measurement, or errors that remain constant no matter what the concentration, are small. The average interassay coefficient of variation (CV) across the whole concentration range assayed was 6.0% for secoisolariciresinol and shonanin, and 6.1% for matairesinol. The range of interassay CV was from 2 to 12% (high to low concentration) for the former two compounds and 2 to 15% for matairesinol.

Application of the assay procedure, optimized for milled flaxseed, to other foods and synthetic glucosides of the lignans indicated that the optimal time of hydrolysis varied markedly between different foods. Figure 3 illustrates the yield of secoisolariciresinol and shonanin combined, from secoisolariciresinol diglucoside together with yields from four foodstuffs containing different concentrations of lignan following hydrolysis over a variety of times (note that similar results were obtained for matairesinol, data not shown). The results indicate that the matrix in which the lignans naturally occur has a significant effect on the optimal time of hydrolysis. Synthetic secoisolariciresinol diglucoside, even in the presence of bleached white wheat flour, was rapidly hydrolyzed by the acid to its aglycone and shonanin.

Acid dehydration of enterodiol, by acid, largely formed a compound which upon derivatization with TMS gave mass ions similar to those reported for enterofuran (26). Derivatization of the enterofuran product with TBDMS produced a chromatographic peak with the same retention time and mass ions as detected in urine. Figure 4 illustrates the typical mass spectra of that chromatographic peak. The two main mass ions illustrated in Fig. 4 (456 and 399) indicate that this peak could be enterofuran. The mass to charge ratio (m/z) of 456 could have been formed from

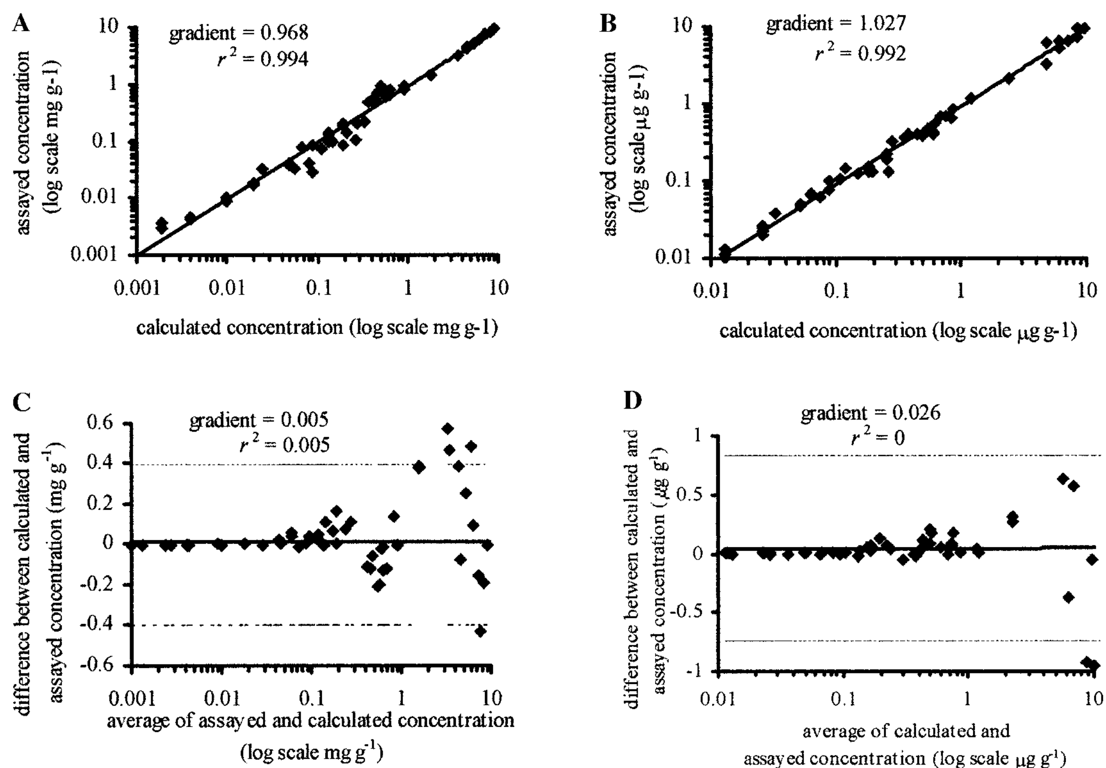


FIG. 2. Recoveries of lignans in a variety of blends of milled linseed and bleached white wheat flour, gradients, and correlation coefficients (r^2) are shown. A and B are regression analyses of assays for secoisolariciresinol and shonanin combined and matairesinol, respectively. C and D are Bland and Altman plots of the data in A and B, respectively; 95% limits of agreement are plotted with hashed lines (30). Each test food was prepared by accurately weighing each of the two pure ingredients into the extraction vessel. The concentration expected to be in the blend was calculated from that of the two flours and the relative proportion of the combination.

the loss of *tert*-butyl group (*m/z* 57) from the molecular ion of fully derivatized enterofuran (*m/z* 513). Similarly *m/z* 399 could have formed from the loss of the other *tert*-butyl group, or alternatively through derivatization of only one of the two hydroxyl groups on enterofuran (see Fig. 1). Confirmation of the compound identified in urine as enterofuran was sought by spiking the urine with the dehydration product of enterodiol. The chromatographic peak identified as enterofuran altered in height upon spiking but not shape; similarly, the mass spectra of the peak only changed in terms of the intensity of the ions.

Seventeen foodstuffs were assayed for their lignan content using the assay procedure described above. Table 1 illustrates the content as the combined concentration of secoisolariciresinol and shonanin, alongside the concentration of matairesinol. The foods are ranked in descending order of lignan concentration. The results illustrated in Fig. 3 indicate that the optimum time of hydrolysis for maximum yield of lignans varies between foodstuffs. Therefore, for each food three concentrations of each compound are reported; the yield after 1, 2, and 3 h of hydrolysis. All of the foods analyzed contained the target lignans. The two linseed samples contained the highest concentrations; the concentration in the next richest food (soy products) was approximately 50-fold lower. The other foods contained lower concentrations, but all could contribute dietary lignans that could have a significant effect on health.

DISCUSSION

Enterolactone and enterodiol were identified in the early 1980s as two peaks on gas chromatograms of human blood and urine (13–15). However, other lignans possessing the two phenolic groups common to the phytoestrogens could also be phytoestrogens. Schottner *et al.* described the preparation of enterofuran from enterodiol and its mass spectra (26). Enterofuran is an example of a lignan that is likely a phytoestrogen, in that it possesses the same arrangement of phenolic groups as enterolactone and enterodiol. The gas chromatographic retention time and mass spectra of enterofuran were characteristic of a previously unidentified peak in urine (Fig. 4). However, positive identification of the peak observed in urine samples as enterofuran awaits the synthesis of a pure reference sample of the compound. Samples of synthetic entero-

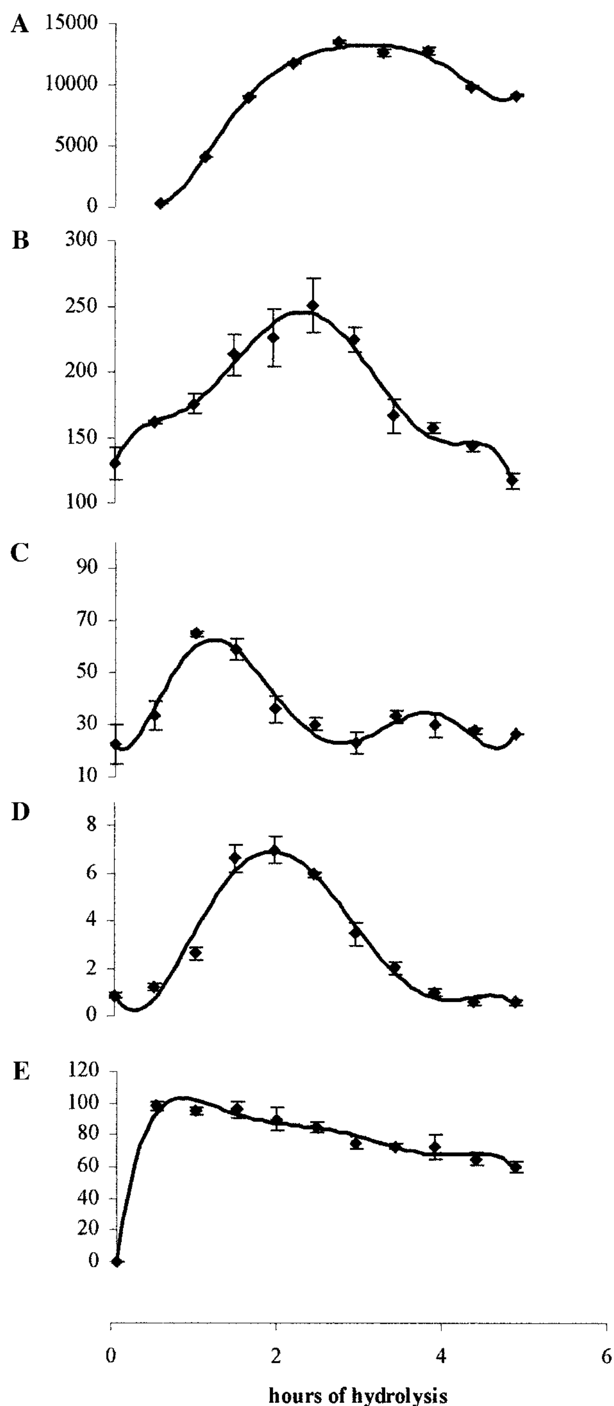


FIG. 3. Yield of secoisolariciresinol and shonanin over time upon acid hydrolysis (1.5 M HCl) of linseed (A), soya flour (B), asparagus (C), and runner beans (D). The units plotted on the *y* axes of A to D represent the combined concentration of secoisolariciresinol and shonanin in micrograms per gram of dried food. The *y* axis of E is plotted as percentage recovery of synthetic secoisolariciresinol diglucoside spiked into bleached white wheat flour.

furan would allow investigation of the estrogenic properties of the compound and its quantification in human samples.

Currently secoisolariciresinol and matairesinol are the most widely known plant lignan phytoestrogens, likely because they are transformed into enterodiol and enterolactone, upon the action of gut bacteria. However, there are many potential lignans that could undergo the removal of hydroxyl groups, conversion of methoxy groups to hydroxyls, and form enterolactone and enterodiol. Furthermore, other lignans with phenolic structures similar to secoisolariciresinol and matairesinol, but with different central structures (e.g., shonanin), could be transformed by gut bacteria into mammalian phytoestrogens similar to enterodiol and enterolactone (e.g., enterofuran). Future research investigating the health effects of lignan phytoestrogens should not preclude the contribution of other lignans, including those that have not yet been identified as endocrine disruptors.

The assay procedure reported in this article has been used to report in Table 1 the concentration of plant lignans in foods which, although are not representative samples, covered a wide concentration range of lignans and constituted a variety of different matrices. The

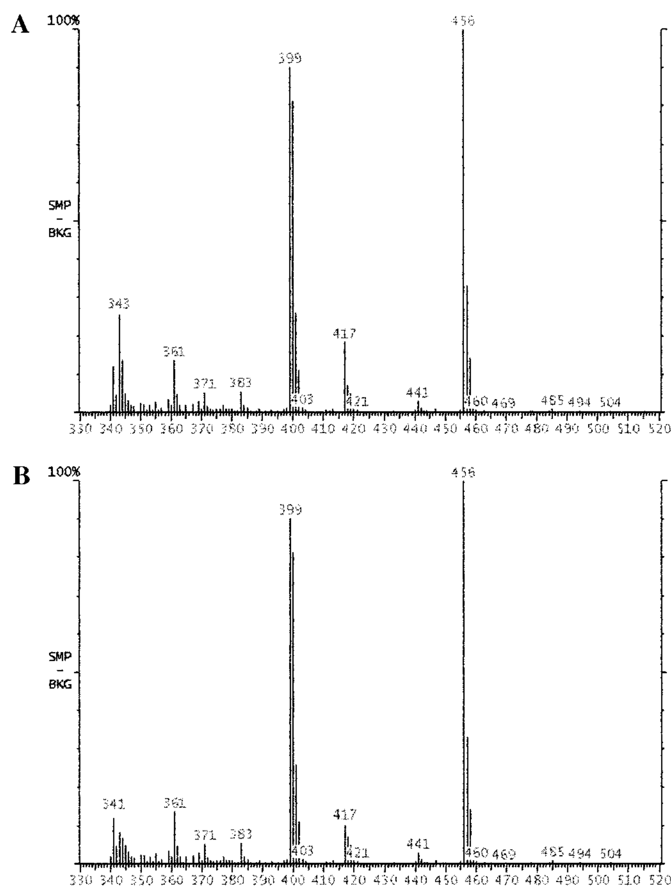


FIG. 4. Mass spectra (EI, background subtracted) of TBDMS ether of enterofuran produced artificially from enterolactone (A) and identified in a urine sample (B).

TABLE 1

Concentration of Lignans Determined to Be in Reference Foods, Used within This Laboratory

Food	Secoisolariciresinol and shonanin			Matairesinol		
	1 h	2 h	3 h	1 h	2 h	3 h
Cambridge linseed	4160	11836	12617	18.5	24.6	58.6
Argentinian linseed	6750	8800	7400	30.0	40.2	90.9
Soy flour	175	226	224	nd	nd	nd
Textured vegetable protein	238	270	247	nd	nd	nd
Coffee beans	108	113	85.5	nd	nd	nd
Asparagus	65.1	36.0	23.3	1.3	1.4	1.0
Brown wheat flour	2.4	2.9	3.8	53.7	89.5	80.5
Tea leaves, dried	26.5	52.5	48.7	0.3	1.8	1.6
Black turtle beans	28.6	30.5	23.5	1.0	1.2	1.2
Courgette	26.8	19.4	10.6	nd	nd	nd
Rye flour	6.3	7.2	5.4	0.9	1.6	1.7
Runner beans	2.6	7.0	3.5	nd	nd	nd
White wheat flour	2.4	2.7	2.8	nd	2.7	3.2
Brown rice	2.9	4.0	3.3	nd	nd	nd
Wild rice	2.6	3.3	2.9	1.3	1.3	0.6
Red rice	3.7	3.9	3.4	1.5	1.4	1.6
American long grain rice	0.4	0.7	0.6	0.9	1.2	1.0

Note. Units are milligrams of lignan(s) per kilogram of dried food and the abbreviation "nd" indicates that the compound was not detected.

results in Table 1 are reported as three concentrations of analyte, secoisolariciresinol, and shonanin combined because after acid hydrolysis the contribution of both is indistinguishable and matairesinol. The results represent the concentration determined after 1, 2, and 3 h of hydrolysis.

The assay reported in this article was developed from one reported by Mazur *et al.* which has been used to produce data indicating the lignan concentration of foods (25). The assay used in this article used fewer steps for the samples prior to analysis than the Mazur method, which led to decreased losses of the target compounds as assessed using the yields of the foods and synthetic secoisolariciresinol diglucoside and matairesinol glucoside (25). However, both methods use acid hydrolysis and assume that the acid hydrolysis liberates all of the lignans in the food from their respective glycosides and the compounds do not significantly degrade after liberation.

Experiments hydrolyzing known quantities of synthetic glucosides of secoisolariciresinol and matairesinol illustrated that in the absence of the food matrix the aglycones were liberated from these glycosides in 20 to 30 min of heating the 1.5 M acid to 100°C (results not shown). Furthermore, with the exception of the degradation of secoisolariciresinol to shonanin, both matairesinol and shonanin were relatively stable in hot acid, with 80% or more remaining after 5 h in 2 M HCl (results not shown). However, the hydrolysis of food, containing the lignans in question (Fig. 3), indicated first that the food matrix had a significant effect on the optimum time of hydrolysis for maximum yield of aglycone lignans. Second, Fig. 3 shows that the yield did not plateau, but that the concentration decreased with further hydrolysis. Concentrations of lignans in foods using the Mazur method, where 2.5 h of hydrolysis was used for different foods, would therefore give different results. The concentration reported would not be the total amount of lignans available in that food, merely the concentration liberated after 2.5 h. Due to the absence of a reference method, and pure reference standards for all possible lignan glycosides, it was not possible to validate the method reported here entirely. In their absence, the correct amount in a particular food remains uncertain.

The assumption that the maximum yield of lignans from a food upon acid hydrolysis is the same as the amount that would be liberated from that food upon ingestion is also imperfect. Acid hydrolysis may liberate more aglycone lignans from a given food than the bacteria in human gut, or the assay may underestimate the amount because of lack of hydrolysis or alternatively degradation of the target compounds by the acid. Additionally, the mammalian lignans produced in the human gut may be formed from not only the known plant lignans but also others yet to be identified. The results reported here are therefore not comparable with results from the Thompson method, which utilizes anaerobic fermentation of the food with bacteria harvested from human feces, to produce enterolactone and enterodiol (24). Nevertheless, until more definitive assays become available for quantification of the phytoestrogen lignans that are available for absorption upon ingestion, the estimates made by the assays de-

veloped to date by both other authors and ourselves remain the sole source of information regarding the lignan content of food (24, 25).

REFERENCES

1. Couse, J. F., Lindzey, J., Grandien, K., Gustafsson, J. A., and Korach, K. S. (1997) *Endocrinology* **138,** 4613–4621.
2. Arts, J., Kuiper, G., Janssen, J., Gustafsson, J. A., Lowik, C., Pols, H. A. P., and VanLeeuwen, J. (1997) *Endocrinology* **138,** 5067–5070.
3. Kuiper, G., Lemmen, J. G., Carlsson, B., Corton, J. C., Safe, S. H., VanderSaag, P. T., VanderBurg, P., and Gustafsson, J. A. (1998) *Endocrinology* **139,** 4252–4263.
4. Wagner, J. D., Cefalu, W. T., Anthony, M. S., Litwak, K. N., Zhang, L., and Clarkson, T. B. (1997) *Metab. Clin. Exp.* **46,** 698–705.
5. Nevala, R., Korpela, R., and Vapaatalo, H. (1998) *Life Sci.* **63,** L95–PL100.
6. Webb, C. M., Rosano, G. M. C., and Collins, P. (1998) *Lancet* **351,** 1556–1557.
7. Cassidy, A., and Griffin, B. (1999) *Proc. Nutr. Soc.* **58,** 193–199.
8. Ghisalberti, E. L. (1997) *Phytomedicine* **4,** 151–166.
9. Murkies, A. L., Wilcox, G., and Davis, S. R. (1998) *J. Clin. Endocrinol. Metab.* **83,** 297–303.
10. Bingham, S. A., Atkinson, C., Liggins, J., Bluck, L., and Coward, A. (1998) *Br. J. Nutr.* **79,** 393–406.
11. Tham, D. M., Gardner, C. D., and Haskell, W. L. (1998) *J. Clin. Endocrinol. Metab.* **83,** 2223–2235.
12. Kurzer, M. S., and Xu, X. (1997) *Annu. Rev. Nutr.* **17,** 353–381.
13. Setchell, K. D. R., Lawson, A. M., Mitchell, F. L., Adlercreutz, H., Kirk, D. N., and Axelson, M. (1980) *Nature* **287,** 740–742.
14. Stitch, S. R., Toumba, J. K., Groen, M. B., Funke, C. W., Leemhuis, J., Vink, J., and Woods, G. F. (1980) *Nature* **287,** 738–740.
15. Setchell, K. D. R., Lawson, A. M., Borrielo, S. P., Harkness, R., Gordon, H., Morgan, D. M. L., Kirk, D. N., Adlercreutz, H., Anderson, M., and Axelson, M. (1981) *Lancet* **i.**
16. Axelson, M., and Setchell, K. D. R. (1981) *FEBS Lett.* **123,** 337–342.
17. Setchell, K. D. R., Lawson, A. M., Conway, E., Taylor, N. F., Kirk, D. N., Cooley, G., Farrant, R. D., Wynn, S., and Axelson, M. (1981) *Biochem. J.* **197,** 447–458.
18. Axelson, M., Sjovall, J., Gustafsson, B. E., and Setchell, K. D. R. (1982) *Nature* **298,** 659–660.
19. Ward, R. S. (1993) *Nat. Prod. Rep.* **10,** 1–28.
20. Ward, R. S. (1995) *Nat. Prod. Rep.* **12,** 183–205.
21. Ward, R. S. (1997) *Nat. Prod. Rep.* **14,** 43–74.
22. Nagatsu, A., Zhang, H. L., Watanabe, T., Taniguchi, N., Hatano, K., Mizukami, H., and Sakakibara, J. (1998) *Chem. Pharm. Bull.* **46,** 1044–1047.
23. Ward, R. S. (1999) *Nat. Prod. Rep.* **16,** 75–96.
24. Thompson, L. U., *et al.* (1991) *Nutr. Cancer* **16,** 43–52.
25. Mazur, W. M., Fotsis, T., Wahala, K., Ojala, S., Salakka, A., and Adlercreutz, H. (1996) *Anal. Biochem.* **233,** 169–180.
26. Schottner, M., Gansser, D., and Spiteller, G. (1997) *Plant. Med.* **63,** 529–532.
27. Schottner, M., Gansser, D., and Spiteller, G. (1997) *Zeitschrift Fur Naturforschung C-a J. Biosci.* **52,** 834–843.
28. Schottner, M., Spiteller, G., and Gansser, D. (1998) *J. Nat. Prod.* **61,** 119–121.
29. Liggins, J., Bluck, L. J. C., Coward, W. A., and Bingham, S. A. (1998) *Anal. Biochem.* **264,** 1–7.
30. Bland, J. M., and Altman, D. G. (1995) *Lancet* **346,** 1085–1087.