British Journal of Nutrition (2005), **93**, 393–402
DOI: 10.1079/BJN20051371

Lignan contents of Dutch plant foods: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol

Ivon E. J. Milder[1,2], Ilja C. W. Arts[1], Betty van de Putte[1], Dini P. Venema[1] and Peter C. H. Hollman[1]*

[1]*RIKILT-Institute of Food Safety, Wageningen University and Research Centre, PO Box 230, 6700 AE Wageningen, The Netherlands*
[2]*Centre for Nutrition and Health, National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands*

(Received 7 July 2004 – Revised 1 November 2004 – Accepted 9 November 2004)

Enterolignans (enterodiol and enterolactone) can potentially reduce the risk of certain cancers and cardiovascular diseases. Enterolignans are formed by the intestinal microflora after the consumption of plant lignans. Until recently, only secoisolariciresinol and matairesinol were considered enterolignan precursors, but now several new precursors have been identified, of which lariciresinol and pinoresinol have a high degree of conversion. Quantitative data on the contents in foods of these new enterolignan precursors are not available. Thus, the aim of this study was to compile a lignan database including all four major enterolignan precursors. Liquid chromatography–tandem mass spectrometry was used to quantify lariciresinol, pinoresinol, secoisolariciresinol and matairesinol in eighty-three solid foods and twenty-six beverages commonly consumed in The Netherlands. The richest source of lignans was flaxseed (301 129 μg/100 g), which contained mainly secoisolariciresinol. Also, lignan concentrations in sesame seeds (29 331 μg/100 g, mainly pinoresinol and lariciresinol) were relatively high. For grain products, which are known to be important sources of lignan, lignan concentrations ranged from 7 to 764 μg/100 g. However, many vegetables and fruits had similar concentrations, because of the contribution of lariciresinol and pinoresinol. *Brassica* vegetables contained unexpectedly high levels of lignans (185–2321 μg/100 g), mainly pinoresinol and lariciresinol. Lignan levels in beverages varied from 0 (cola) to 91 μg/100 ml (red wine). Only four of the 109 foods did not contain a measurable amount of lignans, and in most cases the amount of lariciresinol and pinoresinol was larger than that of secoisolariciresinol and matairesinol. Thus, available databases largely underestimate the amount of enterolignan precursors in foods.

Lignans: Phytoestrogens: Food composition: Secoisolariciresinol: Matairesinol: Pinoresinol: Lariciresinol

Lignans are diphenolic compounds that are widely distributed in the plant kingdom. A large variety of plant lignans exist, but only a few of them are converted into the 'enterolignans' enterodiol and enterolactone by the intestinal microflora. Enterolignans are absorbed into the human body.

Until recently, only secoisolariciresinol and matairesinol were seen as enterolignan precursors, but new precursors have recently been identified, of which lariciresinol and pinoresinol have a high degree of conversion (Heinonen *et al.* 2001). Lignans possess several biological activities, such as antioxidant and (anti)oestrogenic properties, and may thus reduce the risk of certain cancers as well as cardiovascular diseases (Adlercreutz *et al.* 1992; Adlercreutz & Mazur, 1997; Raffaelli *et al.* 2002; Arts & Hollman, 2005).

Secoisolariciresinol and matairesinol have been quantified in a large number of plant foods. Flaxseed (linseed) is the richest known source of these lignans. Other sources are grains, seeds, vegetables, fruits and beverages (Mazur, 1998; Mazur & Adlercreutz, 1998; Horn-Ross *et al.* 2000; Meagher & Beecher, 2000). These data have been incorporated into several phytoestrogen databases (Horn-Ross *et al.* 2000; Keinan Boker *et al.* 2002; Valsta *et al.* 2003). Until now, data on the lariciresinol and pinoresinol contents of foods were not available.

A number of epidemiological studies on the associations between enterolignan concentrations in biological fluids or the intake of plant lignans and chronic disease risk has so far been conducted (reviewed by Arts & Hollman, 2005). In case–control studies (Ingram *et al.* 1997; Pietinen *et al.* 2001; Dai *et al.* 2002), but not in prospective studies (Den Tonkelaar *et al.* 2001; Hulten *et al.* 2002; Grace *et al.* 2004), inverse associations have been found between plasma or urinary lignans and breast cancer risk. For cardiovascular diseases, inverse associations with serum lignans were reported in two Finnish studies (Vanharanta *et al.* 1999, 2003). In addition, studies in which the relation between the dietary intake of secoisolariciresinol and matairesinol and cancer risk was studied gave conflicting results. Protective associations were reported for breast (McCann *et al.* 2002), ovarian (McCann *et al.* 2003), endometrial (Horn-Ross *et al.* 2003) and thyroid (Horn-Ross *et al.* 2002*a*) cancer. In one study, a decreased risk of breast cancer was found only for high intakes of matairesinol, but not for secoisolariciresinol or secoisolariciresinol plus matairesinol (Linseisen *et al.* 2004). In two other studies, increased breast cancer risks were found with a high intake of secoisolariciresinol and matairesinol (Horn-Ross *et al.* 2001; 2002*b*).

To further evaluate the health effects of lignan intake, it is essential that the newly discovered enterolignan precursors are included. Thus, the aim of the present study was to compile a comprehensive database including all four major enterolignan

* **Corresponding author:** Dr Peter C. H. Hollman, fax +31 317 417717, email Peter.Hollman@wur.nl

precursors: lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. Lignans were quantified in 109 plant foods commonly consumed in the Netherlands, including eighty-three solid foods and twenty-six beverages. Additionally, we studied the effect of cooking on the lignan content of a few vegetables.

Materials and methods

Selection of foods

Plant foods were selected for analysis based on data from the Dutch National Food Consumption Survey conducted in 1997–98. This survey was carried out among a sample of households representative of the Dutch population. Two-day dietary records were collected for 6250 persons aged 1–97 years (Hulshof & Van Staveren, 1991; Voedingscentrum, 1998).

In general, plant foods with an average consumption of over 3 g per person per day were selected. Mixed dishes (e.g. apple pie, pizza) were not selected for analysis since their lignan content can be calculated from their ingredients using standard recipes. For fruits, a limit of 1 g per person per day was used. Because of their relatively high consumption, a limit of 10 g per person per day was used for beverages. Waters and soft drinks (except cola) were disregarded. Apple juice was not analysed because the lignan content of apples was very low (see below). Vegetable oils and fats with an average consumption of over 0·5 g per person per day were also added. Using this method, forty-two plant foods were selected. Some important plant foods, for example leek and sweet pepper, were missed because they are ingredients of a large number of foods but are not consumed in large quantities as such. We therefore also used data on the consumption of primary agricultural products. These data were derived from the Food Consumption Survey data using the Conversion Model for Primary Agricultural Products developed at RIKILT (Van Dooren-Flipsen *et al.* 1995). Four extra fruits and eleven extra vegetables were selected, using the same criteria as before. In addition, foods that were high in lignans according to data in the literature, but did not meet the aforementioned consumption criteria, were also selected (e.g. seeds, olives, cashew nuts). Tofu, soya milk and legumes were added since they might be important plant foods for specific population groups (e.g. vegetarians).

Sample collection

Foods were purchased in the form (fresh, canned, in jars, frozen) that was most commonly consumed according to the Food Consumption Survey. In order to enhance the representation of the food samples, products were bought at three different locations (fresh products) or from three major brands (processed foods), and composite samples were assembled. In addition, for fresh products, a minimum of 0·5 kg or 3 units was sampled at each location: an outlet of a nationwide supermarket chain, a local grocery and an open-air street market. The proportion in the composite samples reflected the sales of each food group at each location. For vegetables, the supermarket accounted for 78 %, the grocery for 11 % and the open-air market for 11 %; for fruits, these values were 70 %, 12 % and 18 %, respectively. Potatoes were bought at two supermarkets (each contributing 45 %) and a local grocery store (contributing 10 %). Breads were also bought at two supermarkets (each contributing 37·5 %) and a local bakery (contributing 25 %). For processed foods, composite samples were assembled by mixing the three brands in equal proportions.

Sample preparation

Non-edible parts were removed from the fruit and vegetables. Vegetables were not prepared further since cooking or frying was considered to have only a small effect on lignan content. This assumption was based on the fact that the first step in the lignan analysis consisted of the alkaline extraction for 1 h at 60°C, which did not lead to a considerable loss of lignans (Milder *et al.* 2004). However, we checked this assumption, because during cooking and frying temperatures are higher and lignans might also be lost by leaching. A few vegetables were therefore analysed both raw and prepared according to standard recipes. Lignans in potatoes, rice and macaroni were determined in the prepared product according to standard recipes or the instructions on the packaging.

Composites of solid foods were either directly chopped under liquid N_2 or cut into smaller pieces prior to freezing in liquid N_2, and stored at −20°C until freeze-drying was started within 1 month. After freeze-drying, samples were ground to a powder using a Tecator Knifetec 1095 sample mill (Rose Scientific Ltd, Edmonton, Canada). Dry products, which did not require freeze-drying, such as seeds, muesli and wheat (meal and wholegrain), were ground before preparing the composite samples. Fruits were frozen in liquid N_2 before grinding. All composites of solid foods were stored at −20°C until analysis.

Beverages were collected within 48 h of analysis. Composites were prepared immediately before analysis by mixing three major brands in equal proportions. Beer was degassed in an ultrasonic bath at room temperature. Since alcohol might influence the activity of the enzyme used in the analytical method, beer and wine were analysed both with and without the evaporation of alcohol (using a Zymark Turbo vap LV Evaporator; Zymark Corp., Hopkinton, MA, USA) at 60°C, under a mild N_2 flow (5–10 psi). Since the evaporation of alcohol produced no substantial changes in lignan concentrations, and increased the variation between duplicate samples, the lignan values of the analyses without the evaporation of alcohol are presented here. Tea infusion was prepared by placing one tea bag (1 or 2 g) in boiling tap water (100 ml/g tea leaves). After 5 min, the tea bag was stirred a few times, after which it was removed. Coffee was prepared according to Dutch custom in a coffee-maker with paper filter: a volume of 275 ml boiling water was dripped on 14 g ground coffee. Chocolate milk was diluted ten times with sodium acetate buffer (0·05 M, pH 5·0), in order to avoid gelling during the ether extraction.

Standards

Pure standards of secoisolariciresinol (purity approximately 92 %) and matairesinol (purity >98 %) were obtained from Plantech (Reading, England). Lariciresinol isolated from the wood of *Abies sachalinensis* and pinoresinol isolated from *Euconmia ulmoides* oliv. bark were kindly provided by Dr Ozawa (Rakuno Gakuen University, Japan), Dr Deyama (Yomeishu Seizo Co. Ltd, Japan) and Dr Nishibe (University of Hokkaido, Japan). Internal standards of secoisolariciresinol-d_8 and matairesinol-d_6 were kindly provided by Dr K. Wähälä (University of Helsinki, Finland).

Analytical method

The four major enterolignan precursors lariciresinol, pinoresinol, secoisolariciresinol and matairesinol were measured in each food sample using a liquid chromatography–tandem mass spectrometry method previously described in detail (Milder *et al.* 2004). In brief, lignans were extracted from solid foods with methanol–water (70:30 v/v) containing 0·3 M NaOH at 60°C for 1 h. After neutralising and centrifuging, an aliquot of the supernatant was transferred to a test-tube, and methanol was evaporated under a mild N_2 flow. The extract obtained, or pure beverage, was incubated overnight with *Helix pomatia* β-glucuronidase/sulphatase in sodium acetate buffer (0·05 M, pH 5·0) at 37°C. After enzymatic hydrolysis, samples were extracted twice with diethyl ether. The organic phases were combined, evaporated to dryness and dissolved in methanol–water (30:70 v/v). Internal deuterated standards were added, and samples were subjected to liquid chromatography–tandem mass spectrometry analysis, with atmospheric pressure chemical ionization in the negative-ion mode. Multiple reaction monitoring was performed with the following precursor/product combinations: lariciresinol (359·1/329·1), and secoisolariciresinol (361·1/165·1) with internal standard secoisolariciresinol-d_8 (369·2/168·3), and pinoresinol (357·1/151·2) and matairesinol (357·1/83·1) with internal standard matairesinol-d_6 (363·2/83·1).

Analytical quality control

Control samples were included at the beginning and end of each series of analyses. Single batches of freeze-dried and ground broccoli and wholegrain wheat bread were used as control samples for solid foods. These samples had been stored at −20°C. Black tea infusion was used as a control sample for beverages. One batch of tea leaves was prepared by passing black tea (from tea bags) through two sieves of 0·8 and 0·355 mm. The middle fraction was stored at room temperature for use as a control product. For each series of analyses, a fresh tea infusion was prepared by adding 100 ml boiling tap water to 1 g tea leaves. After 5 min, this was stirred, and 50 ml was filtered through a 1·2 μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI, USA).

Calibration standards of 20, 100, 200, 600 and 1000 ng/ml of each lignan, with 200 ng/ml of deuterated standards, were injected three times, at the beginning, middle and end of each series of analyses. If the lignan values in the sample extracts exceeded 1000 ng/ml, the sample was reanalysed after diluting the alkaline extract. For solid foods, the limit of detection (signal to noise ratio of 3) for lariciresinol was 4 μg/100 g dry weight, for pinoresinol 10 μg/100 g dry weight, and for secoisolariciresinol and matairesinol 6 μg/100 g dry weight. Depending on the moisture content of the food, this corresponded to 0·2–10·0 μg/100 g fresh weight. Detection limits for beverages were for lariciresinol and matairesinol 0·2 μg/100 ml, for pinoresinol 0·4 μg/100 ml, and for matairesinol 0·3 μg/100 ml. The within-run reproducibility for the analysis of the control samples was 6–21 % and the between-run reproducibility 6–33 % (Milder *et al.* 2004). All samples were analysed in duplicate. If differences between the duplicates were more than 20 %, the analysis was repeated and the mean values of the two duplicate analyses were used.

Results and discussion

We found that almost all of the selected plant foods contained lignans (Tables 1 and 2). Only four out of the 109 products did not contain a measurable amount of lignans. The amount of lignans in plant foods varied widely, from 0 to 301 000 μg/100 g fresh weight. Interestingly, in almost all products the newly discovered enterolignan precursors lariciresinol and pinoresinol were found in higher concentrations than the well-known precursors secoisolariciresinol and matairesinol. Thus, databases with only secoisolariciresinol and matairesinol largely underestimate the amount of enterolignan precursors. Besides flaxseed, grain products have been the main focus of lignan research. However, our results show that the lignan contents of many of the vegetables and fruits are similar to those of the grain products when lariciresinol and pinoresinol are also included.

Any comparison of food composition data from various sources is complicated by geographical differences in foods, such as plant variety, growth conditions and food processing. Additional differences may result from variations in the ripeness or season of collection of the sampled foods. Keeping this in mind, we compared our results primarily with lignan data reported by Mazur *et al.* (1998*a*, *b*) and Horn-Ross *et al.* (2000) since these were the most comprehensive sources.

Mazur and co-workers quantified secoisolariciresinol and matairesinol in a variety of Finnish foods with isotope dilution gas chromatography–mass spectrometry (Mazur *et al.* 1996), and these data have been published in several original and review publications (Adlercreutz & Mazur, 1997; Mazur, 1998; Mazur & Adlercreutz, 1998, 2000; Mazur *et al.* 1998*a*, *b*). However, as they report lignan values only on a dry weight basis, it is not possible to compare our lignan values directly with these results.

Recently, some additional lignan values were published on the occasion of the construction of a Finnish phytoestrogen database (Valsta *et al.* 2003). Since these data were expressed on a fresh weight basis, we used these data in preference for comparison. In general, our results for secoisolariciresinol and matairesinol are in agreement with or slightly lower than those published by Mazur *et al.*, although larger differences exist for a few products, which will be discussed below. Horn-Ross and co-workers measured secoisolariciresinol and matairesinol in foods from California (Horn-Ross *et al.* 2000). They also used an HPLC–mass spectrometry method for quantification. In general, our data for secoisolariciresinol are in agreement with or slightly higher than their results. However, since their limit of detection is relatively high (25 μg/100 g) compared with ours (0·2–10·0 μg/100 g), they found lignan concentrations to be below the detection limit in many products.

The lignan contents reported in this paper are measured after alkaline extraction from solid foods. Under alkaline conditions, ester-linked oligomers of secoisolariciresinol in flax are hydrolysed to give the lignan monomer (Ford *et al.* 2001). Alkaline hydrolysis increased the lignan yield of flax about five-fold, but lignan yields from broccoli and bread were also substantially increased (Milder *et al.* 2004). Although we only tested these products, this indicates that ester-linked lignans also occur in other foods besides flax. In addition, because we showed that our carefully optimised alkaline extraction method gave reproducible results (Milder *et al.* 2004), we decided to use this method for our food analyses.

Table 1. Lignan content (μg/100 g fresh edible weight) of solid foods

(Mean of duplicate analyses of composite samples)

Product	Type, processing	Moisture content (%)*	LARI	PINO	SECO	MAT	Total
Oilseeds and nuts							
Flaxseed (*Linum usitatíssimum* L.)		–†	3041	3324	294 210	553	301 129
Sesame seed (*Sesanum indicum* L.)		–	9470	29 331	66	481	39 348
Sunflower seed (*Helianthus annuus* L.)		–	671	167	53	0	891
Cashew (*Anacardium occidentale* L.)		–	496	0‡	133	0	629
Peanut (*Arachis hypogaea* L.)		–	41	0	53	0	94
Poppy seed (*Papaver somniferum* L.)		–	10	0	0	0	10
Grain products							
Breads							
Whole grain flaxseed bread		34	220	383	11 845	26	12 474
Multi-grain bread		34	185	377	6163	19	6744
Rye bread	Dark	46	122	172	13	14	320
	Light	43	111	163	16	12	301
Wheat bread	Whole grain	41	73	33	15	0	121
	Refined	37	38	28	17	0	83
	White	36	11	7	0	0	18
Currant/raisin bread		29	79	9	9	7	104
Other grain products							
Muesli (granola)§	Jordans, crunchy	–	250	497	17	0	764
	Albert Heijn, basic	–	120	210	13	0	343
	Edah, crunchy	–	63	129	17	0	210
Wheat (*Triticum aestivum* L.)	Wholemeal	–	140	38	31	0	210
	White flour	–	18	9	0	0	27
Rice (*Oryza sativa* L.)	Whole grain, boiled	67	28	7	3	2	40
	White,boiled	67	7	0	0	0	7
Macaroni	White, boiled	68	7	5	4	0	15
Vegetables and legumes							
Brassica vegetables (*Brassica oleracea* L.)							
Curly kale (*cv. acephala D. C.* Alef.)		84	599	1691	19	12	2321
Broccoli (*cv. botyris* L. var. *italica*. Plenk.)		87	972	315	38	0	1325
White cabbage (*cv. capitale* L. Alef. var. *alba. D. C.*)		89	212	568	8	0	787
Brussels sprout (*cv. oleracea* L. var. *gemnifera D. C.*)		83	493	220	34	0	747
Sauerkraut (*cv. capitale* L. Alef. var. *alba. D. C.*)		91	116	133	67	0	316
Red cabbage (*cv. capitale* L. Alef. var. *rubra D. C.*)		91	178	90	9	0	276
Cauliflower (*cv. botrytis* L. Alef. var. *botyris* L.)		92	124	58	4	0	185
Allium vegetables							
Garlic (*Allium sativum* L.)		62	286	200	50	0	536
Leek (*Allium porrum* L. var. *porrum*)		89	37	3	38	0	78
Onion (*Allium cepa* L. var. *cepa*)		90	19	0	18	0	36
Other vegetables							
French bean (*Phaseolus vulgaris* L. ssp. *vulgaris*)		89	220	24	29	0	273
Sweet pepper (*Capsicum annuum* L.)	Green	94	164	1	7	0	172
	Red	91	106	1	7	0	113
Carrot (*Daucus carota* L.)		92	60	19	93	0	171
Courgette (*Cucurbita pepo* L. var. *melopepo*)		94	64	37	18	0	119
Spinach (*Spinacea oleracea* L.)	Frozen	94	68	12	2	0	82
Cucumber (*Cucumus sativus* L. sspp. *sativus*)		96	59	1	8	0	67
Tomato (*Lycopersicum esculentum* Mill.)		94	42	14	2	0	58
Chicory (*Chicorum intybus* L. cv. *foliosum*)		95	6	25	17	0	48
Endive (*Chicorum endivia* L.)		93	15	9	14	0	38
Pea (*Pisum sativum* L.)	In jars	81	14	20	0	0	34
Potato (*Solanum tuberosum* L.)	Nicola, boiled	80	17	0	2	0	20
	Redstar, boiled	76	8	0	1	0	10
Lettuce (*Lactuca sativa* L.)		96	5	4	8	0	16
Iceberg lettuce (*Lactuca sativa* L. cv. *capitata* L.)		95	2	0	9	0	11
Sweet corn (*Zea mays* L.)	In jars	76	2	0	5	0	7
Beetroot (*Beta vulgaris* L. var. *conditiva* Alef.)	Boiled	91	3	0	1	0	3
Mushroom (*Agaricus campestris* Fr.)		93	0	0	0	0	0
Legumes							
Baked beans in tomato sauce (*Phaseolus vulgaris* L.)	In jars	71	21	9	8	0	37
Brown beans (*Phaseolus vulgaris* L.)	In jars	69	13	3	10	0	26
Fruits							
Apricot (*Armeniaca vulgaris* L.)		86	105	314	31	0	450
Strawberry (*Fragaria x ananassa* Duch.)		91	117	212	5	0	334
Peach (*Prunus persica* L. Batch)		89	80	186	27	0	293
Pear (*Pyrus communis* L.)		84	155	34	4	0	193
Nectarine (*Prunus persica* L. Batch)		89	41	131	18	0	190

Table 1. *Continued*

Product	Type, processing	Moisture content (%)*	LARI	PINO	SECO	MAT	Total
Raisins (*Vitis euvitis vinifera* L.)	White	–	153	0	9	19	181
	Blue	–	118	0	8	18	144
Grapefruit (*Citrus paradisi* Macfad.)	Pink	88	95	45	9	2	152
Cherries (*Prunus avium* L.)		79	41	100	6	0	147
Kiwi (*Actinidia chinensis* Planch.)		83	17	0	112	0	129
Plum (*Prunus domestica* L.)		86	4	74	4	0	82
Mandarin (*Citrus reticulata* Blanco)		86	57	21	3	1	81
Olives (*Olea europaea* L.)	Black	65	36	37	7	0	80
	Green	77	5	13	26	0	45
Orange (*Citrus sinensis* L. Osbeck)		86	47	24	5	2	78
Melon (*Cucumis melo* L.)	Galia	89	44	22	5	0	71
Grapes (*Vitis vinifera* L.)	Blue	80	52	0	4	5	60
	White	83	25	4	10	3	42
Pineapple (*Ananas comosus* L. Merr.)	Canned	85	3	5	7	5	20
Apple (*Malus domestica* Borkh.)	Elstar	85	1	0	0	0	1
	Jonagold	86	1	0	0	0	1
Banana (*Musa x paradisciaca* L.)		75	0	0	0	0	0
Vegetable oils and fats							
Olive oil	Extra-virgin	–	4	243	0	0	248
	Regular	–	5	101	0	0	106
Margarine		–	7	0	32	0	39
Soya oil		–	0	0	0	0	0
Sunflower oil		–	0	0	0	0	0
Other							
Tomato paste		70	107	70	9	0	187
Tofu		80	61	61	18	0	140
Cocoa	Powder	–	26	26	8	0	60
Chocolate	Plain	–	20	23	0	0	44

LARI, lariciresinol; PINO, pinoresinol; SECO, secoisolariciresinol; MAT, matairesinol.
* Determined by freeze-drying.
† Not freeze-dried.
‡ 0, below detection limit: 4 μg/100 g dry weight for lariciresinol, 10 μg/100 g dry weight for pinoresinol and 6 μg/100 g dry weight for secoisolariciresinol and matairesinol; corresponding to 0·2–10 μg/100 g fresh weight, depending on the moisture content.
§ Separate samples analysed instead of composite.

Ideally, the amount of lignans extracted by the analytical method should reflect the lignans that are available in the human body. So, can these ester-linked lignans be converted to enterolignans in the human body? Until now, no bioavailability studies have been performed that could answer this question. Andreasen *et al.* (2001) showed that human small intestine mucosa and colonic microflora contain esterase activity able to release diferrulic acids from diferrulate esters, so it is likely that these ester-linked lignans have physiological relevance. Lapierre *et al.* (2001) showed that 8–8′diferrulate is liberated from bran lignins under alkaline conditions similar to ours, implying that our alkaline extract might contain lignans that had been incorporated in lignin structures. A rat study indeed suggested that lignins from wheat and rye bran increased the urinary excretion of enterolignans (Begum *et al.* 2004). However, we think that this paper probably overestimated the contribution of these lignins to the enterolignan production. Thus, alkaline extraction seems to be able to account for physiologically relevant lignans incorporated into lignins. Future human studies should, however, provide insight into the real potential of various kinds of bound lignans as enterolignan precursors.

The recovery of lignan aglycones added to control foods was regarded as satisfactory (73–123 %), except for matairesinol added to solid food (51–55 %) (Milder *et al.* 2004). Thus, the concentrations of matairesinol in solid foods that we report here are somewhat underestimated. The low recovery of added matairesinol is caused by its instability during alkaline extraction. However, the yield of lignans (including matairesinol) from control foods was largely increased by the addition of NaOH during the extraction (Milder *et al.* 2004). Thus, alkaline extraction favours the release of matrix-bound matairesinol, which more than compensates for losses by degradation. Besides, our own and previous results show that the amount of matairesinol in foods is usually relatively low compared with that of the other enterolignan precursors. Thus, the underestimation of matairesinol will have only a small effect on estimations of the total dietary lignan content. In general, our results for matairesinol are lower than those of Mazur (Mazur & Adlercreutz, 1998), but for some products we found higher amounts. Horn-Ross *et al.* (2000) detected matairesinol in only three foods. We measured two of these foods and found lower amounts of matairesinol in both.

Oilseeds and nuts

The richest source of lignans was flaxseed. Flaxseed contained mainly secoisolariciresinol (294 210 μg/100 g), but pinoresinol, lariciresinol and matairesinol were also present in substantial amounts (553–3324 μg/100 g). Most of the previously reported values for secoisolariciresinol were higher than we found: 369 900 μg/100 g dry weight by Mazur & Adlercreutz (1998), 385 000–670 000 μg/100 g (converted from secoisolariciresinol diglucoside) by Johnsson *et al.* (2000), 1 261 700 and 880 000 μg/100 g by Liggins *et al.* (2000), and 495 700–1 006 200 μg/100 g by Kraushofer & Sontag (2002*a*), but lower values were also reported (e.g. 81 700 μg/100 g by Obermeyer *et al.* 1995). In addition, the amount of matairesinol (553 μg/100 g) was relatively low compared

Table 2. Lignan content (μg/100 ml) of beverages
(Mean of duplicate analyses of composite samples)

Product	Type, brand	LARI	PINO	SECO	MAT	Total
Alcoholic beverages						
Wine						
Red wine*	Red, South Africa	15·9	6·3	61·3	7·8	91·3
	Red, France	16·1	9·5	47·5	5·9	78·9
	Red, France	8·6	11·9	41·7	6·9	69·1
White wine*	White, France	11·9	3·0	7·6	3·0	25·5
	White, Germany	7·3	1·7	12·2	2·7	23·8
	White, South Africa	4·6	2·5	5·2	3·1	15·5
Beer (lager)						
Lager*	Grolsch	9·2	22·2	0·8	0·0†	32·2
	Heineken	9·0	21·7	1·0	0·0	31·6
	Bavaria	5·9	12·6	0·0	0·0	18·5
Non-alcoholic beverages						
Tea						
Black tea	Ceylon*	30·4	40·6	5·0	1·1	77·1
	English blend	30·8	33·6	5·4	1·4	71·2
	Earl Grey*	28·9	27·0	6·2	1·5	63·6
Green tea*	(with lemon flavour)	18·7	5·7	12·9	2·0	39·2
Coffee						
Coffee*	Albert Heijn, Perla Robusta	13·1	1·5	16·1	0·7	31·3
	Kanis & Gunnink	9·1	1·3	9·2	0·0	19·6
	Douwe Egberts	9·0	0·4	9·4	0·0	18·7
Juices						
Grape juice	Blue	6·5	3·7	10·8	3·9	24·8
Grape juice	White	3·3	0·7	2·5	1·0	7·4
Tomato juice		9·7	9·9	1·6	0·0	21·2
Orange juice	Regular	7·0	7·5	2·7	0·0	17·2
	With pulp	7·4	6·6	2·7	0·0	16·6
Grapefruit juice	Yellow	5·1	4·8	6·0	0·0	15·9
Grapefruit juice	Pink	5·0	3·3	6·7	0·0	15·0
Other						
Soya milk		6·6	30·0	1·1	0·0	37·7
Chocolate milk	Semi-skimmed	0·9	1·3	0·0	0·0	2·2
Cola		0·0	0·0	0·0	0·0	0·0

LARI, lariciresinol; PINO, pinoresinol; SECO, secoisolariciresinol; MAT, matairesinol.
* Separate samples analysed instead of composite.
† 0, below detection limit: 0·2 μg/100 ml for lariciresinol and secoisolariciresinol, 0·4 μg/100 ml for pinoresinol and 0·3 μg/100 ml for matairesinol.

with values previously reported: 1087 μg/100 g dry weight (Mazur & Aldercruetz, 1998), 5680 and 9090 μg/100 g dry weight (Ziggins *et al.* 2000) and 700–2850 μg/100 g dry weight (Kraushofer & Sontag, 2002*b*) Both pinoresinol (Meagher *et al.* 1999; Qui *et al.* 1999) and lariciresinol (Sicilia *et al.* 2003) have previously been identified in flaxseed, but quantitative data were not reported.

The second highest lignan concentration was found in sesame seeds, but in this case pinoresinol was the main constituent (29 331 μg/100 g), and lariciresinol was also relatively abundant (9470 μg/100 g). The presence of pinoresinol in sesame seeds has previously been described (Katsuzaki *et al.* 1992; Jiao *et al.* 1998; Kato *et al.* 1998); it is a precursor of the sesame lignans piperitol, sesamin and sesamolin (Jiao *et al.* 1998; Kato *et al.* 1998). The total lignan concentrations in sunflower seeds (891 μg/100 g) and cashew nuts (629 μg/100 g) were also relatively high, although the concentrations of secoisolariciresinol were low compared with those reported by Mazur & Adlercreutz (1998). Mazur detected some matairesinol (4 μg/100 g dry weight) in cashew nuts, which we did not find. In poppy seeds, we detected only lariciresinol (10 μg/100 g), whereas Mazur & Adlercreutz (1998) also reported the presence of secoisolariciresinol (14 μg/100 g dry weight) and matairesinol (12 μg/100 g dry weight).

Grain products

Both wholegrain flaxseed bread and multigrain bread had a high lignan content (12 500 and 6700 μg/100 g), which can be attributed to the flaxseed present in these breads. For breads without flaxseed, the highest lignan concentration was found in rye bread, but the levels of secoisolariciresinol and matairesinol were approximately two- to three-fold lower than reported for Finnish rye (Nilsson *et al.* 1997*a*) and rye bread (Juntunen *et al.* 2000). For wheat bread, wheat, and rice, the lignan content decreased with the level of refinement. This is in agreement with previous findings (for rye) that lignans are mainly present in the short and bran of grain, which are removed during the refining of grain products (Nilsson *et al.* 1997*b*).

Vegetables and legumes

Brassica vegetables (cabbages, Brussel sprouts, kale) contained unexpectedly high levels of lignans (185–2321 μg/100 g), mainly due to pinoresinol and lariciresinol. The amount of pinoresinol plus lariciresinol in *Brassica* vegetables was on average forty-five times higher than that of secoisolariciresinol

plus matairesinol. Our results for secoisolariciresinol in broccoli, cabbage and Brussel sprouts agree well with those of Mazur, but in broccoli and Brussel sprouts, they again report the presence of some matairesinol. Horn-Ross *et al.* (2000) reported a similar amount of secoisolariciresinol as we found in Brussel sprouts, but they did not detect secoisolariciresinol or matairesinol in broccoli.

Allium vegetables also contained substantial amounts of lignans (36–536 μg/100 g). The concentration of secoisolariciresinol in garlic reported by Horn-Ross *et al.* (2000) was approximately half of what we found, but they reported 38 μg/100 g matairesinol, which we did not detect in garlic. The amount of secoisolariciresinol in garlic reported by Mazur & Adlercreutz (1998) was approximately twice as high as we found, and they reported only trace amounts of matairesinol. For leeks, the concentration of secoisolariciresinol reported by Valsta *et al.* (2003) was approximately three times lower than we found, and they also did not detect matairesinol.

French beans (273 μg/100 g), sweet peppers (green: 172 μg/100 g; red: 113 μg/100 g), carrots (171 μg/100 g) and courgettes (119 μg/100 g) also had relatively high lignan concentrations. For all other vegetables, the total lignan concentrations were below 100 μg/100 g.

Fruits

Lignan values ranged from 0 for banana to 450 μg/100 g for apricot. Valsta *et al.* (2003) reported the lignan contents of six fruits, of which we also measured five. The concentrations of secoisolariciresinol and matairesinol in pear, grapefruit, olive and kiwi were somewhat higher than we found, whereas we found a higher amount of secoisolariciresinol in grapes. Horn-Ross *et al.* (2000) were able to detect secoisolariciresinol and matairesinol in only a few dried fruits (apricots, prunes, raisins) and in peaches. The concentrations they reported for peaches agree well with ours.

Strawberries had a total lignan concentration of 334 μg/100 g. Mazur *et al.* (2000) found relatively high levels of secoisolariciresinol in berries. Although we did not find a large amount of secoisolariciresinol in strawberries, the amounts of lariciresinol and pinoresinol were relatively large.

Raisins (white and blue) were one of the few products in which we detected matairesinol (19 and 18 μg/100 g, respectively). However, the amount of matairesinol in raisins was low compared with that of lariciresinol (153 and 118 μg/100 g for white and blue raisins respectively). Horn-Ross *et al.* (2000) reported a higher concentration of matairesinol in raisins (52 μg/100 g) and only trace amounts of secoisolariciresinol.

The lignan concentration in apple was only 1 μg/100 g, which is interesting because apple is an important source of other polyphenols such as flavonoids (Hertog *et al.* 1992; Arts *et al.* 2000). In addition, Mazur & Adlercreutz (1998) and Horn-Ross *et al.* (2000) reported only trace amounts of lignans in apple.

Vegetable oils and fats

The amount of pinoresinol in extra-virgin olive oil (243 μg/100 g) was higher than that in regular olive oil (101 μg/100 g). This is in agreement with the data published by Owen *et al.* (2000), who found low levels of lignans in refined olive oil but lignan levels of 65–10 000 μg/100 g in extra-virgin oils. These lignans were identified as pinoresinol, 1-acetoxypinoresinol and 1-hydroxypinoresinol. The amount of pinoresinol that we found in extra-virgin oil (of unknown origin), was much lower than that found in Spanish extra-virgin olive oils (range 2000–4500 μg/100 g; Brenes *et al.* 2002*b*). Possibly, extra-virgin olive oil in the Netherlands is more refined than in Spain. Margarine was also selected for analysis, since it contains vegetable fats. The lignan concentration in margarine was 39 μg/100 g; it contained mainly secoisolariciresinol. No lignans were found in soya and sunflower oil.

Other solid foods

The concentration of lignans in tomato paste was higher than that of tomato, which can of course be explained by the concentration step that takes place during manufacturing. It also shows that lignans can (at least partially) survive the production process of tomato paste.

Soya products have primarily been included in phytoestrogen databases because of the high amount of isoflavones in soya, but soyabeans were additionally shown to contain a relatively high amount of secoisolariciresinol (13–273 μg/100 g; Mazur *et al.* 1998*a*). In tofu, however, we found only 18 μg/100 g. Horn-Ross *et al.* (2000) also detected a relatively high amount of secoisolariciresinol (140 μg/100 g) in soyabeans, but did not detect any secoisolariciresinol or matairesinol in tofu. This is in agreement with our result, because of their high limit of detection of 25 μg/100 g.

Cocoa powder and plain chocolate contained a substantial amount of lignans, although the amount of secoisolariciresinol was approximately 50 % lower than that reported by Valsta *et al.* (2003). Similar to our findings, they did not detect any matairesinol.

Alcoholic beverages

Wine, especially red wine, is an important source of several polyphenols (Manach *et al.* 2004), and we found that it also contained a relatively high concentration of lignans. Red wine contained on average 80 μg/100 ml, whereas white wine contained 22 μg/100 ml. Secoisolariciresinol was the most abundant lignan in wine, except in one white wine in which lariciresinol was more abundant. Our results for secoisolariciresinol and matairesinol were slightly lower than those reported by Mazur (1998). Nurmi *et al.* (2003) analysed lignans in wines using HPLC with coulometric electrode array detection. Besides secoisolariciresinol and matairesinol, they also included other plant lignans. Their results for secoisolariciresinol, matairesinol and lariciresinol were slightly lower than ours. They could not exactly quantify pinoresinol but reported that the amount was similar to that of lariciresinol, which is also in agreement with our results.

On average, lager beer contained more lignans (27 μg/100 ml) than white wine but less than red wine. Beer contained mainly pinoresinol and lariciresinol, and only a little secoisolariciresinol.

Non-alcoholic beverages

For the non-alcoholic beverages, the highest amount of lignans was found in tea, a product that is a rich source of various other polyphenols (Manach *et al.* 2004). The amounts of lignans in the three blends of black tea that were analysed were comparable (64–77 μg/100 ml), but the amount of lignans in green tea was lower (39 μg/100 ml). Mazur *et al.* (1998*b*) expressed the lignan values of tea infusions on a mg/kg tea leaves basis. If we convert our tea infusion values to mg/kg tea leaves, our results for secoisolariciresinol are

Table 3. Comparison of lignan concentrations (μg/100 g) in raw and cooked vegetables
(Mean of duplicate analyses)

Product		Fresh weight					Dry weight	
		LARI	PINO	SECO	Total	Prepared/raw (%)*	Total	Prepared/raw (%)*
Boiled vegetables								
Carrot	Raw	60	19	93	171		2039	
	Boiled	73	27	77	178	104	2058	101
Chicory	Raw	6	25	17	48		895	
	Boiled	3	12	13	29	60	567	63
Endive	Raw	15	9	14	38		528	
	Boiled	6	10	11	27	72	339	64
Potato (Nicola)	Raw	28	0	4	31		149	
	Boiled	17	0	2	20	63	98	65
Fried vegetables								
Onion	Raw	19	0	18	36		349	
	Fried	23	0	27	50	136	288	83
Sweet pepper (red)	Raw	106	1	7	113		1255	
	Fried	143	2	14	159	140	983	78
Sweet pepper (green)	Raw	164	1	7	172		2685	
	Fried	182	3	8	193	112	1747	65

LARI, lariciresinol; PINO, pinoresinol; SECO, secoisolariciresinol. O, below detection limit: 4 μg dry weight lariciresinol, 10 μg/100 g dry weight for pinoresinol, 6 μg/100 g dry weight for secoisolariciresinol and matairesinol.
* % = lignan concentration in boiled or fried vegetable compared with raw vegetable.

approximately 1·5–2·5-fold lower than those of Mazur and co-workers, whereas the results for matairesinol are similar. For the preparation of their tea infusion, both the amount of tea leaves (per ml water) and the extraction time used by Mazur and co-workers were twice that of our method. Since we found lower values of secoisolariciresinol in both black and green tea, this might indicate that a higher proportion of secoisolariciresinol could be extracted with longer extraction times. However, our aim was to analyse tea infusion as it is commonly consumed in the Netherlands.

The amount of lignans in coffee was lower than that in tea (19–31 μg/100 ml), which is consistent with the results of Mazur *et al.* (1998*b*).

The total lignan concentration in juices was 7–25 μg/100 ml. As for red wine, and blue grapes, the amount of lignans was higher in blue grape juice than in white grape juice. In general, therefore, the lignan content of blue grapes seems higher than that of white grapes, although the amount of lignans in white raisins was higher than in blue raisins. We did not find a difference between orange juice with and without pulp. We hypothesised that this could be caused by the fact that lignans were not released from the pulp. We thus also analysed juice with pulp with the method for solid foods (including alkaline extraction). This resulted in slightly lower lignan concentrations compared with the analysis without alkaline extraction (results not shown). Thus, the pulp in orange juice did not contain a measurable amount of lignans.

Soya milk contained 38 μg lignans/100 ml, mainly pinoresinol and lariciresinol. We measured only 1 μg/100 ml of secoisolariciresinol in soya milk, whereas Adlercreutz *et al.* (2000) found 10–20 μg/100 g in soya milk and drinks. Horn-Ross *et al.* (2000) reported 32 μg/100 ml secoisolariciresinol in soya milk.

Effect of cooking of vegetables

Lignan values in boiled vegetables were on average 25 % lower than those in raw vegetables (Table 3), whereas after frying, lignan concentrations were on average 30 % higher. The increased lignan concentrations after frying can be explained mainly by the decreased moisture content of the fried foods. On a dry weight basis, the amount of lignans after frying decreased by 25 %, comparable to what we saw for boiling. The vegetables were fried in margarine, which we have shown also to contain some lignans. However, we calculated that the maximum contribution from lignans in the margarine was less than 1 %.

To our knowledge, effects of food preparation on lignan content have only been reported for baking bread, thermal treatments of olive oil, and roasting of pumpkin seeds. Muir & Westcott (2000) reported that secoisolariciresinol diglucoside (purified or as flaxseed), added to wheat flour before the preparation of bread, was stable in the bread making process. Besides, they found that secoisolariciresinol diglucoside could withstand the higher temperatures in the core during baking. Brenes *et al.* (2002*a*) found that microwave heating of olive oil for 10 min did not change the amount of lignans, including pinoresinol. Even after 25 h (simulated) frying at 180°C, only 20–50 % of the lignans were lost, whereas other phenolic compounds were almost completely destroyed. When olive oil was boiled with water (at pH 4–6) for 30 min, a large proportion of lignans leached into the water phase, but the total decrease in the lignan concentration was only 30 %, irrespective of the pH. Murkovic *et al.* (2004) reported that secoisolariciresinol in pumpkin seeds was completely destroyed after 20 min of roasting. Thus, a further evaluation of the effects of food processing might increase the reliability of lignan intake estimations.

In summary, almost all of the 109 measured products contained lignans, and in most cases the amount of lariciresinol and pinoresinol was larger than that of secoisolariciresinol and matairesinol. Thus, available databases largely underestimate the amount of enterolignan precursors in foods. The database reported here will enable a more comprehensive evaluation of the health effects of lignan intake.

Acknowledgements

Lariciresinol and pinoresinol were kindly provided by Dr Ozawa (Rakuno Gakuen University, Japan), Dr Deyama (Yomeishu Seizo Co. Ltd, Japan) and Dr Nishibe (University of Hokkaido, Japan). Internal standards secoisolariciresinol-d_8 and matairesinol-d_6 were kindly provided by Dr K. Wähälä (University of Helsinki, Finland). This work was financed by the Netherlands Organisation for Health Research and Development (Program Nutrition: Health, Safety and Sustainability; project number 014-12-014).

References

Adlercreutz H & Mazur W (1997) Phyto-oestrogens and Western diseases. *Ann Med* **29**, 95–120.

Adlercreutz H, Mazur W, Bartels P, *et al.* (2000) Phytoestrogens and prostate disease. *J Nutr* **130**, 658S–659S.

Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wähälä K, Makela T & Hase T (1992) Dietary phytoestrogens and cancer – in vitro and in vivo studies. *J Steroid Biochem Mol Biol* **41**, 331–337.

Andreasen MF, Kroon PA, Williamson G & Garcia-Conesa MT (2001) Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radical Biol Med* **31**, 304–314.

Arts ICW & Hollman PCH (2005) Polyphenols and disease risk in epidemiological studies. *Am J Clin Nutr* **81** Suppl., 5317–5325.

Arts ICW, van de Putte B & Hollman PCH (2000) Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* **48**, 1746–1751.

Begum AN, Nicolle C, Mila I, *et al.* (2004) Dietary lignins are precursors of mammalian lignans in rats. *J Nutr* **134**, 120–127.

Brenes M, Garcia A, Dobarganes MC, Velasco J & Romero C (2002*a*) Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. *J Agric Food Chem* **50**, 5962–5967.

Brenes M, Garcia A, Rios JJ, Garcia P & Garrido A (2002*b*) Use of 1-acetoxypinoresinol to authenticate Picual olive oils. *Int J Food Sci Tech* **37**, 615–625.

Dai Q, Franke AA, Jin F, Shu XO, Hebert JR, Custer LJ, Cheng JR, Gan YT & Zheng W (2002) Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. *Cancer Epidemiol Biomarkers Prev* **11**, 815–821.

Den Tonkelaar I, Keinan-Boker L, Van t Veer P, Arts CJM, Adlercreutz H, Thijssen JHH & Peeters PHM (2001) Urinary phytoestrogens and postmenopausal breast cancer risk. *Cancer Epidemiol Biomarkers Prev* **10**, 223–228.

Ford JD, Huang KS, Wang HB, Davin LB & Lewis NG (2001) Biosynthetic pathway to the cancer chemopreventive secoisolariciresinol diglucoside-hydroxymethyl glutaryl ester-linked lignan oligomers in flax (*Linum usitatissimum*) seed. *J Nat Prod* **64**, 1388–1397.

Grace PB, Taylor JI, Low YL, *et al.* (2004) Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European prospective investigation of cancer and nutrition-norfolk. *Cancer Epidemiol Biomarkers Prev* **13**, 698–708.

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

Hertog MGL, Hollman PCH & Katan MB (1992) Content of potentially anticarcinogenic flavanoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* **40**, 2379–2383.

Horn-Ross PL, Barnes S, Lee M, Coward L, Mandel JE, Koo J, John EM & Smith M (2000) Assessing phytoestrogen exposure in epidemiologic studies: development of a database (United States). *Cancer Causes Control* **11**, 289–298.

Horn-Ross PL, Hoggatt KJ & Lee MM (2002*a*) Phytoestrogens and thyroid cancer risk: the San Francisco Bay Area thyroid cancer study. *Cancer Epidemiol Biomarkers Prev* **11**, 43–49.

Horn-Ross PL, Hoggatt KJ, West DW, *et al.* (2002*b*) Recent diet and breast cancer risk: the California teachers study (USA). *Cancer Causes Control* **13**, 407–415.

Horn-Ross PL, John EM, Canchola AJ, Stewart SL & Lee MM (2003) Phytoestrogen intake and endometrial cancer risk. *J Natl Cancer Inst* **95**, 1158–1164.

Horn-Ross PL, John EM, Lee M, *et al.* (2001) Phytoestrogen consumption and breast cancer risk in a multiethnic population – the Bay Area Breast Cancer Study. *Am J Epidemiol* **154**, 434–441.

Hulshof KF & Van Staveren WA (1991) The Dutch National Food Consumption Survey: design, methods and first results. *Food Policy* **16**, 257–260.

Hulten K, Winkvist A, Lenner P, Johansson R, Adlercreutz H & Hallmans G (2002) An incident case-referent study on plasma enterolactone and breast cancer risk. *Eur J Nutr* **41**, 168–176.

Ingram D, Sanders K, Kolybaba M & Lopez D (1997) Case-control study of phyto-oestrogens and breast cancer. *Lancet* **350**, 990–994.

Jiao Y, Davin LB & Lewis NG (1998) Furanofuran lignan metabolism as a function of seed maturation in *Sesamum indicum*: methylenedioxy bridge formation. *Phytochemistry* **49**, 387–394.

Johnsson P, Kamal Eldin A, Lundgren LN & Aman P (2000) HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds. *J Agric Food Chem* **48**, 5216–5219.

Juntunen KS, Mazur WM, Liukkonen KH, Uehara M, Poutanen KS, Adlercreutz HCT & Mykkanen HM (2000) Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women. *Br J Nutr* **84**, 839–846.

Kato MJ, Chu A, Davin LB & Lewis NG (1998) Biosynthesis of antioxidant lignans in *Sesamum indicum* seeds. *Phytochemistry* **47**, 583–591.

Katsuzaki H, Kawasumi M, Kawakishi S & Osawa T (1992) Structure of novel antioxidative lignan glucosides isolated from sesame seed. *Biosci Biotechn Biochem* **56**, 2087–2088.

Keinan Boker L, Van der Schouw YT, De Kleijn MJ, Jacques PF, Grobbee DE & Peeters PH (2002) Intake of dietary phytoestrogens by Dutch women. *J Nutr* **132**, 1319–1328.

Kraushofer T & Sontag G (2002*a*) Determination of some phenolic compounds in flax seed and nettle roots by HPLC with coulometric electrode array detection. *Eur Food Res. Technol.* **215**, 529–533.

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

Lapierre C, Pollet B, Ralet MC & Saulnier L (2001) The phenolic fraction of maize bran: evidence for lignin-heteroxylan association. *Phytochemistry* **57**, 765–772.

Liggins J, Grimwood R & Bingham SA (2000) Extraction and quantification of lignan phytoestrogens in food and human samples. *Anal Biochem* **287**, 102–109.

Linseisen J, Piller R, Hermann S & Chang-Claude J (2004) Dietary phytoestrogen intake and premenopausal breast cancer risk in a German case-control study. *Int J Cancer* **110**, 284–290.

McCann SE, Freudenheim JL, Marshall JR & Graham S (2003) Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. *J Nutr* **133**, 1937–1942.

McCann SE, Moysich KB, Freudenheim JL, Ambrosone CB & Shields PG (2002) The risk of breast cancer associated with dietary lignans differs by CYP17 genotype in women. *J Nutr* **132**, 3036–3041.

Manach C, Scalbert A, Morand C, Remesy C & Jimenez L (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**, 727–747.

Mazur W (1998) Phytoestrogen content in foods. *Baillières Clin Endocrinol Metab* **12**, 729–742.

Mazur W & Adlercreutz H (1998) Naturally occurring oestrogens in food. *Pure Appl Chem* **70**, 1759–1776.

Mazur W & Adlercreutz H (2000) Overview of naturally occurring endocrine-active substances in the human diet in relation to human health. *Nutrition* **16**, 654–658.

Mazur WM, Duke JA, Wähälä K, Rasku S & Adlercreutz H (1998*a*) Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. *J Nutr Biochem* **9**, 193–200.

Mazur W, Fotsis T, Wähälä K, Ojala S, Salakka A & Adlercreutz H (1996) Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. *Anal Biochem* **233**, 169–180.

Mazur WM, Uehara M, Wähälä K & Adlercreutz H (2000) Phyto-oestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. *Br J Nutr* **83**, 381–387.

Mazur WM, Wähälä K, Rasku S, Salakka A, Hase T & Adlercreutz H (1998*b*) Lignan and isoflavonoid concentrations in tea and coffee. *Br J Nutr* **79**, 37–45.

Meagher LP & Beecher GR (2000) Assessment of data on the lignan content of foods. *J Food Compos Anal* **13**, 935–947.

Meagher LP, Beecher GR, Flanagan VP & Li BW (1999) Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. *J Agric Food Chem* **47**, 3173–3180.

Milder IEJ, Arts ICW, Venema DP, Lasaroms JJP, Wähälä K & Holllman PCH (2004) 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* **52**, 4643–4651.

Muir AD & Westcott ND (2000) Quantitation of the lignan secoisolariciresinol diglucoside in baked goods containing flax seed or flax meal. *J Agric Food Chem* **48**, 4048–4052.

Murkovic M, Piironen V, Lampi AM, Kraushofer T & Sontag G (2004) Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil. 1. Non-volatile compounds. *Food Chem* **84**, 359–365.

Nilsson M, Aman P, Harkonen H, Hallmans G, Knudsen KEE, Mazur W & Adlercreutz H (1997*a*) Nutrient and lignan content, dough properties and baking performance of rye samples used in Scandinavia. *Acta Agric Scand Sect B Soil Plant Sci* **47**, 26–34.

Nilsson M, Aman P, Harkonen H, Hallmans G, Knudsen K, Mazur W & Adlercreutz H (1997*b*) Content of nutrients and lignans in roller milled fractions of rye. *J Sci Food Agric* **73**, 143–148.

Nurmi T, Heinonen S, Mazur W, Deyama T, Nishibe S & Adlercreutz H (2003) Lignans in selected wines. *Food Chem* **83**, 303–309.

Obermeyer WR, Musser SM, Betz JM, Casey RE, Pohland AE & Page SW (1995) Chemical studies of phytoestrogens and related compounds in dietary supplements: flax and chaparral. *Proc Soc Exp Biol Med* **208**, 6–12.

Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B & Bartsch H (2000) Identification of lignans as major components in the phenolic fraction of olive oil. *Clin Chem* **46**, 976–988.

Pietinen P, Stumpf K, Mannisto S, Kataja V, Uusitupa M & Adlercreutz H (2001) Serum enterolactone and risk of breast cancer: a case-control study in Eastern Finland. *Cancer Epidemiol Biomarkers Prev* **10**, 339–344.

Qui SX, Lu ZZ, Luyengi L, Lee SK, Pezzuto JM, Farnsworth NR, Thompson LU & Fong HHS (1999) Isolation and characterization of flaxseed (*Linum usitatissimum*) constituents. *Pharm Biol* **37**, 1–7.

Raffaelli B, Hoikkala A, Leppala E & Wähälä K (2002) Enterolignans. *J Chromatogr B Biomed Sci Appl* **777**, 29–43.

Sicilia T, Niemeyer HB, Honig DM & Metzler M (2003) Identification and stereochemical characterization of lignans in flaxseed and pumpkin seeds. *J Agric Food Chem* **51**, 1181–1188.

Valsta LM, Kilkkinen A, Mazur W, Nurmi T, Lampi AM, Ovaskainen ML, Korhonen T, Adlercreutz H & Pietinen P (2003) Phyto-oestrogen database of foods and average intake in Finland. *Br J Nutr* **89**, S31–S38.

Van Dooren-Flipsen MMH, Boeijen I, Van Klaveren JD & Van Donkersgoed G (1995) *Conversie van consumeerbare voedingsmiddelen naar primaire agrarische producten* [in Dutch]. Report-95.17. Wageningen, The Netherlands: RIKILT.

Vanharanta M, Voutilainen S, Lakka TA, van der Lee M, Adlercreutz H & Salonen JT (1999) Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. *Lancet* **354**, 2112–2115.

Vanharanta M, Voutilainen S, Rissanen TH, Adlercreutz H & Salonen JT (2003) Risk of cardiovascular disease-related and all-cause death according to serum concentrations of enterolactone: Kuopio ischaemic heart disease risk factor study. *Arch Intern Med* **163**, 1099–1104.

Voedingscentrum (1998) *Zo eet Nederland; Resultaten van de Voedselconsumptiepeiling 1997–1998* [in Dutch]. The Hague, The Netherlands: Voedingscentrum.