JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quantification of Lignans in Food Using Isotope Dilution Gas Chromatography/Mass Spectrometry

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The optimization and validation of a protocol for the quantification of six dietary lignans, i.e., secoisolariciresinol, matairesinol, lariciresinol, pinoresinol, medioresinol, and syringaresinol, in food are presented. The method incorporates isotope dilution to ensure correct accuracy and precision, introducing the utilization of individual stable $^{13}C_3$-labeled lignans. To demonstrate the potential of this new method, preliminary results of the levels of dietary lignans in selected foods are presented. It is concluded that the method fulfils the reliability criteria and can be applied to the analysis of the most common lignans in human food, being an essential asset to establish the intake of lignans in a determined population and their relation with disease prevention.

KEYWORDS: Lignans; isotope dilution; food; phytoestrogens; secoisolariciresinol; matairesinol; lariciresinol; pinoresinol; medioresinol; syringaresinol

INTRODUCTION

The lignans form a widely distributed group of plant phenols. There has been a growing interest in these compounds first supported by their prescription as traditional remedies in many cultures (*1*) and further expanded after the identification of the lignans enterodiol and enterolactone (enterolignans) in humans and animals and the establishment of their dietary origin (*2*, *3*). The increasing interest on the possible physiological effects of enterolactone (*4*), resulted in extensive efforts to determine the concentration of its precursors in food. Following the study of Thompson et al. (*5*) in which flaxseed was identified as the richest source of enterolignan precursors, Mazur and co-workers published the first quantitative method for dietary lignans (*6*) complemented with a comprehensive food lignan database (*7*, *8*) based on the primarily reported lignans secoisolariciresinol and matairesinol (*3*). The lignans isolariciresinol and pinoresinol were soon after identified as minor lignans in flaxseed (*9*, *10*), and, moreover, it was noticed that the human excretion of enterolignans after rye bread, a rich source of lignans, supplementation was higher than expected considering the consumed amount of secoisolariciresinol and matairesinol, indicating the presence of additional enterolignan precursors in rye (*11*). Pinoresinol, lariciresinol, 7′-hydroxymatairesinol, syringaresinol, and arctigenin were finally found to be completely or partially converted to enterolactone using in vitro fermentation (*11*), and new challenges regarding their quantification in food items emerged.

A number of different approaches have been used to quantify secoisolariciresinol and matairesinol in flaxseed and other food items (*12*−*17*). Since the identification of the new dietary lignans, we have used different variations of the protocol of Mazur et al. (*6*) to determine their concentrations in selected foods, such as whole grain rye and rye fractions (*18*), rye- and wheat-based animal diets (*19*), rye and wheat bran (*20*), soybeans and soy-derived products (*21*), and more recently sesame seeds (*22*). Routine analysis of large batches of samples using this method has been avoided since both the original and the adapted methods involved acid hydrolyses during the sample pretreatment that have been identified as a source of variation by inducing the degradation of certain lignans (*22*). The recent appearance of reports with improved extraction procedures involving alkaline extraction (*14*, *17*, *23*), served as the basis to develop a simple and reliable method based on the utilization of the isotope dilution technique for the quantification of the most common dietary lignans in foods. In this method, stable $^{13}C_3$-labeled standards are added to the samples prior to pretreatment and therefore possible losses are corrected in any situation, avoiding previous concerns regarding the variable recovery results depending on the type of food matrix. The method is meant to be used on a routine basis and to create updated food lignan databases.

MATERIAL AND METHODS

Reference Compounds. Individual lignan standards and their correspondent $^{13}C_3$-labeled (>99.7% isotopic purity) surrogates were used to perform isotope-dilution quantification. All standards were synthesized by Dr. Botting's group at the School of Chemistry, University of St. Andrews, U.K. The utilization of $^{13}C_3$-Seco*1* and $^{13}C_3$-

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10.1021/jf051488w CCC: $30.25
Published on Web 10/26/2005

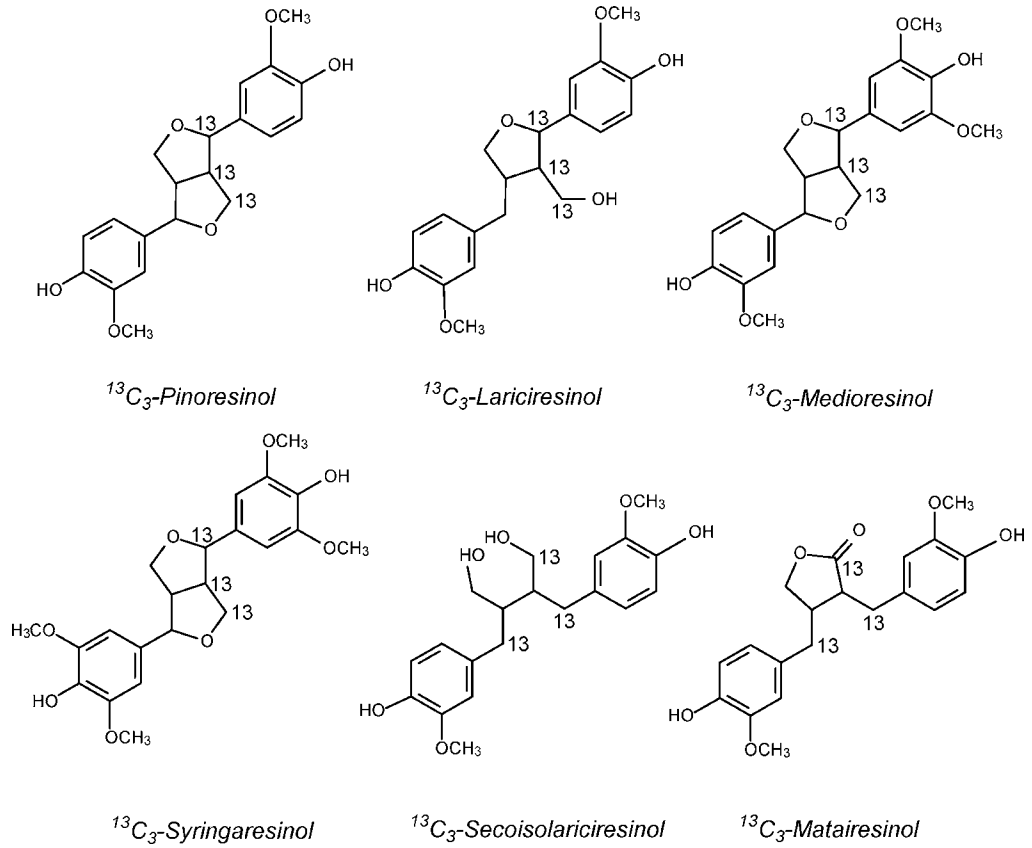

Figure 1. Structures of the $^{13}C_3$-labeled lignans used for quantification.

Mat has been described before (*24*, *25*). The newly synthesized internal standards $^{13}C_3$-Pin, $^{13}C_3$-Syr, $^{13}C_3$-Med, and $^{13}C_3$-Lar were prepared by the radical cyclization method described by Roy and co-workers (*26*) using triply labeled cinnamyl alcohols. The synthesis of the cinnamyl alcohols started from the aromatic halides, and the first of the three ^{13}C atoms was inserted by an aromatic cyanation reaction using ^{13}C-labeled potassium cyanide. The benzonitrile was reduced to the aldehyde, which was further reacted with the Wittig reagent prepared from ethyl [$^{13}C_2$]bromoacetate and triphenylphosphine to add the other two ^{13}C atoms. Reduction of the cinnamic ester gave the corresponding cinnamyl alcohol, which was brominated using PBr_3. To construct the full lignan framework, the ^{13}C-labeled cinnamyl bromide was coupled with an aromatic epoxy alcohol via Williamson ether synthesis. Finally, cyclization using a radical-mediated reaction gave the required furofuran structure. Deprotection of the phenolic groups provided the naturally occurring lignans. The chemical structures of the $^{13}C_3$-labeled lignans are presented in **Figure 1**.

Sample Pretreatment. Prior to sample extraction and purification, a mixture of the $^{13}C_3$-labeled lignan surrogates in methanol (5 μL, amounts ranging from 11.9 ng for Med to 16.1 ng for Pin) were added to each sample to further perform isotope-dilution quantification. For extraction, the protocols of Mazur et al. (*6*) and Milder et al. (*23*) were modified as follows: to 50 mg of sample 1.2 mL of 70% methanol containing 0.3 M NaOH was added, and after incubation at 60 °C for 1 h, the samples were cooled to room temperature and pH was adjusted to 5−6 using glacial acetic acid. Following centrifugation, the residue was washed with 0.5 mL of methanol, and supernatants were pooled and evaporated to dryness under N_2 stream. Hydrolysis of the glycoside forms was carried out by adding to the tubes 2 mL of 0.05 M sodium acetate buffer, pH 5, containing 0.83 mg/mL (equivalent to 374 FU/mL) of β-glucuronidase from purified *Helix pomatia* (*H. pomatia*; Product No G0751, lot 093K1166, Sigma-Aldrich, Steinheim, Germany) and further incubation overnight at 37 °C. The sample extracts were then applied to preconditioned (6 mL of methanol followed by 10 mL of water) C18-cartridges (Waters, Milford, MA). After washing with 5 mL of water, the lignans were recovered with 3 mL of methanol. Sample extracts were again evaporated, redissolved in 500 μL of methanol, and submitted to purification by ion exchange chromatography using DEAE-Sephadex (Pharmacia Biotech AB, Uppsala, Sweden) in the hydroxyl form (*6*). The gel was prepared in methanol and packed into a Pasteur pipet (0.5 × 1.5 cm). Once the sample was applied, the column was washed with methanol and subsequently lignans were eluted with 5 mL of 0.1 M acetic acid in methanol. After evaporation to dryness the sample extracts were derivatized using the silylation mixture pyridine/HMDS/TMCS (9:3:1) to be subsequently injected into the chromatographic system. If not specified, all reagents were from major suppliers and with the highest purity available.

Gas Chromatography/Mass Spectrometry. Instrument consisted of a Fisons GC 8000 chromatograph coupled to a Fisons MD 1000 quadrupole mass spectrometer (Fisons Instrumentation, Inc., Milan, Italy). A capillary column BP-1 (12 m × 0.22 mm i.d., 0.25 mm) (SGE International Pty Ltd., Ringwood, Australia) was used for separation. Setting conditions were 1 mL/min for the flow rate of the carrier gas (He), the injection volume of 1 μL, and program temperature as follows: 150 °C (for 1 min) increased at 40 °C/min to 240 °C, increased at 3 °C/min to 280 °C and kept for 1 min, and finally increased at 10 °C/min to 290 °C and kept for 8 min. The temperature of the injection port was 290 °C, and the ion source and interface were kept at 200 and 250 °C, respectively. The ionization mode was EI+ (70 V), and the detection was performed in the SIM mode.

Instrument Calibration. Calibration curves were prepared by injecting known ascendant concentrations of a mixture of analyte standards with constant concentrations of the internal standards (ca. 3 μg/mL). Linear calibration curves were obtained by plotting the detector response as the area ratio of standard versus $^{13}C_3$-labeled standard against concentrations. Ten concentration levels ranging between 0.025 and 5 μg/mL were obtained in each assay and used for quantification of the reagent blank, and samples and controls. All measurements were performed in duplicate. The repeatability of the instrument was studied in intra- ($N = 10$) and interassay ($N = 5$) experiments by calculation of the variation in the retention times of individual compounds on the chromatographic separation as well as the variation in the detector response. Sensitivity was studied by defining the limits of detection (LODs) for individual compounds, calculated empirically by analyzing

increasing dilutions of the standard solutions and defined as a signal-to-noise ratio of 3:1. Limits of quantification (LOQs) were defined as 5× the LODs.

Precision. Two control samples consisting of millet (low control) and rye bran (high control) were selected on the basis of their different lignan content to study the precision of the method at both low and high lignan concentrations, defined as the variation of the individual values after 10 replicates in a single analysis (intraassay) or during five consecutive duplicate analyses (interassay) and expressing it again as coefficients of variation.

Accuracy. Standard addition method was used to estimate the losses of the lignans attributable to the method. Appropriate aliquots of the lignan standard mixture were added to a lignan-free matrix consisting of refined wheat flour to perform three levels of fortification corresponding approximately to the average LOQ (level 1.60 μg/(100 g), dry basis), 5×LOQ (level 2.300 μg/(100 g), dry basis), and 25×LOQ (level 3, 1500 μg/(100 g), dry basis). No isotope dilution was carried out, and therefore results represent raw recoveries that will be automatically corrected when the complete method is applied.

Experimental Applicability. Different food samples were selected to test the applicability of the method to real samples. Samples were purchased at local markets and include cereals, vegetables, and fruits. The samples were lyophilized (HETO-FD3, Heto Holten, Allerød, Denmark), homogenized (A11 Basic Homogenizer, Ika−Werke Gmbh & Co. KG, Staufen, Germany), and stored at constant temperature (8 °C) and humidity until needed.

RESULTS AND DISCUSSION

In this report we present a unique method to measure dietary lignans in food that will constitute the basis for the creation of complete food lignan databases. The analysis of phytochemicals in food is always challenging due to the great variety of matrix characteristics. In the case of lignans, the unknown conjugation pattern in each food increases this variability, making the creation and validation of a universal method for all lignans in all foods very complicated. Due to the inherent sensitivity and selectivity of the technique, mass spectrometry is the detection method of choice for this type of analysis. It also permits the utilization of isotope-labeled compounds as internal standards to correct for possible losses during the sample pretreatment. In previous mass spectrometry-based assays, deuterated surrogates have been applied to GC-MS analysis (*6*, *23*); however, it has been reported that deuterated standards can suffer from poor stability, which limits their use (*27*, *28*). Triply ^{13}C-labeled standards have been used before for the quantification of isoflavones and enterolignans in serum using LC-MS/MS (*24*) and urine using GC-MS (*25*) and proven to be stabile. This stability permits their introduction prior to sample extraction, and therefore the correction of any possible loss of the compounds of interest independently of the type of food matrix involved. However, the extent of the hydrolysis of the conjugates cannot be determined when adding unconjugated labeled internal standards to the samples, and this aspect has to be optimized by maximizing the yield of lignans from the food matrix. In the method of Milder et al. (*23*), deuterated Seco and Mat are used as internal standards for correct accuracy in the quantification of Seco, Mat, Lar, and Pin, but unfortunately the internal standards are described to be added only prior to injection into the chromatographic system and possible losses during sample pretreatment are, therefore, not considered. Nevertheless, the authors reported extensive work for the optimization of the sample pretreatment conditions to maximize the yield of dietary lignans from different food matrixes. The comparison of the proposed alkaline hydrolysis-based method with our previously reported acid hydrolysis-based method (*21*, *22*) showed similar efficiency in the extraction of lignans from our selected control samples, but, more importantly, previously reported degradations from Lar to isolariciresinol and Seco to anhydrosecoisolariciresinol (*10*, *22*) were not observed with this type of treatment, confirming the idea that these two lignans are not present in food and are only the result of the acidic conditions. The absence of lignan conversions significantly improved the precision of the method, and therefore we decided to adopt the combination of methanolic extraction and alkaline hydrolysis as the step prior to enzymatic hydrolysis. Other modifications were also introduced to adapt and improve the methodology. The amount of sample was reduced and proportionally was the volume of the alkaline reagent, avoiding large volumes of solvents. Commercially available purified *H. pomatia* was used as the source of enzymes for the hydrolysis of lignan glucosides, avoiding extra purification steps (*6*). After incubation, the released aglycones were extracted by means of C18-cartridges that were as effective as the previously reported liquid extraction with diethyl ether, improving the extraction step with additional cleanup of the samples. Subsequent purification was performed by ion exchange chromatography with DEAE-Sephadex in the hydroxyl form (*6*). The combination of C18-cartridges and DEAE-Sephadex was proven sufficient for the purification of the extracts prior to GC-MS. By combining alkaline hydrolysis and the above steps, the number of purifications from our previous methods (*6*, *21*) was considerably reduced.

Specificity, repeatability and sensitivity were the parameters selected to validate the method; these are especially important for a routine method such as the one reported here. Specificity is assured by the utilization of the SIM mode using the ion pairs described in **Table 1**. In the same table it can be observed the low variation in the detector response both in intra- and interassay experiments, as well as the minimum variation in the chromatographic separation. The method sensitivity was found to be appropriate for the analysis of lignans in different types of food. LODs varied from 3.08 pg on column (6.16 μg/(100 g), dry basis) for Seco to 8.02 pg on column (16.0 μg/(100 g), dry basis) for Pin (**Table 2**). These results are slightly

Table 1. Chromatographic Parameters

	separation params		detection params	
lignan	RT (min)	RT intra-[a]/interassay[b] (CV)	selected ions $M^+/M+3$	detector response[c] intra-[a]/interassay[b] (CV)
Seco	11.00	0.13/0.29	560.4/563.4	2.12 /15.0
Mat	12.13	0.16/0.30	502.4/505.4	1.09 /10.6
Lar	13.49	0.14/0.27	486.4/489.4	0.55 /14.3
Pin	14.71	0.10/0.30	502.4/505.4	0.58 /8.89
Med	16.81	0.10/0.24	532.4/535.4	0.48 /7.88
Syr	18.93	0.06/0.23	562.4/565.4	0.50 /9.82

[a] Intraassay variation ($N = 10$). [b] Interassay variation ($N = 5$). [c] Isotope dilution ratio (standard area/surrogate area).

Table 2. Quantification Parameters

	LOD[a]		LOQ[b]	linearity range	
lignan	pg on column	μg/(100 g), dry basis	μg/(100 g), dry basis	pg on column	r^2
Seco	3.08	6.16	30.8	4920	0.9993
Mat	6.13	12.26	61.3	4900	0.9991
Lar	6.46	12.92	64.6	5150	0.9984
Pin	8.02	16.04	80.2	6400	0.9977
Med	5.97	11.94	59.7	4775	0.9986
Syr	6.44	12.88	64.4	5150	0.9984

[a] Limits of detection, studied on column and estimated in food. [b] Limits of quantification, estimated as 5 × LODs.

Table 3. Precision of the Method

control[a]	lignan	concn[b] (μg/(100 g))	intraassay (CV)[c]	interassay (CV)[d]
low	Seco	67.5 ± 2.26	8.39	10.22
	Mat	2.72 ± 0.11	4.12	10.75
	Lar	19.9 ± 0.69	2.95	10.85
	Pin	85.8 ± 1.70	9.66	4.25
	Med	7.97 ± 0.21	8.62	7.02
	Syr	62.7 ± 2.84	10.72	11.08
high	Seco	130 ± 2.98	8.11	7.23
	Mat	131 ± 4.21	6.83	10.10
	Lar	786 ± 16.3	4.27	6.56
	Pin	778 ± 20.9	8.33	8.50
	Med	381 ± 7.10	7.03	5.89
	Syr	3800 ± 108	13.44	9.02

[a] Control sample, low = millet, high = rye bran. [b] Concentrations in the control samples, mean values ± SEM ($N = 10$). [c] Intraassay variation ($N = 10$). [d] Interassay variation ($N = 5$).

Table 4. Evaluation of the Losses of Lignan Standards during the Method by Means of Recovery Tests

	% recovery per level of fortification[a]			mean recovery[b]	
	level 1	level 2	level 3		
lignan	~60 μg/(100 g)	~300 μg/(100 g)	~1500 μg/(100 g)	%	CV
Seco	71	101	91	88	4.43
Mat	41	50	61	50	3.71
Lar	71	89	97	86	7.73
Pin	71	96	101	90	4.91
Med	64	93	100	86	6.81
Syr	78	92	94	88	16.10

[a] Mean values of triplicate analyses of three increasing levels of fortification; see text for details. [b] Mean recovery for individual lignans.

higher that those published in other methods (*6*, *23*), although other recent papers have reported higher values (*15*, *30*). Regarding the study of the precision of the method, the variation of the analyses for low- and high-control samples is presented in **Table 3**. Variation was acceptable (CVs < 14%) for all of the lignans in both intra- and most importantly interassay experiments, indicating that the method is repeatable, a compulsory feature in a method that is indicated for routine analyses. Once characterized, the average concentrations of the control samples were used afterward to ensure the quality of the data between batches, by comparison of duplicate analyses with previous results.

Accuracy of the method is assured by the use of individual lignan surrogates as internal standards. Nevertheless, recovery tests were carried out to evaluate the actual losses during the analytical method. Three different levels of fortification were chosen to represent a wide range of possible concentrations in food. As reported in **Table 4**, losses do occur to a small extent and even recoveries at level 1, with concentrations as low as the LOQ, were acceptable with the exception of Mat. The degradation of Mat under alkaline conditions has been reported already, and our results only confirm previous findings (*23*) that suggested the compromise between the need of the alkaline conditions to liberate Mat from the food matrix and its lability in the aglycone form. In the method proposed here, this possible degradation of Mat is corrected with the use of internal standards, and accurate results can be provided.

Six different plant lignans are included in the method representing, to our knowledge, the most abundant lignans in the diet. Other lignans, for instance 7′-hydroxymatairesinol and sesamin that have been previously quantified in sesame seeds (*22*), were initially included in the method development, but the decision was made to remove them afterward from the routine method development since their absence in the foods analyzed so far suggests that these lignans are not common in dietary sources other than sesame seeds. The concentrations of plant lignans were determined in 17 selected food items, including a wide range of concentrations as presented in **Table 5**. Average coefficients of variation for triplicate analysis varied from 5.5 (Pin) to 11.2% (Mat). Comparisons between values obtained in previous studies for similar foods must be taken cautiously since conclusions cannot be drawn without considering key aspects such as plant variety and growth conditions. However, it is important to point out that, although the number of samples analyzed is small, it can be seen how Med and specially Syr are very abundant lignans in foods. When our results for Seco, Lar, and Pin were compared to the values recently published in the Dutch lignan database (*31*), they were found to be similar, but when the values for Syr and Med were added, the total amount increased considerably. In fact Syr was the most abundant lignan in the analyzed cereals with the exception of flaxseed, which presented by far the highest content of lignans, mainly Seco (323 mg/(100 g), 97% of the total). In the case of Mat, our concentrations are higher than those reported by Milder et al. (*31*). If not attributable to variety differences, this discrepancy could be explained by the reported

Table 5. Amounts (μg/(100 g)) of Lignans in Selected Foods, Mean Values ($N = 3$), Wet Basis

lignan	% moisture	Seco	Mat	Lar	Pin	Med	Syr	Total
		Cereals (Whole Grain)						
flaxseed (*Linum usitatissimum* L.)		323670	5202	3670	2460	0	0	335000
rye (*Secale cereale* L.)		38	27	324	381	148	973	1891
buckwheat (*Fagopyrum esculentum* Moench.)		131	1	362	92	33	248	867
oats (*Avena sativa* L.)		19	71	183	194	40	352	859
wheat (*Triticum aestivum* L.)		35	3	62	37	30	372	507
barley (*Hordeum vulgare* L.)		30	3	85	72	11	169	370
millet (*Panicum miliaceum* L.)		67	3	20	85	8	62	245
		Vegetables and Fruits						
asparagus (*Asparagus officinalis* L.)	93	183	2	47	49	5	58	344
lemon (*Citrus lemon* L.)	89	4	0	25	185	64	57	336
pineapple (*Ananas comosus* L.)	86	7	10	67	4	3	81	171
kiwi (*Actinidia chinensis* Planchon)	82	116	0	10	8	5	8	148
grape (*Vitis vinifera* L.)	89	32	0	37	28	8	21	126
orange (*Citrus sinensis* L.)	85	11	0	19	9	6	77	123
eggplant (*Solanum melongena* L.)	93	5	0	68	28	4	2	109
tomato (*Lycopersicon esculentum* Mill.)	95	1	0	11	5	2	2	23
radish (*Raphanus sativus* L.)	96	1	1	14	2	1	2	22
cucumber (*Cucumis sativus* L.)	96	2	0	1	1	0	0	4

low matairesinol recoveries that, in our case, have been corrected with the use of surrogate standards.

Some dietary lignans, for instance Syr, seem to be converted to enterolactone to a very small extent (*11*); however the relation of enterolactone with the prevention of chronic diseases is still not clear, and more work is needed to establish any specific link. Furthermore, plant lignans may possess individual biological actions that must be taken into consideration besides the possible role of enterolignans. Therefore, the creation of a lignan database based on the most abundant lignans in foods will cover all the future outcomes in this rapidly growing area of research. In conclusion, the proposed protocol aims to serve as a reference analytical method and a unique tool for building accurate food lignan databases that will assist in the studies of the relation between dietary lignans and the prevention of chronic diseases.

ABREVIATIONS USED

HMDS, hexamethyldisilazane; Lar, lariciresinol; Mat, matairesinol; Med, medioresinol; Pin, pinoresinol; Seco, secoisolariciresinol; Syr, syringaresinol; TMCS, trimethylclorosilane.

ACKNOWLEDGMENT

The authors would like to thank Anja Koskela and Inga Wiik for exceptional technical assistance.

LITERATURE CITED

(1) *Lignans. Chemical, biological and clinical properties*; Ayres, D. C., Loike, J. D., Eds.; Cambridge University Press: Cambridge, U.K., 1990; p 402.

(2) Setchell, K. D. R.; Lawson, A. M.; Borriello, S. P.; Harkness, R.; Gordon, H.; Morgan, D. M. L.; Kirk, D. N.; Adlercreutz, H.; Anderson, L. C.; Axelson, M. Lignan formation in man-microbial involvement and possible roles in relation to cancer. *Lancet* **1981**, *2*, 4−7.

(3) Axelson, M.; Sjövall, J.; Gustafsson, B. E.; Setchell, K. D. R. Origin of lignans in mammals and identification of a precursor from plants. *Nature* **1982**, *298*, 659−660.

(4) Adlercreutz, H. Western diet and Western diseases: Some hormonal and biochemical mechanisms and associations. *Scand. J. Clin. Lab. Invest.* **1990**, *50* (Suppl. 201), 3−23.

(5) Thompson, L. U.; Robb, P.; Serraino, M.; Cheung, F. Mammalian lignan production from various foods. *Nutr. Cancer* **1991**, *16*, 43−52.

(6) Mazur, W.; Fotsis, T.; Wähälä, K.; Ojala, S.; Salakka, A. Isotope dilution gas chromatographic−mass spectrometric method for the determination of isoflavonoids, coumesterol, and lignans in food samples. *Anal. Biochem.* **1996**, *233*, 169−180.

(7) Mazur, W. M.; Duke, J. A.; Wähälä, K.; Rasku, S.; Adlercreutz, H. Isoflavonoids and lignans in legumes: Nutritional and health aspects in humans. *Nutr. Biochem.* **1998**, *9*, 193−200.

(8) Mazur, W. M.; Adlercreutz, H. Naturally occurring oestrogens in food. *Pure Appl. Chem.* **1998**, *70*, 1759−1776.

(9) Meagher, L. P.; Beecher, G. R.; Flanagan, V. P.; Li, B. W. Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. *J. Agric. Food Chem.* **1999**, *47*, 3173−3180.

(10) Sicilia, T.; Niemeyer, H.; Honig, D.; Metzler, M. Identification and stereochemical characterization of lignans in flaxseed and pumpkin seeds. *J. Agric. Food Chem.* **2003**, *51*, 1181−1188.

(11) Heinonen, S.; Nurmi, T.; Liukkonen, K.; Poutanen, K.; Wähälä,K.; Deyama, T.; Nishibe, S.; Adlercreutz, H. In vitro metabolism of plant Lignans: New precursors of mammalian lignans enterolactone and enterodiol. *J. Agric. Food Chem.* **2001**, *49*, 3178−3186.

(12) Setchell, D. R. K.; Childress, C.; Zimmer-Nechemias, L.; Cai, J. Method for measurement of dietary secoisolariciresinol using HPLC with multichannel electrochemical detection. *J. Med. Food* **1999**, *2*, 193−198.

(13) Liggins, J.; Grimwood, R.; Bingham, S. A. Extraction and quantification of lignan phytoestrogens in food and human samples. *Anal. Biochem.* **2000**, *287*, 102−109.

(14) Kraushofer, T.; Sontag, G. Determination of some phenolic compounds in flax seed and nettle roots by HPLC with coulometric electrode array detection. *Eur. Food Res. Technol.* **2002**, *215*.

(15) Kraushofer, T.; Sontag, G. Determination of matairesinol in flax seed by HPLC with coulometric electrode array detection. *J. Chromatogr., B: Biomed. Sci. Appl.* **2002**, *777*, 61−66.

(16) Johnson, P.; Kamal-Eldin, A.; Lundgren, L. N.; Aman, P. HPLC method for the analysis of secoisolariciresinol diglucoside in flaxseeds. *J. Agric. Food Chem.* **2000**, *48*, 4643−4351.

(17) Eliasson, C.; Kamal-Eldin, A.; Andersson, R.; Aman, P. High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction. *J. Chromatogr., A* **2003**, *1012*, 151−159.

(18) Liukkonen, K.; Katina, K.; Wilhelmsson, A.; Myllymaki, O.; Lampi, A. M.; Kariluoto, S.; Piironen, V.; Heinonen, S.-M.; Nurmi, T.; Adlercreutz, H.; Peltoketo, A.; Pihlava, J.-M.; Hietaniemi, V.; Poutanen, K. Process-induced changes on bioactive compounds in whole grain rye. *Proc. Nutr. Soc.* **2003**, *62*, 117−122.

(19) Bach Knudsen, K. E.; Serena, A.; Kjaer, A. K. B.; Tetens, I.; Heinonen, S.-M.; Nurmi, T.; Adlercreutz, H. Rye bread in the diet of pigs enhances the formation of enterolactone and increases its levels in plasma, urine and feces. *J. Nutr.* **2003**, *133*, 1368−1375.

(20) Begum, A. N.; Nicolle, C.; Mila, I.; Lapierre, C.; Nagano, K.; Fukushima, K.; Heinonen, S.-M.; Adlercreutz, H.; Remesy, C.; Scalbert, A. Dietary lignins are precursors of mammalian lignans in rats. *J. Nutr.* **2004**, *134*, 120−127.

(21) Peñalvo, J. L.; Heinonen, S.-M.; Nurmi, T.; Deyama, T.; Nishibe, S.; Adlercreutz, H. Plant lignans in soy-based health supplements. *J. Agric. Food Chem.* **2004**, *52*, 4133−4138.

(22) Peñalvo, J. L.; Heinonen, S.-M.; Aura, A.-M.; Adlercreutz, H. Dietary sesamin is converted to enterolactone in humans. *J. Nutr.* **2005**, *153*, 1056−1062.

(23) Milder, I. E.; Arts, I. C.; Venema, D. P.; Lasaroms, J. J.; Wahala, K.; Hollman, P. C. Optimization of a liquid chromatography-tandem mass spectrometry method for quantification of the plant lignans secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol in foods. *J. Agric. Food Chem.* **2004**, *52*, 4643−4651.

(24) Grace, P. B.; Taylor, J. I.; Botting, N. P.; Fryatt, T.; Oldfield, M. F.; Al-Maharik, N.; Bingham, S. Quantification of isoflavones and lignans in serum using isotope dilution liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1350−1357.

(25) Grace, P. B.; Taylor, J. I.; Botting, N. P.; Fryatt, T.; Oldfield, M. F.; Bingham, S. Quantification of isoflavones and lignans in urine using gas chromatography/mass spectrometry. *Anal. Biochem.* **2003**, *315*, 114−121.

(26) Roy, S. C.; Rana, K. K.; Guin, C. Short and stereoselective total synthesis of furano lignans (±)-dihydrosesamin, (±)-lariciresinol dimethyl ether, (±)-acuminatin methyl ether, (±)-sanshodiol methyl ether, (±)-lariciresinol, (±)-acuminatin, and (±)-lariciresinol monomethyl ether and furofuran lignans (±)-sesamin, (±)-eudesmin, (±)-piperitol methyl ether, (±)-pinoresinol, (±)-piperitol, and (±)-pinoresinol monomethyl ether by radical cyclization of epoxides using a transition-metal radical source. *J. Org. Chem.* **2002**, *67*, 3242−3248.

(27) Wahala, K.; Rasku, S.; Parikka, K. Deuterated phytoestrogen flavonoids and isoflavonoids for quantitation. *J. Chromatogr., B: Biomed. Sci. Appl.* **2002**, *777*, 111−122.
(28) Clarke, D. B.; Lloyd, A. S.; Botting, N. P.; Oldfield, M. F.; Needs, P. W.; Wiseman, H. Measurement of intact sulfate and glucuronide phytoestrogen conjugates in human urine using isotope dilution liquid chromatography-tandem mass spectrometry with [13C3]isoflavone internal standards. *Anal. Biochem.* **2002**, *309*, 158−172.
(29) Nurmi, T.; Heinonen, S.; Mazur, W.; Deyama, T.; Nishibe, S.; Adlercreutz, H. Lignans in selected wines. *Food Chem.* **2003**, *83*, 303−309.
(30) Horn-Ross, P. L.; Barnes, S.; Lee, M.; Coward, L.; Mandel, J. E.; Koo, J.; John, E. M.; Smith, M. Assessing phytoestrogen exposure in epidemiologic studies: Development of a database (United States). *Cancer Causes Control* **2000**, *11*, 289−298.
(31) Milder, I. E.; Arts, I. C.; Putte, B.; Venema, D. P.; Hollman, P. C. Lignan contents of Dutch plant foods: A database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. *Br. J. Nutr.* **2005**, *93*, 393−402.

Received for review June 23, 2005. Revised manuscript received September 6, 2005. Accepted September 7, 2005. Financial support was provided by the Phytoprevent project, "The Role of Dietary Phytoestrogens in the Prevention of Breast and Prostate Cancer" (EU Grant QLK1-2000-00266). This study does not necessarily reflect the views of the Commission and in no way anticipates the Commission's future policy in this area. Additional support was obtained from the Sigrid Juselius Foundation (Helsinki) and The Nordic Joint Committee for Agricultural Research (NKJ).

JF051488W