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In Vitro Fermentative Production of Plant Lignans from Cereal Products in Relationship with Constituents of Non-Starch Polysaccharides

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Summary

Recently special attention has been paid to dietary fibre-associated phytoestrogens such as plant lignans, which are related to the prevention of different hormone-dependent diseases. Therefore, phytoestrogens associated with dietary fibre and their metabolites are of interest for investigation. The aim of this work is to investigate the formation of enterolignans: enterolactone (ENL) and enterodiol (END) from their precursors by the action of intestinal microflora and their relationship with non-starch polysaccharides (NSP) in various cereal products from wheat, rye, barley and oats. For the investigation of the bioconversion of plant lignans, a technique of *in vitro* fermentation was used and the quantitative analysis of their metabolites ENL and END was performed by high-performance liquid chromatography (HPLC) with coulometric electrode array detection. The enterolignan formation in various cereal products ranged from 78.3 to 321.9 nmol/g depending on the product type: END from 8.7 to 149.3 nmol/g and ENL from 64.4 to 278.3 nmol/g. The lignan production in bran was about two times higher than that in whole flour of the same kind of cereals. Close correlations were found between the total NSP content and the total amount of enterolignans and ENL; between pentoses and the total amount of enterolignans and ENL; between arabinose or xylose and ENL; and between galactose and END values. Considering the correlations between hexoses and END as well as between pentoses and ENL found in cereals, it can be assumed that pentoses are closely related to the quantities of plant lignans in cereal products and their conversion to enterolignans.

Key words: enterolignans, enterolactone, enterodiol, non-starch polysaccharides, *in vitro* fermentation, cereals

Introduction

Dietary fibre plays a very important role in human nutrition, improving digestion and decreasing the level of glucose and cholesterol in blood, and also decreasing the risk of diabetes, obesity and stomach cancer. Recently special attention has been paid to dietary fibre-associated phytochemicals such as plant lignans. Epidemiological and *in vitro* fermentation studies and experiments with animals have suggested that the biological activity of these compounds is associated with beneficial health effects, such as antitumour, antioxidant, both estrogenic and antiestrogenic activities (*1*), or protection against coronary heart disease (*2*). Flaxseeds were recognized as one of the reachest dietary sources of lignans (*3,4*), in which glycosides of secoisolariciresinol (SECO) and matairesinol (MAT) are the major components, followed by traces of pinoresinol (PIN), lariciresinol (LAR) and isolariciresinol

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(I-LAR) (*1*). The content of SECO in flaxseeds has been reported to vary from 2900 to 12 600 mg/kg, and levels of MAT were found in a range from 5.5 to 58.6 mg/kg (*5*), thus many studies have been conducted on flaxseed lignans (*6*). However, the low consumption of flaxseeds in most human populations cannot explain the general occurrence of mammalian lignans in human tissues. A large variety of legumes, whole cereals, fruits, vegetables and beverages such as tea or coffee were also found to contain small amounts of SECO (<35 mg/kg) and traces of MAT (*7,8*). Nevertheless, cereals are a staple food in the Western diet and therefore an important source of bioavailable lignans.

Despite the potential importance of lignans in disease risk reduction, little is known concerning their dietary origin. Lignans are a dimeric natural product derived from the combination of two phenylpropanoid C6-C3 units at β carbon atoms (*9*). Lignans are structurally related to estrogens, and they may function as weak estrogens or estrogen antagonists. They are generally glycosidically linked to carbohydrates and in the large intestine are deconjugated from the carbohydrate portion by the human intestinal bacteria to the enterodiol (END) and enterolactone (ENL), known as enterolignans. These metabolites are considered to be responsible for the biological effects in humans (*10*). High ENL and END levels in urine or plasma are generally associated with a high intake of dietary fibre and with the consumption of whole grain foods, and fruits and vegetables (*11*). It has been suggested that the lignans contained in these food sources are the precursors of ENL and END. A positive association was found between urinary lignan excretion and dietary fibre intake in a group of 98 North Americans, the best correlation being observed for dietary fibre originating from grains (*12*). However, two studies in which rats or humans were fed wholemeal cereals or cereal bran showed that the content of SECO and MAT, the two main lignans identified in cereals, was too low by a factor of 5–25 to explain the levels of ENL and END excreted in urine (*13,14*). The recent identification of a number of lignans in rye and other cereals still does not completely explain the urinary excretion of ENL and END (*15*). This suggests the existence of other precursors. Among the lignans present in cereals, SECO and MAT are the best known precursors of enterolignans; nevertheless, other components such as PIN and LAR have also been identified as precursors of cereal lignan (*15*). Furthermore, syringaresinol (SYR), the major lignan in rye and wheat bran (3 times more abundant than SECO, MAT, PIN and LAR taken together), can also be converted to ENL and END.

The recently obtained data about lignans have revealed that lignins, which cannot be easily extracted by solvents – unless they are chemically or physically degraded into smaller fragments, could be major dietary precursors of enterolignans. Their dietary origin and that they are metabolized by the gut microflora to form part of the ENL excreted in urine was demonstrated in rats (*16*). Precursors are essentially present in the cereal bran (*17,18*), and whole cereals may thus significantly contribute to their intake. There is, however, a considerable difference in lignan content between different cereals and breads, as most lignans are found in the bran and hardly any in the starchy core of the grain. Wheat and rye bran had the highest lignan content of all cereals; however, flaxseeds and sesame seeds were by far the most lignan-rich of the studied species. White wheat flour thus has a very low lignan content compared to whole grain cereals. Rye, wholegrain and flour, also contains more lignans than wheat. Rye bread, largely consumed in Nordic countries, is a good source of enterolignans (*13,19*). As a consequence, it is the cereals and whole grain products, particularly rye and barley that provide the most important dietary source of lignan precursors.

Juntunen *et al*. (*13*), and Jacobs Jr. *et al*. (*20*) have observed a positive correlation between total dietary fibre intake and lignan exrection. Because lignans are closely associated with the dietary fibre matrix of plant food, it is possible that their composition might influence lignan availability; however, there are no data available in this field. Furthermore, the composition of dietary fibre has been suspected to influence the growth of certain species of intestinal bacteria and thus may affect the enterolignan formation. Therefore, dietary fibre and with it the associated phytoestrogens and their metabolites are relevant as the object of investigation. In view of this deficiency of research in the field of dietary fibre and enterolignan formation, it is of importance to know more about the possible correlations between lignan bioconversion and the dietary fibre composition in cereal products.

The aim of this work is to investigate the formation of enterolignans: enterolactone (ENL) and enterodiol (ENL) from their precursors by using *in vitro* fermentation with human faecal microbiota and their relationship with non-starch polysaccharides (NSP) in various cereal products from barley, oats, rye and wheat.

Materials and Methods

Cereal products

The quantities of the constituent sugars of non-starch polysaccharides (NSP), such as arabinose, xylose, mannose, glucose and galactose, and the formation of enterodiol (END) and enterolactone (ENL) from their precursors were analyzed in wholemeal and bran of barley, oats, rye and wheat, as well as in white wheat flour. Additionally, flaxseed was investigated, which according to literature, contains the highest quantity of lignans.

Components of dietary fibre: cellulose, hemicelluloses, lignins, cutin and β-glucans have been analyzed in cereals of different varieties grown in Lithuania. Registered traditional cereal cultivars and six up-and-coming lines developed at the Plant Breeding Department of the Lithuanian Institute of Agriculture have been analyzed: barley (*Hordeum vulgare* L.) cultivars Aidas, Ula, Rolandas and Auksiniai 3; oat (*Avena sativa* L.) cultivars Javor, Dragon, German, Radius, Jaugila and Celsia; rye (*Secale cereale* L.) cultivars Duoniai, Rukai, Tolovskaja, Kustro, SW 870493, Hybrid 346, Hybrid 341, Hybrid 347, Hybrid 345, Hybrid 339 and Hybrid 343; and wheat (*Triticum aestivum* L.) cultivars Sirvinta, Alba, Kosack, LZI 2828-47, LZI 2804-8, LZI 2905-1, LZI 2901-26, LZI 2804-24, LZI 2804-33 and LZI 3182. All samples were collected in Lithuania during the year 2009.

For analysis, barley, rye and wheat grains, as well as flaxseed were milled in Laboratory Mill 3100 (Perten

Instruments AB, Kungens Kurva, Sweden) at a particle size of 0.8 mm, and oat grains in Retsch Mill (Retsch GmbH, Haan, Germany) at a particle size of 0.75 mm and stored at –20 °C.

In vitro fermentation of cereal products

In vitro fermentation was performed according to a modified incubation method of Karppinen *et al.* (*21*). The *in vitro* colon model assists in the elucidation of the role of microbiota in the metabolic network of human digestive system and it helps in the identification of the crucial reactions.

The culture medium was made of a carbonate-phosphate buffer solution containing (in g/L): $NaHCO_3$ 9.240, $Na_2HPO_4{\cdot}2H_2O$ 3.542, NaCl 0.470, KCl 0.450, $Na_2SO_4{\cdot}10H_2O$ 0.227, $CaCl_2$ (anhydrous) 0.055, $MgCl_2{\cdot}6H_2O$ 0.100, urea 0.400 with added trace elements (10 mL of the following solution (in mg/L): $FeSO_4{\cdot}7H_2O$ 3680, $MnSO_4{\cdot}H_2O$ 1159, $ZnSO_4{\cdot}7H_2O$ 440, $CoCl_2{\cdot}6H_2O$ 120, $CuSO{\cdot}5H_2O$ 98, $Mo_7(NH_4)_6O_{24}{\cdot}4H_2O$ 17.4). The culture medium was reduced in an anaerobic chamber for 2 days prior to fermentation.

Faeces were collected from three healthy human volunteers who suffered no digestive disease and had not received antibiotics for at least 3 months. Freshly passed faeces were immediately taken in an anaerobic chamber, pooled, and homogenized with an equal mass of culture medium using a Warring blender. The slurry was diluted to 16.7 % (volume) with the culture medium, filtered through a 1-mm sieve, and used immediately as inocula. A mass of 0.1 g of analyzed food sample was weighed into 50-mL glass vials, 10 mL of the inoculum were added, and then stored in anaerobic chamber at 30 °C. The vials were sealed with rubber stoppers and shaken in a water bath at 37 °C for 24 h. Fermentation was stopped by plunging the vials into the ice water, after which the vial contents were freeze dried. Duplicate incubations were carried out for each sample. Also, duplicate blanks, containing only culture medium and inoculum, were incubated for 0 and 24 h.

Determination of enterolignans by HPLC with coulometric electrode array detection

Quantitative analyses of END and ENL were conducted using high-performance liquid chromatography (HPLC) with the coulometric electrode array detection (CEAD) according to Heinonen *et al.* (*15*). The HPLC system consisted of a pump model 580 (ESA, Chelmsford, MA, USA) and an automatic injector model 540 (ESA). An intersil ODS-3 (GL Science Inc., Tokyo, Japan) column (3.0×150 mm, 3.3 μm, 9LI 500 10) in combination with precolumn Quick Relate C_{18} (Upchurch Scientific Inc., Oak Habor, WA, USA) was used for the separation of compounds, and for detection of END and ENL, a Coulochem Electrode Array Detector (ESA) equipped with eight electrodes was used.

The freeze-dried incubated samples were weighed (approx. 20 mg), and 500 μL of water and 10 μL of 6 M HCl were added. The samples were extracted twice with 5 mL of diethyl ether. The extracts were combined and evaporated to dryness under N_2 flow. The samples were dissolved in 500 μL of MeOH and subsequently diluted in the mobile phase.

Pure standards of END and ENL used for quantitative analyses were obtained from Fluka Chemie (Buchs, Switzerland). Quantification was performed with the standard solutions of END (7.0 to 350.0 μg/L) and ENL (10.8 to 541.7 μg/L) dissolved in MeOH and diluted with mobile phase prior to HPLC analysis. The mobile phase consisted of 20 % solution B (50 mM NaOAc (pH=5)/MeOH/ACN, 40:40:20 volume ratio) and 80 % solution A (50 mM NaOAc (pH=5)/MeOH, 80:20 volume ratio).

Enterolignans were separated (flow rate of 1.2 mL/min) on the reversed phase column and were detected at potentials from 180 mV (channel 1) to 720 mV (channel 8). The END and ENL were quantified using calibration curves and determined by evaluation of their quantities in a faecal blank sample. The amounts of END and ENL produced in the faecal blank samples in 24 h were subtracted from the results obtained from the incubations carried out with the food samples. Duplicate samples were analyzed for each incubation sample and the standard deviations were calculated. The coefficients of variation (CV) between replicates ranged from 0.2 to 17.5 % for END and from 0.2 to 18.5 % for ENL. The observed values of CV are very similar to the findings of Heinonen *et al.* (*15*).

Determination of dietary fibre components

The analysis of the dietary fibre components: neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) was carried out using the Van Soest and Wine acid detergent method (*22*). The NDF fraction contains hemicelluloses, cellulose, cutin and lignins; the ADF cellulose, cutin and lignins; and the ADL cutin and lignins. The hemicellulose and cellulose contents were calculated as the difference between NDF and ADF fractions, and ADF and ADL fractions, respectively. Using this method, lignin could be determined together with cutin by means of the quantity of the ADL fraction. Before extraction of the dietary fibre components, starch was removed enzymatically with α-amylase from *Bacillus licheniformis* (Termamyl 120L, Novozymes A/S, Bagsvaerd, Denmark) and amyloglucosidase 102857 from *Aspergillus niger* (Roche Diagnostics GmbH, Mannheim, Germany). For NDF analysis, starch-free material was extracted with a neutral detergent, and the residue was filtered and weighed after washing, drying and ashing. By sample extraction with acid detergent, hemicelluloses were removed and the ADF fraction was precipitated. The quantity of lignins and cutin was determined by extraction of the ADF fraction with sulphuric acid.

The content of β-glucans was determined enzymatically according to ICC Standard No. 166 (*23*) using a Megazyme kit K-BGLU (Megazyme International Ireland Ltd., Wicklow, Ireland).

Determination of constituent sugars of NSP by GLC

Modified Englyst and Cummings method (*24*) was used for the determination of the constituent sugars of NSP. In this method, starch, including that resistant to gelatinization in boiling water, was dispersed with dimethyl sulphoxide and removed from the sample matrices

enzymatically with α-amylase from *Bacillus licheniformis* (Termamyl 120L, Novozymes A/S) and amyloglucosidase 102857 from *Aspergillus niger* (Roche Diagnostics GmbH). NSP was precipitated with ethanol and then hydrolyzed with sulphuric acid for 2 h until monosaccharides were obtained. The constituent sugars of NSP: arabinose, xylose, mannose, glucose and galactose were determined by gas liquid chromatography (GLC) using *myo*-inositol (Sigma-Aldrich Co, Saint Louis, MO, USA) as internal standard. Alditol acetates prepared by using *N*-methylimidazole to catalyze the acetylation reaction were used for GLC determination of the released monosaccharides. The recoveries (in %) of sugars after acetylation as described under this method are as follows: arabinose 98.7± 2.2, xylose 99.7±1.0, mannose 98.2±0.5, glucose 98.3±0.6, and galactose 97.4±0.6. GLC was performed by using a Shimadzu-GC-17 AAF FID gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector (FID) and an RTX 50 Fused Silica (Restek Corporation, Bellefonte, PA, USA) analytical column (30 m× 0.25 mm, 0.25 μm) and an automatic sample injector AOC-17. The column was maintained at 230 °C and the injector and detector were kept at 300 °C. The carrier gas (helium) flow rate was 1.67 mL/min. The content of monosaccharides was determined by using a compatible computer provided with CLASS-GC10 ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The total NSP were expressed as the sum of constituent sugars. Duplicate samples were analyzed for each food sample and the standard deviations were calculated. For arabinose, xylose, mannose, glucose and galactose, the coefficients of variation between replicates were 2.1–22.8, 1.1–25.0, 1.3–29.0, 1.4–31.2 and 1.3–27.6 %, respectively.

Results and Discussion

Formation of enterodiol and enterolactone from cereal products using in vitro fermentation

The quantitative results of enterodiol (END) and enterolactone (ENL) formed during the 24 h of faecal incubation of cereal products and flaxseed are summarized in Table 1.

Table 1. Enterolignan formation from their precursors in different cereal products and flaxseed

Sample	Incubation time	*b*(END)/(nmol/g)		*b*(ENL)/(nmol/g)		*b*(total enterolignans)/(nmol/g)	
	h	wb	db	wb	db	wb	db
Cereal products							
barley bran	0	1.1	1.3±0.2	1.0	1.2±0.3	2.1	2.5
	24	121.5	141.2±6.7	172.6	200.7±4.8	294.1	341.9
oat bran	0	0.9	1.0±0.3	2.4	2.8±0.4	3.3	3.8
	24	149.3	173.7±3.3	171.4	198.9±0.8	320.7	372.6
rye bran	0	1.0	1.2±0.4	2.3	2.7±0.2	3.3	3.9
	24	34.6	40.2±0.2	278.3	323.6±1.1	312.9	363.8
wheat bran	0	0.9	1.0±0.3	1.0	1.2±0.3	1.9	2.2
	24	141.3	164.3±2.6	180.6	210.0±0.1	321.9	374.3
barley wholemeal	0	1.1	1.3±0.4	1.2	1.4±0.2	2.3	2.7
	24	62.5	72.7±4.2	64.4	74.9±3.6	126.9	147.6
oat wholemeal	0	1.0	1.1±0.2	1.6	1.8±0.4	2.6	2.9
	24	65.9	76.6±3.5	71.8	83.5±1.2	137.7	160.1
rye wholemeal	0	1.0	1.2±0.3	2.4	2.8±0.5	3.4	4.0
	2	119.3	138.7±5.7	111.3	129.4±1.5	230.6	268.1
wheat wholemeal	0	1.1	1.3±0.4	0.6	0.7±0.1	1.7	2.0
	24	66.1	76.8±5.1	76.8	89.2±4.9	142.9	166.0
white wheat flour	0	1.1	1.3±0.2	1.2	1.4±0.2	2.3	2.7
	24	8.7	10.1±0.2	69.6	80.9±1.1	78.3	91.0
min	0	0.9	1.0	0.6	0.7	1.7	2.0
	24	8.7	10.1	64.4	74.9	78.3	91.0
mean±S.D.	0	1.0	1.2±0.1	1.5	1.9±0.7	2.5	2.9±0.8
	24	85.5	99.4±57.3	133.0	154.6±84.8	218.4	253.9±113.5
max	0	1.1	1.3	2.4	2.8	3.4	4.0
	24	149.3	164.3	278.3	323.6	321.9	374.3
Flaxseed							
mean±S.D.	0	9.6	11.2±1.1	10.1	11.7±1.5	19.7	22.9±2.3
	24	4350.8	5059.1±41.0	6082.4	7072.6±41.0	10433.2	12131.7±82.1

END=enterodiol, ENL=enterolactone, wb=wet basis, db=dry basis, S.D.=standard deviation

The total enterolignan formation in various cereal products ranged from 78.3 to 321.9 nmol/g depending on the product type, for END from 8.7 to 149.3 nmol/g and for ENL from 64.4 to 278.3 nmol/g. The cereal species can be placed in the following order with respect to total enterolignan content: rye>wheat>oat>barley. Bran cereals were the highest producers of enterolignans (294.1–321.9 nmol/g). The proportion of total lignans in whole grain as compared to rye bran was 74 % and ranged between 43 and 44 % in wheat, oat and barley.

In most of the analyzed cereal products, slightly more ENL was formed than END, and in rye bran and white wheat flour the ratio of ENL to END was even 8:1, and the proportion of ENL in total enterolignans was 41–52 %, with the exception of rye bran and white wheat flour where this proportion was 11 %.

How much the different foods contribute to the total enterolignan formation in humans depends also on their level in the individual food and its use in the diet. Flaxseed is by far the richest source of enterolignan precursors, because it contains the highest amount of SECO, some MAT and also PIN and LAR (*25,26*). Our investigation also confirms this. The amount of enterolignans formed in flaxseed (10 433.2 nmol/g) was of different magnitude than in cereal products (Table 1). However, currently its dietary contribution is low because it is not widely used as staple food. Therefore, foods such as whole grain products, especially rye products, due to their higher level of intake could be important sources of lignans in the Nordic type of diet.

Smeds *et al.* (*27*) quantified seven dietary lignans (7-hydroxymatairesinol, secoisolariciresinol, matairesinol, lariciresinol, pinoresinol, medioresinol, and syringaresinol) to characterize the variation in lignan content and composition of winter rye, spring wheat and spring oat. They found that grain lignan content and composition were significantly different in different cereal species, with the highest total content in winter rye, the second highest in spring oat, and the lowest content in spring wheat. Syringaresinol was the dominant lignan in all three species, especially in wheat and rye, in which the contribution to the total lignan content was approx. 80 %. In oat, the contribution was only 42 %, with larger contributions of LAR and PIN than the other species. Similar order was obtained by Peñalvo *et al.* (*28*) in whole grain: rye>wheat>oat>barley. Our results of enterolignan analysis in cereal products are in agreement with those of Smeds *et al*. (*27*) and Peñalvo *et al*. (*28*). It has been shown that lignans are concentrated in the bran layer of the grains; wheat and rye bran had the highest lignan content of all cereals (*2,11*). These findings are also supported by the present study.

Microbial metabolism of plant lignans to enterolignans END and ENL has been under continuous investigation during recent years. Despite the structural diversity and different sources of plant lignans, they undergo conversion to END and ENL by human gut microbiota. In the colon, MAT is converted directly to ENL, while other lignans such as SECO, PIN and LAR are transformed *via* another route. The latter lignans are first converted to END and further to ENL. This can explain the higher concentration of END in comparison with ENL found during the experiment with flaxseed, which is the richest source of SECO. If we compare our results with lignan data reported by Mazur and Adlercreutz (*11*), Heinonen *et al*. (*15*), Mazur (*25*), Milder *et al*. (*26*) and Horn-Ross *et al*. (*29*), they confirm that other lignans such as PIN and LAR have a high degree of conversion and could be metabolized in high yields into ENL and END when incubated *in vitro* with a human faecal microflora.

Experiments with flaxseed and rye bran by Aura *et al*. (*30*) showed that rapid decrease in pH, lack of carbohydrate nutrient and accumulation of metabolite products may have caused external stress to the microbiota shown by a delay in END production and a suppression of ENL production in *in vitro* samples. It is also possible that when readily fermentable carbohydrates are present, the metabolism of the faecal microbiota is directed to the fermentation of readily available substrates rather than to bioconversion of phenolic compounds.

Complex analysis of the cereal dietary fibre

The quantities of hemicelluloses, cellulose, β-glucans, and lignins and cutin also determined in Lithuanian barley, oat, rye and wheat are presented in Table 2.

The highest quantities of total dietary fibre and most of its fractions were found in oats. In this type of cereals the hemicellulose and cellulose content was, respectively, 1.3 and 2.7 times higher than in barley, 1.6 and 5.5 times higher than in rye and 1.8 and 5.1 higher than in wheat. The lignin and cutin content together in oats was from 1.8 to 2.4 times higher in comparison with other cereals.

Among the tested cereals, barley was specific for the highest content of β-glucans in percent on dry basis (db) 5.1±0.4, which was 1.4 times higher than in oats, 2.8 times higher than in rye and 6.4 times higher than in wheat. In comparison with rye and wheat, the content of hemicellulose ((11.2±0.7) %) and cellulose ((4.9±0.8) %) in barley was also higher. In rye, only the content of β-glucans ((1.8±0.3) %) was higher than in wheat ((0.8±0.1) %). The quantities of hemicelluloses, cellulose and lignins in rye and wheat were equivalent.

By comparing different genotypes of cereals, it was noticed that the variation in the composition of dietary fibre fractions depends not only on the kind of cereals but also on the genotype.

Non-starch polysaccharides and their constituent sugars in cereal products

The results of the investigation of non-starch polysaccharides (NSP) of cereal products and flaxseed are presented in Table 3. In all analyzed cereal products, the total NSP content ranged from 31.0 to 153.8 mg/g. The main constituent sugar was glucose (12.6–47.9 mg/g), then xylose (4.9–66.3 mg/g) and arabinose (5.0–34.5 mg/g). The contents of other constituent sugars, *i.e.* galactose (1.4–6.3 mg/g) and mannose (2.2–3.6 mg/g) were substantially lower.

The highest total NSP content was in rye bran (153.8 mg/g), of which the highest were arabinose and xylose content (34.5 and 66.3 mg/g, respectively). The reason could be that during cereal processing the outer layers of the grains, containing the highest amount of dietary fibre, are passed to the rye bran fraction. Therefore,

Table 2. Composition of cereal dietary fibre

Cultivar	*w*(hemicelluloses) mg/g	*w*(cellulose) mg/g	*w*(β-glucans) mg/g	*w*(lignins and cutin) mg/g
Barley				
Aidas	108	58	53	13
Ula	122	48	54	17
Rolandas	107	40	46	16
Auksiniai 3	112	51	49	17
min	107	40	46	13
mean±S.D.	112±7	49±8	51±4	16±2
max	122	58	54	17
Oat				
Javor	136	122	35	27
Dragon	98	99	30	21
German	157	149	41	34
Radius	163	147	39	32
Jaugila	147	148	37	31
Celsia	141	134	36	31
min	98	99	30	21
mean±S.D.	140±23	133±20	36±4	29±5
max	163	149	41	34
Rye				
Duoniai	90	20	20	15
Rukai	103	22	17	15
Hybrid 346	85	21	20	14
Hybrid 341	93	31	18	10
Hybrid 347	88	29	21	11
Hybrid 345	89	26	20	11
Hybrid 339	86	22	20	18
Hybrid 343	85	26	20	14
SW 870493	87	27	11	15
Tolovskaja	87	18	12	16
Kustro	67	22	17	18
min	67	18	11	10
mean±S.D.	88±9	24±4	18±3	14±3
max	103	31	21	18
Wheat				
Sirvinta	79	19	10	15
Kosack	84	24	6	11
Alba	71	23	8	09
LZI 2828-47	86	29	8	12
LZI 2804-8	76	26	10	13
LZI 2905-1	83	31	9	12
LZI 2901-26	66	27	8	10
LZI 2804-24	73	31	9	11
LZI 2804-33	78	32	8	11
LZI 3182	73	25	9	12
min	66	19	6	9
mean±S.D.	78±6	26±4	8±1	12±2
max	86	32	10	15

S.D.=standard deviation

Table 3. Composition of NSP constituent sugars in cereal products and flaxseed

Sample	*w*(NSP constituent sugars)/(mg/g)										*w*(total NSP) mg/g	
	Ara		Xyl		Man		Glu		Gal			
	wb	db	wb	db	wb	db	wb	db	wb	db	wb	db
Cereal products												
rye bran	34.5	40.1±1.1	66.3	77.1±2.2	3.6	4.2±0.1	44.8	52.1±0.8	4.6	5.3±0.2	153.8	178.8
barley wholemeal	10.0	11.6±0.4	10.9	12.7±0.4	3.0	3.5±0.3	47.9	55.7±2.8	1.4	1.6±0.1	73.2	85.1
oat wholemeal	8.0	9.3±0.7	7.8	9.1±0.8	2.3	2.7±1.2	44.2	51.4±5.0	2.4	2.8±0.2	64.7	75.3
white wheat flour	5.0	5.8±0.5	4.9	5.7±0.5	2.2	2.6±0.1	12.6	14.7±2.9	6.3	7.3±0.7	31.0	36.1
min	5.0	5.8	4.9	5.7	2.2	2.6	12.6	14.7	1.4	1.6	31.0	36.1
mean±S.D.	14.4±14.0	16.7±16.2	22.5±29.4	26.2±34.4	2.8±1.3	3.2± 1.4	37.4±17.5	43.5±19.7	3.7±2.3	4.2±3.6	80.8±52.1	93.8±61.3
max	34.5	40.1	66.3	77.1	3.6	4.2	47.9	55.7	6.3	7.3	153.8	178.8
Flaxseed												
mean±S.D.	22.1±0.6	25.7±0.7	23.7± 1.5	27.6±1.8	1.5±0.3	1.8±0.4	45.0±0.6	52.3±0.8	18.7±0.3	21.7±0.4	111.0±5.3	129.1±6.2

NSP=non-starch polysaccharides, Ara=arabinose, Xyl=xylose, Man=mannose, Glu=glucose, Gal=galactose, wb=wet basis, db=dry basis, S.D.=standard deviation

lower amounts of NSP are passed to the flour, especially with low ash content. For example, white wheat flour contained the lowest amount of total NSP (31.0 mg/g) and specific proportion of constituent sugars: the highest amount of galactose and the lowest amounts of other monosaccharides. In comparison with white flour, grain wholemeal produced using simple milling processes contained higher amounts of NSP, xylose, glucose and arabinose.

By comparing the analyzed products, after rye bran, flaxseed also contains a high amount of NSP. Also, galactose content was the highest in this product (18.7 mg/g). The amount of this monosaccharide in flaxseed was 5 times higher than in cereal products. Mannose content in flaxseed (1.5 mg/g) was lower than in cereal products.

Analysis of NSP composition showed that the main constituent sugars of NSP in cereals are glucose, xylose, and arabinose, and that the amounts of hexoses and pentoses were almost similar (content of hexoses compared to pentoses was only about 1.25 times higher). This confirms that higher quantities of cellulose, β-glucans and arabinoxylans are present in cereals. Cellulose, β-glucans, arabinoxylans and galactans are the main NSP components in flaxseed.

Correlation between dietary fibre components and plant lignan metabolites

Despite the potential importance of lignans in reducing disease risk, little is known concerning their dietary origin. High ENL and END levels in urine or plasma are generally associated with a high intake of dietary fibre (*13,20*) and its composition (*30*).

According to Begum *et al*. (*16*), lignins are structurally related to lignans and may also be metabolized into enterolignans. They are cell wall polymers made of phenylpropane units (*31*), therefore structurally closely related to SECO and MAT. Lignins, because of their polymeric nature and because they are embedded in the cell wall, are usually considered inert in the digestive tract. However, the results presented by Begum *et al*. (*16*) showed that they are metabolized by the gut microflora to form part of the ENL excreted in urine.

In the present study, no significant relationships were determined between the amounts of enterolignans formed in cereal products and the amount of lignins or other fractions of dietary fibre. The most likely explanation is a difference in the structure of the synthetic lignin used by Begum *et al*. (*16*) compared to the native lignin. It is difficult to obtain pure lignins from complex plant materials and more particularly from materials such as bran, which has a relatively low lignin content compared to other lignocellulosic materials such as wood. Indeed, the analytical method differs from the one used here by the mode of extraction of lignins and also gives differences in results.

By analyzing the NSP composition of selected cereal products and enterolignans formed during their fermentation, it was noticed that some correlations existed between the quantities of total NSP, their constituent sugars and enterolignans (Table 4).

In cereal products, close correlations were found between the total NSP and the total amount of enterolignans and ENL; between pentoses and the total amount of enterolignans and ENL; between arabinose or xylose and ENL; and between galactose and END values. Considering the correlations between hexoses and END, as well as between pentoses and ENL found in cereals, it can be assumed that in cereals lignans can occur as different glycosides, *e.g.* not only conjugated with glucose but also with other NSP constituent sugars such as xylose or arabinose. This indicates that pentoses are closely related to the quantities of plant lignans in cereal products. Obviously, the lignan formation depends more on the specific composition and structure of dietary fibres, which varies between different kinds of cereals, than on their amount.

In general, our results are in agreement with the data about phenolic compounds that occur naturally in glycosylated and conjugated forms. Therefore, the nature,

Table 4. Squared correlation coefficient (R^2) between total NSP, their constituent sugars and enterolignans in cereal products

Enterolignans	NSP constituent sugars							
	Ara	Xyl	Man	Glu	Gal	Pen	Hex	Total
END	0.0052	0.0193	0.0143	0.7425	0.9382	0.0139	0.6444	0.0191
ENL	0.9710	0.9887	0.6797	0.0836	0.0850	0.9841	0.1537	0.8687
Total	0.9772	0.9610	0.7640	0.2709	0.0021	0.9671	0.3724	0.9780

NSP=non starch polysaccharides, END=enterodiol, ENL=enterolactone, Ara=arabinose, Xyl=xylose, Man=mannose, Glu=glucose, Gal=galactose, Hex=hexoses, Pen=pentoses

size, structure, solubility, degree and position of glycosylation and conjugation with other compounds can influence their bioavailability, absorption, distribution, metabolism and excretion in humans (*32*,*33*). Only aglycones and some glycosides can be absorbed in the small intestine, whereas polyphenols linked to other sugars (arabinose, xylose or rhamnose) often reach the colon and are hydrolyzed before absorption. Our study suggests that the content of lignans in cereals and their bioconversion to enterolignans depends on their associations with the constituent sugars of NSP. Therefore, much research is still needed in this area to understand plant lignan origin, metabolism and absorption in humans in order to create recommendations for their intake.

Conclusions

The amount of enterolignans produced during 24 h by *in vitro* fermentation of various cereal products such as barely, oat, rye and wheat wholemeal or bran ranged from 78.3 to 321.9 nmol/g, the amount of END from 8.7 to 149.3 nmol/g, and of ENL from 64.4 to 278.3 nmol/g. The levels of ENL were higher than of END. The main constituent sugars of NSP in cereals were glucose, xylose and arabinose (37.4, 22.5 and 14.4 mg/g, respectively). The quantities of hexoses and pentoses in cereals were similar, they contained more arabinoxylans. Quantitative relationships between hexoses and ENL (R^2=0.9841), and also between pentoses and END (R^2=0.6444) in cereals indicate that pentoses are closely related to the quantities of plant lignans in cereal products and their conversion to enterolignans.

The results show that cereal products are important sources of enterolignan precursors. The results of the investigation contribute to the information for a database of bioactive compounds in plant foods.

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