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Quantification of a Broad Spectrum of Lignans in Cereals, Oilseeds, and Nuts

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Twenty-four plant lignans were analyzed by high-performance liquid chromatography−tandem mass spectrometry in bran extracts of 16 cereal species, in four nut species, and in two oilseed species (sesame seeds and linseeds). Eighteen of these were lignans previously unidentified in these species, and of these, 16 were identified in the analyzed samples. Four different extraction methods were applied as follows: alkaline extraction, mild acid extraction, a combination of alkaline and mild acid extraction, or accelerated solvent extraction. The extraction method was of great importance for the lignan yield. 7-Hydroxymatairesinol, which has not previously been detected in cereals because of destructive extraction methods, was the dominant lignan in wheat, triticale, oat, barley, millet, corn bran, and amaranth whole grain. Syringaresinol was the other dominant cereal lignan. Wheat and rye bran had the highest lignan content of all cereals; however, linseeds and sesame seeds were by far the most lignan-rich of the studied species.

KEYWORDS: 7-Hydroxymatairesinol; syringaresinol; lignans; cereals; rye bran; wheat bran; HPLC-MS/MS; accelerated solvent extraction

INTRODUCTION

Lignans are natural polyphenols widely distributed in the plant kingdom as natural defense substances. They have been suggested to induce a wide range of biological effects, such as antioxidant, antitumor, antiviral, antibacterial, insecticidal, fungistatic, estrogenic, and antiestrogenic activities, and to protect against coronary heart disease (*1*−*10*).

It has been known for a long time that the lignans secoisolariciresinol (**10**) and matairesinol (**15**) (**Figure 1**) are present in our diet, e.g., in oilseeds, nuts, grains, cereals, legumes, berries, and fruits (*11*). Very recently, the identification of other lignans was reported in various kinds of foods, including cereals. Milder et al. (*12*) reported quantities of pinoresinol (**1**) and lariciresinol (**6**), and the list was soon extended with mediores-inol (**2**) and syringaresinol (**3**), as reported by Peñalvo et al. (*13*). Linseeds and sesame seeds seem to be the richest dietary sources of lignans (*12*); however, knots of Norway spruce are by far the richest natural known sources of lignans (*14*). 7-Hydroxymatairesinol (**16**) is an abundant spruce knot lignan (*14*, *15*), and sesame seed is the only foodstuff in which this lignan has been previously identified (*16*).

The role of the extraction method for the yield of lignans from food matrices is an important issue that has practically been ignored in previous studies. Destructive methods, i.e., alkaline or acid extraction under severe conditions, have been applied in most of the previous studies (*11*−*13*, *17*−*19*). It is known that **10** occurs in linseeds as diglucoside-hydroxymethyl glutaryl ester-linked oligomers (*20*). Milder et al. (*19*) discovered that alkaline hydrolysis substantially increases the lignan yield from linseeds but also from broccoli and bread, indicating that ester-linked lignans occur in other foods besides linseeds. The structures of these ester-linked lignans are, however, not known, at least yet. They may have a similar structure as the ester-linked oligomers of **10** occurring in linseeds. Strong acid hydrolysis breaks both ester linkages and glycosidic bonds, whereas alkaline hydrolysis breaks ester linkages but requires an additional acid or enzymatic hydrolysis step to release lignan glycosides. Unfortunately, both strong alkaline and strong acid treatment are destructive to some lignans or cause transformation reactions from one lignan to another lignan. In **Figure 2**, the

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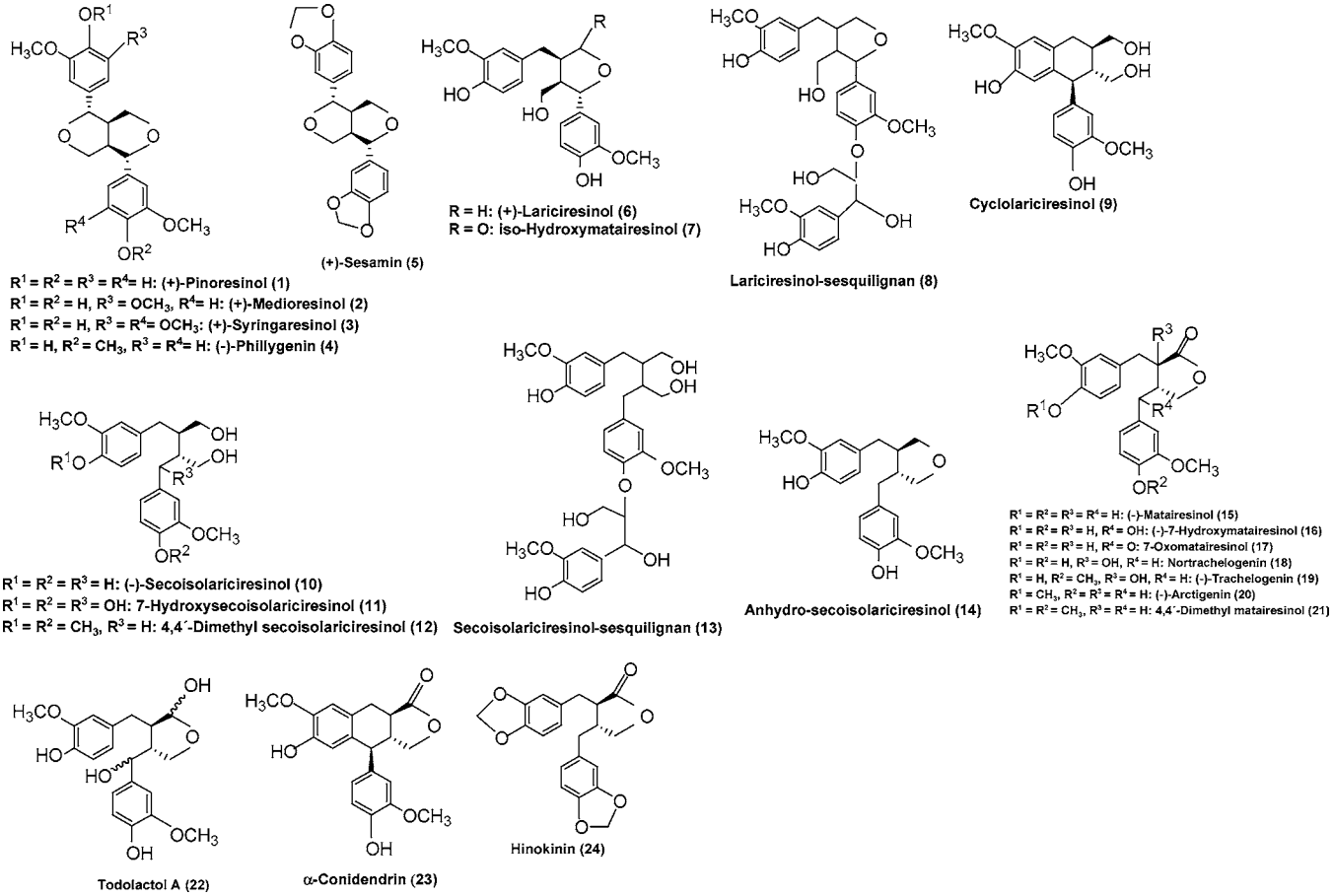

Figure 1. Molecular structures of the lignans determined in the present study.

known conversions are presented. Under alkaline conditions, **16** forms α-conidendrin (**23**), hydroxymatairesinol acid, and **7** (*21*), and **23** is (further) converted to α-conidendric acid (*21*−*23*), which is the main product of **16** in alkaline solution (*21*). Also, **15** is unstable during alkaline extraction (*12*). Furthermore, under acid conditions, both **16** and α-conidendric acid are converted to **23** (*21*, *24*), **6** is converted to cyclolariciresinol (**9**) (*25*−*28*), and **10** is converted to anhydro secoisolariciresinol (**14**) (*17*) (**Figure 2**).

Lignans **7**, **9**, **16**, **23**, and α-conidendric acid are known constituents in Norway spruce knotwood (*14*, *15*, *29*) and, therefore, also potential constituents in other plants such as cereals. Consequently, application of strong alkaline or acid conditions during the extraction of these lignans from the plant matrix gives misleading results. Very recently, a milder method was developed for analysis of **6**, **9**, and **10** in foodstuffs, applying methanolysis by sonication with sodium methoxide to cleave possible ester bonds of esterified lignan glycosides, with subsequent enzymatic hydrolysis to cleave glycosidic bonds (*30*).

One limiting factor for identifying and quantifying lignans has been the lack of pure reference standards. We have in our laboratories, due to isolation from wood (*1*, *31*, *32*) and herbal species (*33*−*37*) and to chemical modification of lignans (*25*, *29*, *38*, *39*), a collection of more than 40 pure lignan and lignan glycoside preparations. Therefore, it is now possible for us to quantify a broader spectrum of lignans than previously reported. In this study, we analyzed 18 plant lignans previously unidentified in cereals by high-performance liquid chromatography−tandem mass spectrometry (HPLC-MS/MS) in bran extracts of 16 cereal species and, additionally, in four nut species and in oilseeds (sesame seeds and linseeds).

The main objective of the present study was to carry out a comprehensive survey on the lignan content and composition of some agricultural products with a special emphasis on cereals. The simplest way to determine lignans is in their aglycone form, wherefore some kinds of hydrolysis methods are necessary in order to release ether- and/or ester-linked lignans. This led us to investigate the role of extraction method and of enzymatic hydrolysis on the lignan yield.

MATERIALS AND METHODS

Chemicals and Reference Compounds. All solvents were of analytical grade and purchased from commercial sources. β-Glucuronidase/sulfatase (type H-1, from *Helix pomatia*) (Sigma-Aldrich Co., St. Louis, MO) containing 492 units/mg of β-glucuronidase and 10 units/mg of sulfatase was used for the enzymatic hydrolysis.

Lignan **16** was isolated from knots of *Picea abies* as described previously (*24*). The preparation was a mixture of two stereoisomers, differing in the stereochemistry at C-7 [(−)-*allo*-7-hydroxymatairesinol (minor isomer) and (−)-7-hydroxymatairesinol (major isomer)] with a ratio of about 30/70. Lignans **6** and **10** were isolated from knots of *Abies balsamea* and *Araucaria angustifolia*, respectively, as described previously (*1*, *40*). Lignan **1** was isolated from the excudated resin of *P. abies*. Lignan **7** (*29*), 7-hydroxysecoisolariciresinol (**11**) (*25*), **15**, **23** (*24*), and 7-oxomatairesinol (**17**) (*38*) were prepared from **16** according to methods described previously. Lignans **2**, **3** (*33*), phillygenin (**4**) (*34*), trachelogenin (**19**) (*35*), arctigenin (**20**) (*36*), and sesamin (**5**) (*37*) were isolated from herbal species as described previously. Lignan **9** was prepared by treating **6** with concentrated formic acid. Sesquilignans of **6** and **10** (**8** and **13**), nortrachelogenin (**18**), and todolactol A (**22**) were prepared as described by Willför et al. (*1*, *31*, *32*). Also 4,4′-dimethyl secoisolariciresinol (**12**), **14**, 4,4′-dimethyl matairesinol (**21**), hinokinin (**24**), and 3,3′-diOCD_3-pinoresinol (*d*-labeled dimethylated **1**), which was used as an internal standard, were

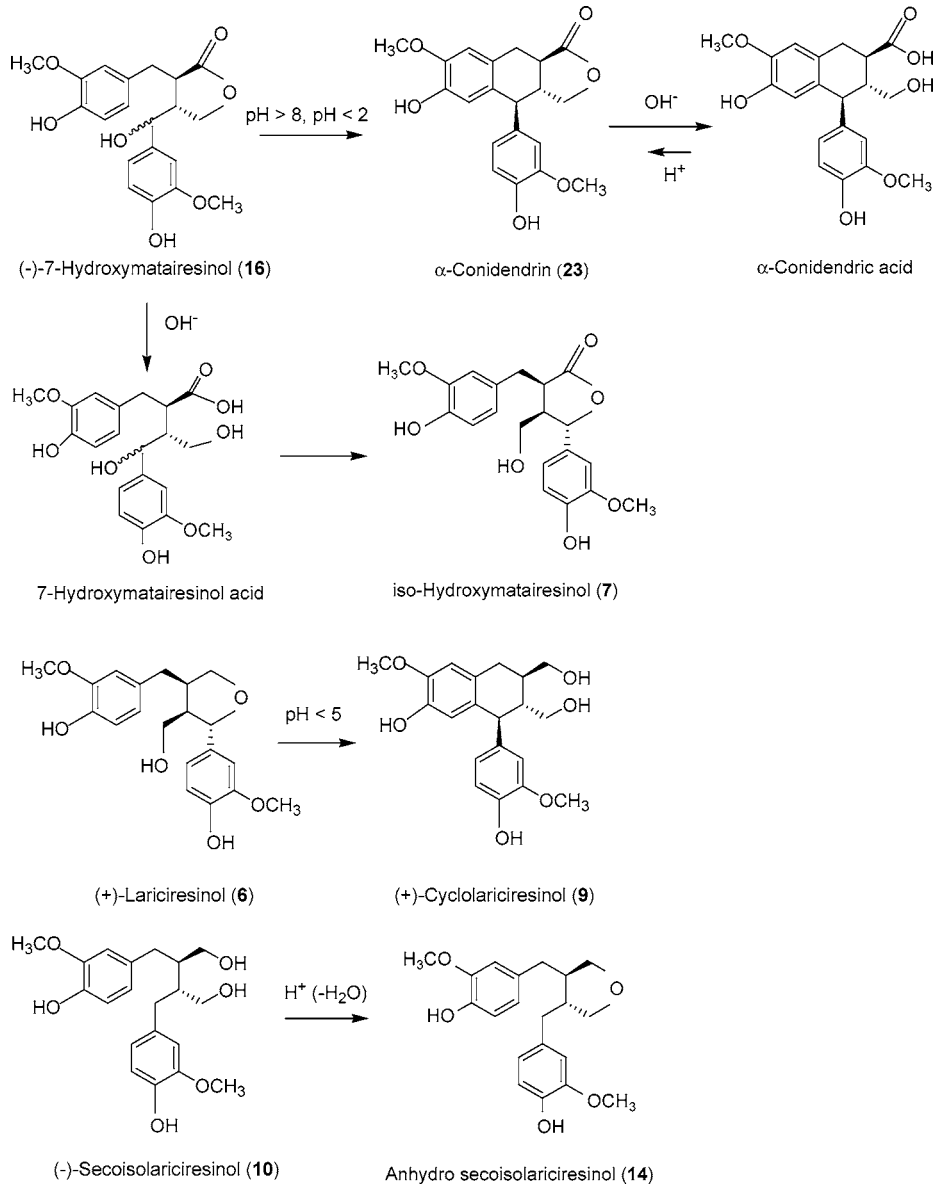

Figure 2. Known conversions of lignans during treatment with bases or acids in aqueous solutions.

prepared in our laboratory. Lignan **14** was prepared by acidic cyclization of **10**. Lignan **24** was prepared from didemethylated matairesinol [prepared in our laboratory from **15** according to a modification of a previously described method (*41*)] by reaction with diiodomethane and with potassium carbonate in acetone. Lignans **12** and **21** were prepared from **10** and **15**, respectively, by treating with methyl iodide and with potassium carbonate in acetone. *d*-Labeled dimethylated **1** was prepared analogously from **1** using trideuterated methyl iodide. Matairesinol-d_6 (*d*-labeled **15**, deuterated at positions C-2, C-2′, C-5, C-5′, C-6, and C-6′), which was used as the other internal standard, was prepared as described previously (*42*).

The purities of the lignans as determined by gas chromatography−mass spectrometry (GC-MS) (in silylated form) were the following (%): **1**, **24**, and *d*-labeled dimethylated **1**, 99.8; **2**, **3**, **5**, **12**, and **19**−**21**, 100.0; **4**, 97.5; **6**, **7**, and **16**, 97.0; **8**, 95.5; **9**, 92.3; **10** and **23**, 98.1; **11**, 93.7; **13**, 90.5; **15**, 99.9; **17** and **22**, 98.0; **18**, 95.8; and *d*-labeled **15**, 99.5. The purities were taken into account in the quantifications. The purity of **14** was 69.2%, and the preparation contained 12.3% unreacted **10**. Therefore, **10** and **14** were determined separately.

Equipment. Cereal grains of eight cereal species, i.e., rye, wheat, oat, barley, triticale, spelt wheat, buckwheat, and millet, were ground to whole-grain flour using a Fidibus 21 stone mill (Komo GmbH, Germany). The bran was separated from the cereal grains of all 16 cereal species using a Faribon 300 grain mill (Gensaco, Inc., NY). Nuts and oilseeds were milled using a Polymix analytical mill A 10 (Kinematica AG, Switzerland). The bran was further milled using an IKA MF 10 mill with a cutting grinding head (IKA Group).

The ground samples were extracted using an Accelerated Solvent Extractor apparatus (Dionex Corp., Sunnyvale, CA). Quartz sand (granular 1−2 mm) used in ASE was purchased from J.T. Baker (Mallinckrodt Baker B.V., Deventer, The Netherlands). Deionized tap water was purified using a Simplicity 185 water purification system (Millipore Corp., Billerica, MA) and then by eluting through a glass column packed with approximately 50 g of Bondesil 40 μm RP-18 material (Varian Inc., Harbor City, CA). This lignan-free water was used in the HPLC eluent.

Lignans were quantified in cereal, oilseed, and nut samples by HPLC−electrospray ionization−MS/MS using an Agilent 1100 series HPLC (Agilent Technologies, Inc., Palo Alto, CA) system and a Micromass Quattro Micro instrument (Micromass Ltd., Manchester, United Kingdom) in the multiple reaction monitoring (MRM) mode. The analysis data were collected and analyzed using MassLynx V4.0 software, and the quantification was performed using QuanLynx V4.0 software (Micromass Ltd.).

The purities of **22** and the two sesquilignans were determined on a 10 m × 0.25 mm i.d. HP-1 GC column, temperature programmed from 100 to 340 °C, using an HP 6890-5973 GC-quadrupole-MSD (Hewlett-Packard, Palo Alto, CA). The purities of the other lignans were

determined on a 30 m × 0.20 mm i.d. HP-1 column, temperature programmed from 180 to 300 °C, using an HP 5890 GC coupled to an HP 5971A series MSD.

Samples. Dried grains of rye, wheat, oat, barley, spelt wheat, buckwheat, millet, quinoa, amaranth, brown rice, wild rice, and red rice, as well as corncobs, sesame seeds, linseeds, peanuts, almonds, cashew nuts, and walnuts, were purchased from local stores. Dhurra grains and Japanese rice bran were obtained from Japan. The grains of corncobs were gathered, frozen, and freeze-dried. Grains of triticale, var. Prego, were obtained from Boreal Kasvinjalostus Oy (Jokioinen, Finland).

Sample Preparation Methods. *ASE.* Approximately 1 g of the bran or whole-grain flour and 0.5 g of milled oilseeds or nuts were weighed, thoroughly mixed with quartz sand, and poured into ASE tubes. ASE was performed as described previously (*15*) with slight modifications. The material was extracted first with *n*-hexane, then with acetone, and finally with acetone−water (70:30, v/v). During the hexane and the acetone extraction, the solvent temperature was 90 °C, the pressure was 14.2 MPa, and 2 × 5 min static cycles were applied. During the acetone−water extraction, the same parameters were used, except that the temperature was 100 °C. The three fractions were collected into separate glass tubes. The acetone and acetone−water fractions were combined, and the solvent was evaporated to dryness using a rotary evaporator. The extract was weighed and then redissolved in a known volume of 7−10 mL of acetone−water (70:30, v/v). Two aliquots of 0.5 mL each were transferred into test tubes, and the solvent was evaporated using a stream of nitrogen gas. Enzymatic hydrolysis was performed on one of the aliquots by adding 1 mg of the enzyme preparation dissolved in 1 mL of 0.01 M acetate buffer, pH 5.0, and keeping it at 37 °C for 19 h, i.e., according to a slight modification of the method optimized by Milder et al. (*19*). To the other aliquot, which was not hydrolyzed, 1 mL of 0.01 M acetate buffer, pH 5.0, was added immediately before extraction. A 250 ng amount of *d*-labeled **15** and 212 ng of *d*-labeled dimethylated **1** were added to all samples, and the solutions were liquid−liquid extracted with 2 × 0.75 mL of ethyl acetate. The ethyl acetate phase was evaporated to dryness under nitrogen gas, and 0.5 mL of methanol/acetonitrile/0.1% acetic acid (15:15:70 v/v/v) was added. The samples were placed in an ultrasonic bath for 5 min and then centrifuged for 10 min at 4000 rpm.

Alkaline and Acid Extraction. The alkaline extraction was performed as described by Milder et al. (*19*) and Peñalvo et al. (*13*). The acid extraction was performed similarly as the alkaline extraction, except that the NaOH in the methanol solution was replaced by 0.01 M HCl. The alkaline and acid extracts were enzymatically hydrolyzed (acid extracts in which **7** was determined were not enzymatically hydrolyzed), extracted with ethyl acetate, and reconstituted as the ASE extracts.

For determination of total content of **16**, i.e., including possible esterified or other alkaline-extractable forms of **16**, alkaline-treated extracts of rye, wheat, oat, triticale, spelt wheat, and millet bran were acidified to pH 1 with HCl and kept for 3 h at room temperature. The extracts were neutralized and treated as other alkaline extracts, and the total content of **16** was determined as excess **23**.

HPLC-MS/MS Method and Quantification. The HPLC-MS/MS methods and quantification used were modifications of previously described methods (*26*, *43*, *44*). Lignans **3**−**5**, **12**, **21**, **24**, and *d*-labeled dimethylated **1** were analyzed in the positive mode, and all of the other compounds were analyzed in the negative mode. The HPLC method was modified by using a 100 mm × 2.1 mm i.d., 3.5 μm, Agilent Zorbax SB-C8 column (Agilent Technologies, Inc.) and methanol−acetonitrile 1:1 (v/v) as eluent B. As eluent A, 0.1% acetic acid−isopropanol 99:1 (v/v) was used in the negative mode and 0.05% trifluoroacetic acid was used in the positive mode. The gradient used in negative mode was from 10 to 30% B in 6 min, then to 50% B in 10 min, and to 80% B in 9 min. The initial composition was then reached after 27 min and held for 5 min, rendering a total analysis time of 32 min. The gradient used in the positive mode was similar except that the final composition was 95% B. The initial composition was reached after 28 min and held for 7 min, rendering a total analysis time of 35 min.

The MS/MS conditions used for **6**, **9**, **10** (*26*), **7**, **11** (*44*), **15**, *d*-labeled **15**, **16** (*26*, *43*), **17**, and **23** (*43*, *44*) have been described previously. The MS/MS transitions monitored for **1**, **2**, **8**, **13**, **14**, **18**, **19**, **20**, and **22** were the deprotonated molecular ions to the predominant fragment ions of *m*/*z* 151, 151, 195, 165, 328, 179, 99, 83, and 191, respectively (negative ionization mode). The cone voltages ranged from 22 to 40 V, and collision energy voltages ranged from 16 to 37 eV. The MS/MS transitions monitored for *d*-labeled dimethylated **1**, **3**, **4**, **5**, **12**, **21**, and **24** were the protonated molecular ions to the predominant fragment ions of *m*/*z* 154, 401, 355, 185, 151, 369, and 161, respectively (positive ionization mode). The cone voltages ranged from 9 to 20 V, and collision energy voltages ranged from 7 to 24 eV. The conditions were optimized by syringe infusion of the pure compounds.

The quantification was performed using calibration curves of standard solutions at six different concentration levels. Three different standard mixes were prepared in methanol with known amounts of pure compounds. One mix consisted of the lignans analyzed in positive mode; the lignans analyzed in negative mode were divided into two separate mixes. The calibration range of the analytes was usually from 5−10 to 1000−2000 ng/mL. For analysis, 0.5 mL of each standard solution was withdrawn and the solvent was evaporated to dryness. One milliliter of 0.01 M acetate buffer, pH 5.0, and internal standards were added, in the same amount as used in the real samples. The standard solutions were extracted with ethyl acetate and reconstituted as the real samples, but they were not enzymatically hydrolyzed.

All lignans analyzed in negative mode were quantified against *d*-labeled **15**; the lignans analyzed in positive mode were quantified against *d*-labeled dimethylated **1**. The analyte/internal standard concentration ratio was plotted against the analyte/internal standard peak area ratio as a linear regression curve with 1/× weighting and the origin excluded. The obtained concentration at each concentration level of the calibration curve was allowed to deviate from the nominal concentration by no more than 15%; otherwise, the calibration point was omitted. At least four out of the six calibration points were within the 15% deviation. Samples giving a concentration above the highest point of the calibration curve were diluted and reanalyzed. Intra- and interassay variations of the ASE method were determined by analyzing three rye bran extracts prepared in parallel and three extracts prepared at different days in different sample batches.

RESULTS AND DISCUSSION

Analytical Method. Figure 3A,**B** shows typical MRM chromatograms of some of the detected lignans in the ASE-extracted rye bran. The two stereoisomers of **16** are partly coeluting. The reproducible HPLC retention times relative to *d*-labeled **15** (RT 20.0 min) were as follows: **11**, 0.59; **9**, 0.69; **22**, 0.70; **16**, 0.73; **7**, 0.76; **10**, 0.78; **6**, 0.80; **8** and **13**, 0.82; **18**, 0.85; **2** and **17**, 0.91; **1**, 0.92; **23**, 0.95; **15** and **19**, 1.00; **14** and **20**, 1.13; and relative to *d*-labeled dimethylated **1** (RT 24.7 min): **3**, 0.77; **12**, 0.88; **4**, 0.94; **21**, 1.01; and **24** and **5**, 1.09.

The ASE method showed acceptable intra- and interassay variation for all of the lignans that could be detected in rye bran ($n = 3$, RSD < 20%) both in enzymatically hydrolyzed and in unhydrolyzed extracts. The lignans seemed to be quantitatively extracted by ASE from the cereal matrix, because when an ASE-extracted rye bran sample was further extracted with acetone and acetone:water 70:30 (v/v), only 1% or less of the previously obtained lignan concentration was detected.

Importance of Enzymatic Hydrolysis. Table 1 shows the proportion of lignan aglycones of total lignans, which in the case of ASE extraction means the sum of lignan aglycones and unesterified glycosides, of some cereal brans, sesame seeds, and linseeds, i.e., the effect of enzymatic hydrolysis. Lignans **1**−**3**, **6**, and **9**, which are probably located at the beginning of the biosynthetic pathway (*45*), seem to occur mainly in glycoside form in cereal brans. Lignans **10**, **15**−**17**, and **23** are probably later in this pathway, and they seem to occur mainly in aglycone form. Consequently, it seems that when determining furofuran and furano type lignans such as **1**−**3** and **6**, enzymatic hydrolysis

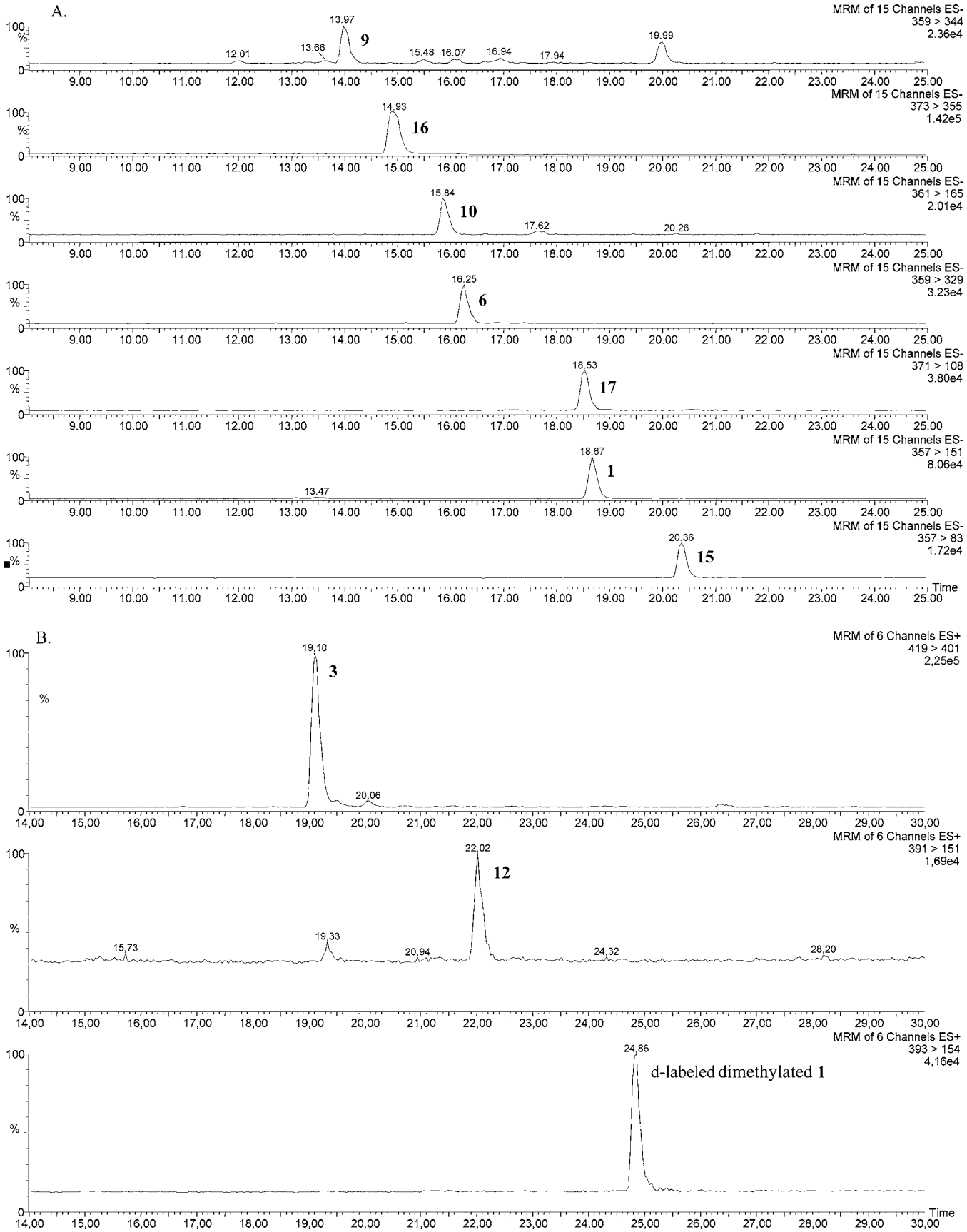

Figure 3. MRM chromatograms of an ASE-extracted rye bran sample. (**A**) Negative ionization mode (only seven of 18 detected lignans shown); (**B**) positive ionization mode.

is necessary, whereas when determining dibenzylbutanediol and dibenzylbutyrolactone type lignans such as **10**, **15**−**17**, and **23**, enzymatic hydrolysis is not always necessary. Lignan **7** was degraded during the enzymatic hydrolysis and was therefore determined only in unhydrolyzed extracts. It should also be noted that if pH values below 5 are used in the enzymatic

Table 1. Proportions (%) of Lignan Aglycones of Total Lignan Content (=Aglycones + Unesterified Glycosides) in ASE Extracts of Brans of Some Cereal Species and in Sesame and Linseeds

	lignan									
foodstuff	1	2	3	6	9	10	15	16	17	23
rye	22	16	10	18	42	38	26	100	93	89
wheat	26	0	24	53	88	100	100	100	100	100
oat	7	0	9	16	74	92	34	100	84	99
barley	66	0	13	3	43	100	100	100	100	100
buckwheat	19	0	16	16	16	86	100	92	100	0
corn	0	0	26	0	58	59	100	100	97	76
brown rice	0	0	49	16	37	69	78	77	86	0
Japanese rice	40	0	23	2	0	85	0	0	100	0
sesame seeds	32	99	26	23	100	85	34	86	86	79
linseeds	6	0	0	7	16	0	41	58	0	0

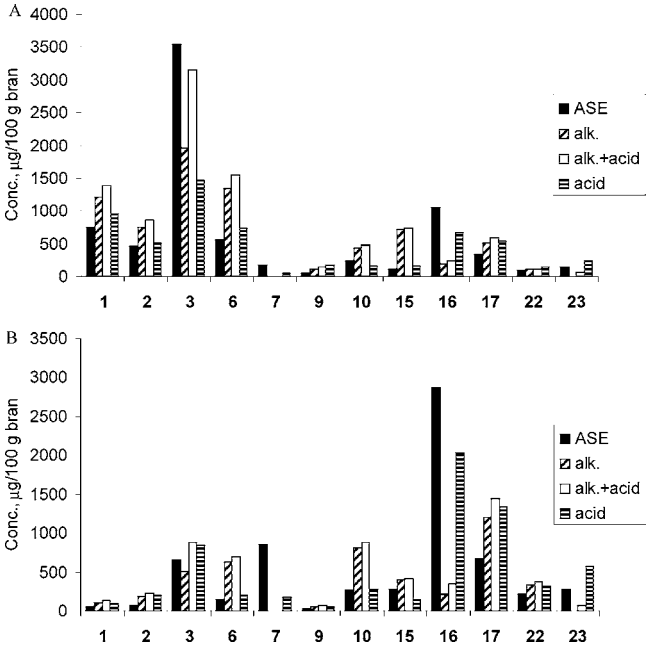

Figure 4. Concentrations (μg/100 g) of main lignans following ASE, mere alkaline extraction (alk.), alkaline followed by acid extraction (alk. + acid), or acid extraction. (**A**) Rye bran and (**B**) wheat bran.

hydrolysis, **6** is partly converted to **9** (*26*). In linseeds, most of the lignans seem to be mainly in glycoside form. The proportions of lignan aglycones in sesame seeds obtained in this study do not correspond with those obtained by Peñalvo et al. (*16*). It is possible that this proportion varies between different sesame seed samples.

Effect of the Extraction Method on Lignan Yield. Figure 4A shows the concentrations of the main lignans in a rye bran extract, and **Figure 4B** shows the concentrations in a wheat bran extract after using different extraction methods. The results show that in these two cereal brans (and also on average in all 16 cereals), a combination of alkaline and acid extraction gives the highest yield of **1**, **2**, **6**, **10**, **15**, and **17**. Consequently, these lignans seem to occur more in esterified forms in cereals than other lignans. This extraction method also gives the highest yield of **3** and **22** in wheat bran; however, in rye bran and on average in all 16 cereals, ASE gives the highest yield of **3**. Mere acid extraction seemed not to be as efficient in releasing the esterified forms of these lignans as alkaline extraction. This may be due to the mild conditions used, i.e., 0.01 M HCl, 37 °C, and 1 h as compared to 2 M HCl, 100 °C, and 2.5 h used in previous studies (*11*, *17*, *18*). However, an acid extraction after the alkaline extraction results in an increased yield of most of the lignans, indicating that lignans possibly linked to the lignin structures in the bran are released. Begum et al. (*46*) discovered that lignins account for approximately 5% of the weight in rye and wheat bran. Moreover, they found that the urinary excretion of the plant lignan metabolite enterolactone increases in rats fed with extractive-free bran or with lignin dehydrogenation polymers. Structures of **1** and **6** are incorporated in lignin (*46*), and it is possible that other lignans are also bound to lignin. These lignans are probably released under the acid conditions in the stomach, after which they can act as enterolactone precursors. The lignan fraction extracted during the mild acid treatment may correspond to the biologically available plant lignan fraction.

Surprisingly, no conversion of **10** to **14** could be observed during acid extraction, unlike previous observations using strong acid treatment (*17*). It is possible that this is due to the considerably milder acid hydrolysis conditions used in the present study as compared to the previous studies. Acid extraction gives a higher yield of **9** than alkaline extraction (rye bran) (**Figure 4A**); however, this is partly caused by conversion of **6** to **9** during the acid conditions (*25*−*28*), and **9** should therefore be extracted using mere alkaline extraction.

It seems that esterified lignan forms are partly extracted using ASE, because when the ASE extracts are subjected to alkaline hydrolysis, the lignan concentration occasionally rises (results not shown). However, our ASE method allowed the determination of only lignan aglycones and unesterified lignan glycosides. As pointed out above, ASE gives on average the highest yield of **3** of all extraction methods, contrary to the other lignans located at the beginning of the biosynthetic pathway. It seems that **3** does not usually occur in esterified form, which may be due to steric hindrance of the phenolic hydroxyl groups.

ASE gives the highest yield of both **7** and **16**, because of the alkaline and acid instability of these compounds (**Figure 4**). Lignan **16** forms **23** and conidendric acid, among other compounds, during alkaline or acid treatment (*21*, *24*). The instability of **16** in strong acid and alkali is the reason why this compound has not been detected in cereals previously. The highest yield of **23** is achieved by acid extraction (**Figure 4**). However, this compound should be extracted using ASE because **16** is partly converted to **23** in acid solution (*21*, *24*).

The degree of esterification of these alkaline- and acid-labile compounds cannot be directly determined using the conventional methods (strong alkaline or acid extraction). However, the esterified portion of **16** can be indirectly determined by acidifying the alkaline extract and determining **16** as excess **23**. This concentration of **23** includes also the possible released esterified portion of **23** itself, but because the levels of **23** are usually much lower than the levels of **16**, the obtained concentration probably reflects the approximate total concentration of **16**. The results indicated that compared to ASE, the alkaline treatment would give approximately twice as high concentrations of **16** in rye, spelt wheat, and triticale bran, and approximately three times as high concentrations in wheat, oat, and millet bran if **16** would be stable during this treatment. This indicates that a larger portion of **16** in cereal brans occurs as esterified glycosides or in other forms released only by alkaline treatment than in aglycone and unesterified glycoside form, implying that the concentrations of **16** listed in **Table 2** are underestimated. In the case of the minor cereal lignans **8**, **13**, **18**, and **22**, there were (on average) no obvious differences in lignan yield between the different extraction methods (results

Table 2. Concentrations of Lignans in Extracts of Cereal Brans, Oilseeds, and Nuts, μg/100 g[a]

(A) Major Lignans (1–3, 5, 6, 9, 10, 13, and 15–17)

foodstuff	**10**	**5**	**1**	**6**	**16**	**3**	**13**	**9**	**2**	**17**	**15**
linseeds	165759[b]	ND	871[b]	1780[b]	35[c]	48[d]	6326[c]	3726[e]	ND	9.8[b]	529[b]
sesame seeds	240[b]	62724[d]	47136[c]	13060[c]	7209[c]	205[b]	19[c]	1775[e]	4153[c]	707[b]	1137[b]
rye	462[b]	ND	1547[c]	1503[b]	1017[c]	3540[c]	37[b]	99[e]	858[b]	585[b]	729[b]
wheat	868[b]	ND	138[b]	672[b]	2787[c]	882[b]	77[b]	54[e]	232[b]	1428[b]	410[b]
triticale	165[b]	ND	518[b]	402[b]	886[c]	899[b]	28[b]	53[e]	165[b]	432[b]	34[b]
oat	90[c]	ND	567[b]	766[b]	712[d]	297[b]	27[c]	60[e]	112[b]	167[b]	440[b]
spelt wheat	155[b]	ND	494[b]	583[b]	136[d]	1126[b,c]	33[b]	61[e]	291[b]	135[b]	8.6[b]
Japanese rice	39[b]	ND	503[b]	757[b]	17[d]	967[b]	3.5[b]	66[e]	74[b]	2.9[c]	ND
wild rice	46[b]	ND	194[b]	55[b]	ND	1116[b]	ND	3.5[c]	92[b,c]	ND	1.22[c]
buckwheat	350[b]	ND	63[b]	505[b]	142[c]	145[b,c]	43[c]	145[c]	16[b]	20[c]	39[b]
barley	42[b]	ND	71[b]	133[b]	541[c]	140[b]	24[c]	28[c]	22[b]	28[c]	42[c]
amaranth	98[b]	ND	53[b]	45[b]	519[d]	47[b]	3.7[c]	8.4[e]	114[c]	207[d]	33[b]
corn	125[b]	ND	33[b]	69[b]	407[d]	220[b]	2.4[b]	11[c]	ND	161[b]	21[b]
cashew nuts	316[b]	ND	19[b]	307[b]	31[c]	26[b]	8.8[b]	147[e]	ND	2.2[d]	55[c]
almonds	159[b]	82[d]	208[b]	233[b,d]	27[c]	40[d]	8.2[b]	103[e]	ND	ND	24[c]
millet	80[b]	ND	216[b]	34[b]	160[d]	96[b]	6.0[c]	18[e]	12[b]	62[d]	82[c]
quinoa	30[b]	ND	54[b]	125[b]	163[d]	180[b]	ND	14[e]	ND	71[d]	15[b]
peanuts	116[b]	ND	32[b]	98[b]	20[c]	81[b]	32[c]	43[c]	ND	1.9[c]	19[c]
walnuts	99[b]	ND	34[b]	47[b]	5.6[d]	24[d]	15[b]	57[e]	ND	3.2[b]	60[c]
red rice	66[b]	ND	60[b]	71[b]	4.0[c]	160[b]	2.1[c]	11[e]	24[b]	1.3[c]	87[b]
brown rice	20[b]	ND	66[b]	153[b]	13[c]	99[b]	0.36[c]	8.3[e]	27[b]	2.1[c]	1.6[c]
dhurra	13[b]	ND	33[b,d]	3.8[b]	16[c]	90[b]	ND	5.6[c]	ND	0.98[c]	1.9[c]
total	169338	62806	52910	21402	14848	10428	6696	6497	5419	4027	3769

(B) Minor Lignans (**7**, **8**, **11**, **12**, **14**, and **18**–**23**)

foodstuff	**22**	**7**	**23**	**11**	**18**	**8**	**14**	**21**	**20**	**12**	**19**
linseeds	22[c]	ND	ND	958[b]	33[c]	186[b]	ND	0.8[d]	ND	ND	ND
sesame seeds	2476[c]	1611[c]	756[c]	17[b]	83[c]	83[c]	ND	397[b]	19[c]	ND	ND
rye	117[b]	177[c]	138[c]	7.8[c]	36[d]	38[b]	270[e]	ND	ND	24[c]	ND
wheat	366[b]	833[c]	271[c]	17[c]	80[d]	75[b]	34[e]	ND	ND	ND	ND
triticale	103[b]	181[c]	130[c]	5.1[c]	32[d]	16[b]	33[e]	ND	ND	1.9[c]	4.6[c]
oat	48[c]	73[c]	65[c]	ND	11[d]	28[c]	24[e]	ND	7.0[c]	ND	ND
spelt wheat	20[b]	13[c]	70[c]	21[c]	ND	10[b]	45[e]	ND	48[c]	ND	ND
Japanese rice	2.9[b]	4.7[c]	5.4[c]	ND	ND	9.4[b]	ND	ND	ND	ND	10[c]
wild rice	ND	ND	ND	ND	12[c]	ND	ND	ND	ND	ND	ND
buckwheat	87[c]	ND	ND	ND	68[c]	12[c]	8.2[e]	ND	ND	0.96[c]	ND
barley	127[c]	ND	33[c]	ND	15[b]	6.6[c]	ND	ND	ND	ND	ND
amaranth	19[b]	20[d]	5.9[c]	ND	15[d]	21[c]	ND	ND	8.2[c]	ND	ND
corn	18[c]	19[c]	32[c]	ND	11[c,d]	1.3[b]	ND	ND	ND	ND	ND
cashew nuts	ND	ND	ND	ND	12[c]	5.8[e]	ND	ND	ND	ND	ND
almonds	ND	ND	ND	ND	10[c]	ND	ND	ND	ND	ND	ND
millet	7.7[c]	13[c]	17[c]	ND	11[d]	24[c]	ND	ND	ND	15[c]	ND
quinoa	5.9[b]	9.7[c]	11[c]	ND	7.6[c]	1.3[e]	ND	ND	ND	ND	ND
peanuts	ND	ND	ND	ND	93[c]	2.8[e]	ND	ND	ND	ND	ND
walnuts	ND	ND	ND	65[c]	ND	0.29[e]	ND	ND	127[c]	ND	ND
red rice	ND	ND	ND	ND	8.0[c]	9.4[c]	ND	ND	ND	ND	ND
brown rice	ND	ND	ND	ND	2.9[c]	ND	ND	ND	ND	ND	3.2[c]
dhurra	ND	ND	4.3[c]	ND	1.3[b]	ND	ND	ND	ND	ND	ND
total	3419	2954	1539	1291	542	530	414	398	209	42	18

[a] The extraction method giving the highest yield was chosen. The compounds were quantified in enzymatically hydrolyzed samples, except for **7**, which was quantified in unhydrolyzed samples. ND, not detected. [b] Alkaline extraction followed by acid extraction. [c] ASE extraction. [d] Acid extraction. [e] Alkaline extraction.

not shown), indicating that these lignans do not occur in esterified form at all.

Figure 5 shows the total concentration (sum of all lignans) in 16 cereal species after using different extraction methods. In the case of rye, wheat, and barley bran, ASE gives the highest total lignan yield, which is due to the relatively high content of **3** and **16** of these cereals. With corn, amaranth, millet, and quinoa, mere acid extraction gives the highest total lignan yield. A combination of alkaline and acid extraction gives the highest yield in case of almost all other cereal species. On average, for all 16 cereal species, this extraction method gives approximately an 11% higher yield than ASE or acid extraction. Wheat bran seems to contain the highest lignan content accessible for the human body (i.e., lignan yield after acid extraction), followed by rye and triticale/oat/spelt wheat bran.

To conclude, when determining several different types of lignans, including alkaline- and acid-labile lignans, at least two different extraction methods should be applied, e.g., alkaline extraction followed by acid extraction combined with a mild extraction method such as ASE.

Proportion of Lignans in Bran and in Whole Grain. It has been shown previously that **10** and **15** are concentrated in the bran layer of the grain of rye, wheat, and oat (*11*). These findings are supported by the present study. The proportion of total lignans in whole grain as compared to bran of wheat was only 9.6% and ranged between 25 and 40% in rye, oat, barley, and spelt wheat. However, in triticale, buckwheat, and millet, the lignans seemed not to be concentrated in the bran layer. The proportion of lignans in whole grain as compared to bran ranged between 62 and 78% in these species.

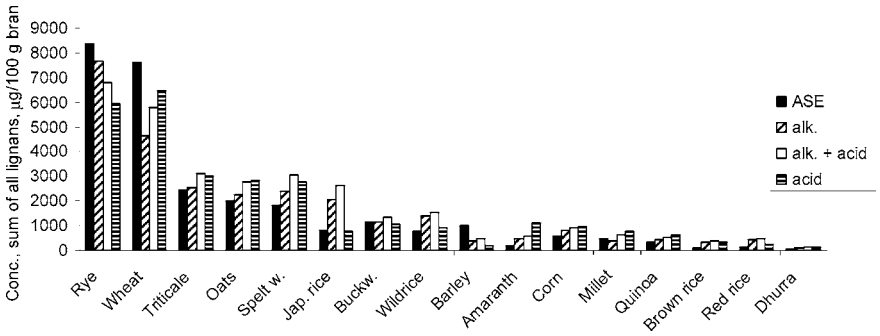

Figure 5. Total lignan concentration (μg/100 g) (sum of all lignans) in 16 cereal species following ASE, mere alkaline extraction (alk.), alkaline followed by acid extraction (alk. + acid), or acid extraction.

The average concentration of eight cereal species (rye, wheat, oat, barley, triticale, spelt wheat, buckwheat, and millet) in whole grain of the concentration in the bran ranged between 37 and 89% in the case of **1**−**3**, **6**, **10**, and **15** and between 1.4 and 30% in the case of **7**−**9**, **13**, **16**, **17**, **22**, and **23**. The results indicate that the lignans probably located at later steps of the biosynthetic pathway are more concentrated in the bran layer and, as pointed out previously, occur more in aglycone form than those located at the beginning of the pathway.

Extensive Survey of the Lignan Content and Composition of Cereals, Oilseeds, and Nuts. In the most comprehensive previous study of cereal lignans, six lignans (**1**−**3**, **6**, **10**, and **15**) were identified and quantified in whole-grain alkaline extracts of six cereal species (*13*). In the present work, 14 more lignans were identified and quantified in the bran of 16 cereal species and in both bran and whole grains of eight species. In alkaline extracts of whole grain, we obtained very similar concentrations as those reported in the previous work (results not shown).

The "optimal" lignan concentrations, i.e., the concentrations obtained using the extraction method giving the highest yield of each lignan, are presented in **Table 2**. The "new" cereal lignans are **7**−**9**, **11**−**14**, **16**−**20**, **22**, and **23**. Lignan **16** is the dominant lignan in wheat (containing the highest amount of **16** of all cereal species), barley, corn, and amaranth. Furthermore, taking into account the alkaline-extractable portion, **16** is the dominant lignan also in triticale, oat, and millet bran and possibly also in quinoa bran. In the other cereal species, **1**, **3**, or **6** are the dominating lignans. Also, **17** is an abundant lignan in cereals, in some cereals more abundant than **10** and **15**. The overall abundance of cereal lignans can be placed in the following order according to the obtained results: **3** > **16** > **6** > **1** > **17** > **10** > **2** > **15** > **7** > **22** > **23** > **9** > **14** > **18** > **13** > **8** > **20** > **11** > **12** > **19**. However, the abundance of **16** exceeds that of **3** when the alkaline-extractable portion of **16** is considered.

The "new" cereal lignans account for approximately 50% of the total lignan content in cereals (on average). This means that even though knowledge on lignan content in cereals has very recently increased (*12*, *13*), these data were still limited. The previously unidentified lignans dominate in wheat, triticale, barley, corn bran, and amaranth. In millet bran, the previously unidentified lignans account for nearly 50% of the total lignan content, and in rye, buckwheat, quinoa, and oat bran, the proportion is 30−45%. In spelt wheat, dhurra, and all rice species, the proportion is 1−18%. The lowest proportion of "new" lignans is found in wild rice, in which **3** is dominant, accounting for 73% of the total lignan content.

The cereal species can be placed in the following order with respect to total lignan content (**Table 2**): rye > wheat > triticale > oat > spelt wheat > Japanese rice > wild rice > buckwheat > barley > amaranth > corn > millet > quinoa > red rice > brown rice > dhurra. However, the lignan content in wheat exceeds that in rye when the alkaline-extractable portion of **16** is considered.

When the total lignan contents of sesame seeds, linseeds, and the nut species are compared to that in cereal species, it is obvious that linseeds and sesame seeds still remain by far the most lignan-rich species used as foodstuffs. It should be noted that when the lipophilic sesamin type lignans (*47*) are included, the known lignan content of sesame seeds is higher than that of linseeds. The lignan content of the four nut species was comparable to the content in corn, millet, quinoa, red rice, and brown rice bran. The concentrations of some lignans have been determined previously in nuts and oilseeds (among other foodstuff) by Mazur and Adlercreutz (*11*) and Milder et al. (*12*). Mazur and Adlercreutz analyzed **10** and **15** and applied strong acid treatment, whereas Milder et al. determined **1**, **6**, **10**, and **15** and applied alkaline extraction. The concentrations of **10** obtained by Mazur and Adlercreutz in cashew nuts, peanuts, almonds, and walnuts were within the same ranges as those obtained in the present study. However, the concentrations of **15** were much lower in their study, which is also true for the level of **15** in cashew nuts and peanuts in the study by Milder et al. (*12*). The reason for this may be that **15** does not occur in esterified form in these species; therefore, the compound is more effectively extracted using ASE than acid extraction. According to Schwartz and Sontag (*30*), the degree of esterification of lignan glycosides depends on the plant species, but it is possible that the degree of esterification also depends on other factors. This should be confirmed by further studies analyzing several samples of the same species. The same authors determined concentrations of **6**, **9**, and **10** in cashew nuts and sesame seeds, among other foods, using a two-step method applying combined methanolysis to release esterified glycosidic forms and enzymatic hydrolysis. The results were compared to those obtained after mere enzymatic hydrolysis, after aqueous ethanolic extraction by sonication, and it was shown that in cashew nuts, the degree of esterification of the analyzed lignans was small, which is consistent with the results obtained in the present study, i.e., the difference in the obtained concentration in ASE extracts and alkaline-treated extracts. However, for sesame seeds, the results were not consistent, which may be due to the large differences in analytical methods applied in the two studies or to a varying degree of esterification in different sesame seed samples.

In addition to the levels of **15**, the levels of **1**, **6**, and **10** in peanuts and cashew nuts were lower in the study by Milder et al. (*12*) than in the present study, also when comparing concentrations obtained with the same extraction method. On

the other hand, our obtained concentrations of **1**, **6**, **10**, and **15** in linseeds were lower than the concentrations obtained previously (*12*, *13*). The differences may at least partly be due to differences in analytical methods; however, it is also possible that the content of some lignans may vary within the same species depending on, e.g., genetic factors or growth conditions. For example, it has been shown that the concentration of sesamin in sesame seeds varies greatly in different samples of sesame seeds, ranging between 7 and 712 mg/100 g in 65 different samples (*47*). Moreover, as pointed out above, it is also possible that the degree of esterification of the same lignan glycosides varies within the same species, and comparisons of lignan levels between different studies may be pointless as long as these variation factors are unknown.

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