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ORIGINAL PAPER

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Cell wall hydroxycinnamates in wild rice (*Zizania aquatica* L.) insoluble dietary fibre

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Abstract The contents of ester-linked phenolic acids in wild rice (*Zizania aquatica* L.) dietary fibre were quantified by HPLC analysis, and oligosaccharide hydroxycinnamates were isolated and identified to investigate the linkages of hydroxycinnamic acids to cell wall polymers. In wild rice insoluble dietary fibre (WRIDF), ferulic acid was the most abundant phenolic acid (3942 µg g^{-1}), but significant amounts of sinapic acid (518 µg g^{-1}) and *p*-coumaric acid (142 µg g^{-1}) were also detected. Treatment of WRIDF with carbohydrate hydrolases or trifluoroacetic acid released several oligosaccharide hydroxycinnamates. After fractionation with Amberlite XAD-2, five feruloylated oligosaccharides were isolated by gel chromatography and semi-preparative HPLC and identified as arabinoxylan ferulate fragments. The feruloylated tetrasaccharide {[5-*O*-(*trans*-feruloyl)][*O*-β-D-Xyl*p*-(1→2)]-*O*-α-L-Ara*f*-(1→3)}-*O*-β-D-Xyl*p*-(1→4)-D-Xyl*p* was isolated for the first time from edible plant material. Although some results indicated that sinapic acid is also (at least partially) linked through an ester bond to polysaccharides, isolation of defined oligosaccharide sinapates was not achieved.

Keywords *Zizania* sp. · Dietary fibre · Arabinoxylan · Ferulic acid · Sinapic acid

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Introduction

Wild rice (*Zizania* sp.) is an annual aquatic grass which, along with rice (*Oryza sativa* L.), belongs to the cereals (*Poaceae*, tribe *Oryzoidae*). Four species of wild rice, also known as Indian rice, Canadian rice or wild oats, have been identified [1]: *Zizania aquatica* L. and *Zizania palustris* L. grow in certain areas in the northern USA and Canada, *Zizania texana* A.S. Hitchc. grows in a small area in Texas, and *Zizania latifolia* Turcz. is an Asian species. In comparison with other cereals the commercial importance of wild rice is currently rather low, but the consumers are increasingly becoming aware of the many uses of wild rice in various food preparations. For example, it can be used as a main meal ingredient, pure or blended with rice, in soups, after preparation as breakfast cereal, for pasta, croutons, pancakes, waffles etc. Due to its processing wild rice creates a pleasant, nutty taste, which distinguish wild rice from other cereals. Consumer interest in wild rice has led to increased commercialisation. Methods of growing wild rice under controlled conditions in paddies and industrial methods of harvesting and processing were developed. For these reasons, Oelke et al. [2] foretell that "the future outlook for increased wild rice use is bright".

The nutritional quality of wild rice is equal to or perhaps higher than that of other cereals due to its relatively high protein and low fat content [3]. Like other cereals, wild rice is a source of dietary fibre. Dietary fibre, which is known to be beneficial for human nutrition, is defined as follows [4]: "Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibre promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation". Cereal dietary fibre mainly consists of plant cell walls. In graminaceous plant cell walls various xylans, cellulose and mixed-linked β-glucans are the

dominant polysaccharides. Phenolic acids such as ferulic acid (FA) and *p*-coumaric acid (*p*CA) are minor compounds of the monocot plant cell wall [5], where they are bound to polysaccharides and lignin [6]. The attachment of FA and *p*CA has been carefully analysed in only a few plant species [7]. In monocot plants FA can be attached to arabinoxylans and xyloglucans. However, most investigations were carried out using forage grass stems as the source of plant cell walls. Cereal grains for human nutrition were investigated to a much lesser degree.

The presence of ferulates attached to polysaccharides provides a convenient mechanism to cross-link polysaccharides through diferulates [8]. Diferulates were recently investigated in various cereal grains, including wild rice grains [9]. It was concluded that diferulates might play an important role in influencing the physicochemical properties of dietary fibre that may influence their physiological effects. Besides diferulates, disinapates, which are formed from sinapates in an analogous radical coupling process, were detected in wild rice dietary fibre [10]. Up to now, no information exists on the attachment of monomeric sinapic acid (SA) to cell wall polymers.

In this paper we describe the analysis of monomeric phenolic acids and their attachment to polymers in WRIDF.

Materials and methods

General

*Trans-p*CA, *trans*-FA, vanillic acid, protocatechuic acid and protocatechuic aldehyde were purchased from Sigma (St. Louis, USA). Sinapic acid, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, 4-hydroxyphenylacetic acid, syringic acid, DMSO and $NaBH_4$ were from Fluka (Buchs, Switzerland). Acetonitrile and methanol (HPLC grade) were from Baker (Deventer, Holland). Amberlite XAD-2 was obtained from Serva (Heidelberg, Germany), and Sephadex LH-20 was from Amersham Pharmacia (Freiburg, Germany). All other chemicals were from Merck (Darmstadt, Germany). Heat-stable α-amylase Termamyl 120 L (EC 3.2.1.1, from *Bacillus licheniformis*, 120 KNU g^{-1}), the protease Alcalase 2.4 L FG (EC 3.4.21.62, from *Bacillus licheniformis*, 2.4 AU g^{-1}) and the amyloglucosidase AMG 300 L (EC 3.2.1.3, from *Aspergillus niger*, 300 AGU g^{-1}) were from Novo Nordisk (Bagsvaerd, Denmark), and Driselase (from Basidiomycetes) was from Sigma (St. Louis, USA).

All extracts and eluates were concentrated under reduced pressure using a rotary evaporator at <40 °C. Standard deviations are based on triple determinations.

Wild rice

Whole grains of wild rice (*Zizania aquatica* L.), harvested in North-Ontario and Saskatchewan (Canada), were obtained from a local German supplier.

Preparation of insoluble dietary fibre

Wild rice flour (10 g, particle size <0.5 mm) was suspended in sodium phosphate buffer (0.08 M, pH 6.0, 300 mL), and α-amylase (750 µL) was added. Beakers were placed in a boiling water bath for 40 min and shaken gently every 5 min. The pH was adjusted to 7.5 with 0.275 M NaOH (ca. 60 mL), and samples were incubated with protease (300 µL) at 60 °C for 30 min with continuous agitation. After adjusting the pH to 4.5 with 0.325 M HCl (ca. 60 mL), amyloglucosidase (350 µL) was added and the mixture was incubated at 60 °C for 30 min with continuous agitation. The suspension was centrifuged. The residue was washed twice with hot water, 95% (v/v) ethanol and acetone and finally dried at 60 °C overnight in a vacuum oven. For analysis of monomeric phenolics, WRIDF was corrected for residual protein and ash contents. The process described was repeated several times to get sufficient WRIDF for the isolation of oligosaccharide hydroxycinnamates.

Determination of residual protein and ash contents

Nitrogen content of WRIDF was determined by the Kjeldahl method. Protein was calculated as $N \times 6.25$. Ash content was determined gravimetrically by incineration of WRIDF at 525 °C for 8 h.

Extraction of ester-linked phenolics from insoluble dietary fibre

WRIDF (100 mg) was weighed into a screw-cap tube, 4-hydroxyphenylacetic acid as internal standard dissolved in MeOH/H_2O (50/50 v/v) was added and saponification with NaOH (2 M, 5 mL) was carried out under nitrogen and protected from light for 18 h at room temperature. Samples were acidified (pH <2) with concentrated HCl (0.95 mL) and extracted into diethyl ether (three times; 8, 6 and 4 mL). Extracts were combined and evaporated under a stream of nitrogen. Samples were redissolved in MeOH/H_2O (50/50 v/v, 1 mL), sonicated and membrane filtered prior to HPLC analysis.

HPLC analysis

A method to separate monomeric phenolic acids and aldehydes was developed. The following phenolics were separated within 30 min (in their order of elution) using the method described below: protocatechuic acid, protocatechuic aldehyde, 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid (internal standard), vanillic acid, 4-hydroxybenzaldehyde, syringic acid, vanillin, *trans-p*CA, *cis-p*CA, *trans*-FA, *trans*-SA and *cis*-FA. Isomers c*is*-FA and *cis-p*CA were prepared by dissolving their *trans*-isomers in MeOH (FA) or 0.5 M ammonia (*p*CA) and irradiation with UV-light for 14 h. All other phenolics as standard substances were purchased.

Separation was performed by HPLC (L-6200 Intelligent pump, T-6300 Column thermostat (Merck/Hitachi, Darmstadt, Germany)) using a Nucleosil 100–5 C18 HD column (250×4 mm i.d., 5 µm, Macherey-Nagel, Düren, Germany). Elution was carried out using a ternary gradient system consisting of aqueous 1 mM trifluoroacetic acid (TFA), MeOH and acetonitrile (MeCN): initially MeCN 7%, MeOH 0%, TFA 93%, linear over 20 min to MeCN 9%, MeOH 6%, TFA 85%, linear over 15 min to MeCN 9%, MeOH 15%, TFA 76%, following a rinsing and equilibration step. The injection volume was 20 µL, the column temperature 45 °C, and the flow rate was maintained at 1 mL min^{-1}. Phenolic acids and aldehydes were detected using a Waters 994 programmable photodiode array detector (DAD) (Waters, Eschborn, Germany). Phenolics were identified by comparison of their relative retention times and UV-spectra with those of standard compounds. UV-spectra were also used to judge peak purity. Quantitation was carried out at 280 nm using the internal standard.

Enzymatic degradation of insoluble wild rice dietary fibre

WRIDF (10 g) was suspended in H_2O (900 mL) and incubated with Driselase (1 g) at 37 °C for 48 h. After heat inactivation of the enzymes (10 min, 100 °C), centrifugation and filtration, the solution was concentrated to 50 mL. A 1 mL-aliquot was removed to analyse the phenolics in the hydrolysate by HPLC after saponi-

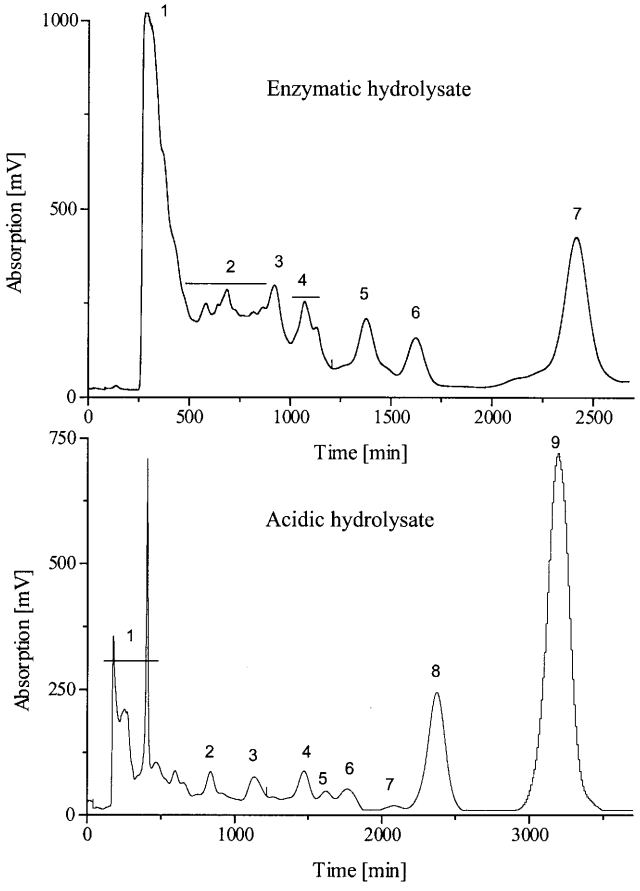

Fig. 1 Sephadex LH-20 chromatograms of the enzymatic (*top*, fractions E1–7) and acidic (*bottom*, fractions A1–9) hydrolysates (MeOH/H_2O-fractions after Amberlite XAD-2 fractionation)

fication (as detailed below for soluble fractions). The centrifugation residue was dried in a vacuum oven and phenolics were determined by HPLC as described for WRIDF.

Chemical degradation of insoluble wild rice dietary fibre

WRIDF (15 g) was suspended in aqueous 50 mM trifluoroacetic acid (1 L) and heated at reflux (100 °C) for 3 h [11]. After centrifugation and filtration the solution was adjusted to pH 5.0 and centrifuged again. The solution was concentrated to 100 mL. As described for the enzymatic degradation, phenolics were determined in the hydrolysate as well as in the residue.

Fractionation of the hydrolysates

The hydrolysates were applied to a column (42×2 cm) of Amberlite XAD-2. Elution was carried out with H_2O (350 mL), MeOH/H_2O (50/50 v/v, 500 mL) and MeOH (350 mL). All fractions were concentrated to 10 mL and 100 µL were removed for the determination of phenolic compounds. The MeOH/H_2O fraction was further concentrated to 5 mL and applied to a column (100×2.5 cm) of Sephadex LH-20. The elution was performed with water (flow rate: enzymatic hydrolysate 0.5 mL min^{-1}, acidic hydrolysate 0.7 mL min^{-1}; L-6000 Pump from Merck/Hitachi, Darmstadt, Germany), a two-wavelength UV-detector (280 and 325 nm; Variable Wavelength Monitor 2141 from LKB/Pharmacia, Freiburg, Germany) was used for the detection. Fractions were collected every 12 min. Further purification was carried out by semi-preparative HPLC using the HPLC system described above and a Nucleosil 100–5 C18 column (250×8 mm i.d., 5 µm, CS-Chromatographie Service, Langerwehe, Germany). Elution was performed isocratically with MeCN and H_2O at room temperature and a flow rate of 4 mL min^{-1}. The injection volume was 50 µL, using a 100 µL injection loop. The MeCN/H_2O ratio and the elution time varied for the different fractions (Fig. 1): E5 11/89 (30 min), E6 12/88 (45 min), E7 14/86 (45 min), A8 12/88 (30 min), A9 11/89 (45 min).

Identification of oligosaccharide hydroxycinnamates

Molecular weight was determined using flow injection analysis-mass spectrometry (FIA-MS) (HP Series 1100: autosampler G1313, pump G1312A, mass spectrometer G1946A (ion-source AP-ESI), Hewlett-Packard, Waldbronn, Germany). MeOH/H_2O (50/50 v/v) was used as solvent.

Phenolic compounds were determined by HPLC (as described above) after hydrolysis. Extraction of ester-linked phenolics from oligosaccharide hydroxycinnamates (and from other soluble fractions) was carried out by adding 2 M NaOH (100 µL) to the evaporated sample and stirring for two hours in the dark at room temperature. The reaction was stopped by adding 2 M H_3PO_4 (150 µL). This solution was used directly for the HPLC analysis.

Neutral carbohydrate compounds were determined as alditol acetates after hydrolysis. Hydrolysis of oligosaccharide hydroxycinnamates was performed with 2 M TFA for 30 min at 120 °C. To determine the carbohydrate composition of WRIDF, hydrolysis was carried out according to Englyst et al. [12]. Reduction and acetylation was according to Blakeney et al. [13]. Alditol acetates were separated by GLC (Hewlett-Packard 5890 Series II, Waldbronn, Germany) using a 0.25 mm×30 m HP-5MS capillary column (0.25 µm film thickness) (Hewlett-Packard, Waldbronn, Germany). GLC conditions were as follows: initial column temperature: 65 °C, held for 1 min, ramped at 30 °C min^{-1} to 165 °C, held for 12 min, ramped at 10 °C min^{-1} to 220 °C, held for 3 min, ramped at 20 °C min^{-1} to 240 °C and held for 3 min; cold on-column injection, flame ionisation detection (detector temperature 300 °C). He (1.5 mL min^{-1}) was used as carrier gas.

One-dimensional (^{1}H, ^{13}C-decoupled, DEPT) and two-dimensional NMR experiments (H,H-COSY, TOCSY, HMQC, HMBC) were performed on a Bruker DRX 500 spectrometer (Bruker, Rheinstetten, Germany). Samples (4–9 mg) were deuterium exchanged and dissolved in D_2O (0.7 mL). Chemical shifts (δ) were referenced to internal acetone (0.5 µL) by setting the ^{1}H signal to δ 2.20 ppm and the methyl signal to δ 30.89 ppm.

Results and discussion

Dietary fibre content and neutral sugar composition

The content of WRIDF was 3.34±0.10 g $100g^{-1}$ wild rice flour after correction for residual protein and ash content. After ethanol precipitation the content of soluble wild rice dietary fibre was determined to 0.79± 0.04 g $100g^{-1}$ wild rice flour after correction for residual protein and ash content. Crude fibre contents of wild rice have been published at 0.65–1.95 g $100g^{-1}$ [1] and 1.1–3.3 g $100g^{-1}$ [3]. Values for the content of "fibre" are mentioned in Oelke EA [2] (1.2–4.5 g $100g^{-1}$) without stating the methods used.

The relative neutral sugar composition of WRIDF polysaccharides was determined: 52.7% glucose, 17.7% arabinose, 17.7% xylose, 6.5% galactose and 5.4% mannose. Trace amounts of rhamnose and fucose were also detected. Assuming arabinose and xylose are derived

mainly from arabinoxylans, wild rice arabinoxylans exhibit a very high arabinose/xylose ratio of 1.0. Comparably high arabinose/xylose ratios were determined for arabinoxylans from rice endosperm (0.80) or from rice bran (0.93) [14] as well as from sorghum millet (0.83) [15]. Arabinoxylans from other cereal grains normally have ratios of 0.40–0.60. However, arabinose and xylose may have origins other than arabinoxylans. Rice endosperm cell walls have a polysaccharide composition different from other cereals, e.g. significant amounts of pectins and xyloglucans have been found [16]. Therefore, it is possible that pectins and xyloglucans may also influence the arabinose/xylose ratio of the wild rice.

Phenolic compounds

From WRIDF, the hydroxycinnamic acids *trans*-FA, *cis*-FA, *trans*-*p*CA and *trans*-SA, the benzoic acids 4-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid as well as the phenolic aldehydes 4-hydroxybenzoic aldehyde, protocatechuic aldehyde and vanillin were identified by comparing their relative retention times and UV-spectra with those of authentic standards. As expected, FA was identified as the major low temperature alkali-extractable phenolic acid with approximately 4 mg g^{-1} WRIDF (Table 1). *p*CA, which dominates in grass stems [17], was found in relatively small amounts of about 140 µg g^{-1} WRIDF. A relatively high amount of SA, approximately 520 µg g^{-1} WRIDF, was determined. To date, only trace amounts of SA have been found in grasses. An exception is in rice cell walls, where significant amounts of SA were determined, but the authors speculated that it might be a degradation product of lignin following the extraction procedure [18]. We also identified SA in the insoluble dietary fibre of other cereal grains, e.g. wheat, rye, corn and barley, but only in amounts below 100 µg g^{-1} (data not shown). Besides WRIDF, a higher amount of SA (185 µg g^{-1}) was determined merely in insoluble rice dietary fibre. The relatively high level of SA in WRIDF is consistent with the finding of significant amounts of 8–8'-coupled disinapates, newly discovered dehydrodimers analogous of two of the 8–8′-coupled diferulates [10].

Levels of identified benzoic acids were generally low and, with the exception of protocatechuic acid, below 40 µg g^{-1} WRIDF.

The identification of phenolic aldehydes released upon alkaline hydrolysis was not predictable. 4-Hydroxybenzaldehyde, protocatechuic aldehyde and vanillin were found in small amounts below 90 µg g^{-1}. It seems unlikely that the detected aldehydes were extraction residues. Therefore, they may be bound to cell wall polymers of WRIDF or are degradation products that were set free from other phenolic compounds during alkaline hydrolysis. Aldehydes may be linked through an alkali-labile bond to nitrogen bases, e.g. from structural proteins, or they may be linked with their phenolic hydroxyl group through an ester bond to some acids in the cell wall, e.g. uronic acids from polysaccharides. Alkali-extractable vanillin was also found in cell walls of wheat straw [19] and vanillin as well as 4-hydroxybenzaldehyde was detected in cell walls of different tissues of quinoa [20].

Isolation of oligosaccharide hydroxycinnamates

Driselase is an enzyme mixture containing several carbohydrate hydrolases including cellulase, xylanase, galactanase, arabinanase and polygalacturonase but is devoid of feruloyl esterases [21]. Driselase hydrolysed 46% of WRIDF and 71% of the *trans*-FA, 61% of the *cis*-FA, but only about 15% of the SA and *p*CA were released in soluble form. The low levels of released SA and *p*CA indicate either that these phenolic acids are bound to a smaller degree to polysaccharides, or that these acids bound to polysaccharides inhibit local polysaccharide degradation. Table 2 shows the distribution of FA and SA following Amberlite XAD-2 fractionation. The fact that 75% of the *trans*-FA was eluted with H_2O, but only 20% with MeOH/H_2O, means that the enzymatic hydrolysis liberates predominantly feruloylated oligosaccharides with a high degree of polymerisation (dp). This is in contrast to enzymatic hydrolysates of insoluble rye dietary fibre where only 9% of the *trans*-FA was eluted with H_2O as opposed to 89% with MeOH/H_2O (as will be detailed elsewhere). The MeOH/H_2O fraction should contain low molecular weight feruloylated oligosaccharides. Therefore, wild rice arabinoxylans seem to contain structures which hinder the enzymes from degrading the arabinoxylans to low molecular weight feruloylated oligosaccharides.

Using the conditions described for trifluoroacetic acid hydrolysis, hydroxycinnamic acids are released in esterified form with minimal release of free hydroxycinnamic

Table 1 Contents of alkaline extractable monomeric phenolics from insoluble wild rice dietary fibre

Hydroxycinnamic acids	(µg g^{-1})	Benzoic acids	(µg g^{-1})	Phenolic aldehydes	(µg g^{-1})
trans-ferulic acid	3744±35	4-OH-benzoic acid	26±4	4-OH-benzaldehyde	56±14
cis-ferulic acid	198±36	protocatechuic acid	128±37	protocatechuic aldehyde	89±23
trans-*p*-coumaric acid	142±2	vanillic acid	32±7	vanillin	27±4
cis-*p*-coumaric acid	n.d.	syringic acid	34±12		
trans-sinapic acid	518±18				

±standard deviation (*n*=3), n.d. not detected

Table 2 Distribution of ferulic acid and sinapic acid following Amberlite XAD-2 fractionation (as a percentage of original ferulic acid and sinapic acid in the hydrolysate)

Fraction	Enzymatic hydrolysate			Acidic hydrolysate		
	trans-FA	*cis*-FA	*trans*-SA	*trans*-FA	*cis*-FA	*trans*-SA
H_2O	75	57	52	7	8	26
MeOH/H_2O 50/50	20	24	33	81	65	62
MeOH	5	19	15	12	27	12

acids [11]. Acidic treatment hydrolysed 47% of WRIDF, but significantly more of the SA (41%) and *p*CA (43%) were released in soluble form compared to enzymatic hydrolysis. In addition, 74% of the *trans*-FA and 61% of the *cis*-FA were hydrolysed. The MeOH/H_2O fraction on Amberlite XAD-2 fractionation contained 81% of the FA (Table 2) indicating that after acidic hydrolysis most FA was bound to monosaccharides or oligosaccharides with a low dp.

The hydrolysates were fractionated on a Sephadex LH-20 column (Fig. 1). Fractions were screened by HPLC-DAD and the enzymatically hydrolysed fractions E5, E6, E7 as well as the acid hydrolysed fractions A8 and A9 were further purified by semi-preparative RP-HPLC. Fractions E5, E6 and E7 eluted in two peaks (ratio approximately 2:1) which were connected by a plateau representing two isomers (α- and β-anomers of the reducing xylose as shown below).

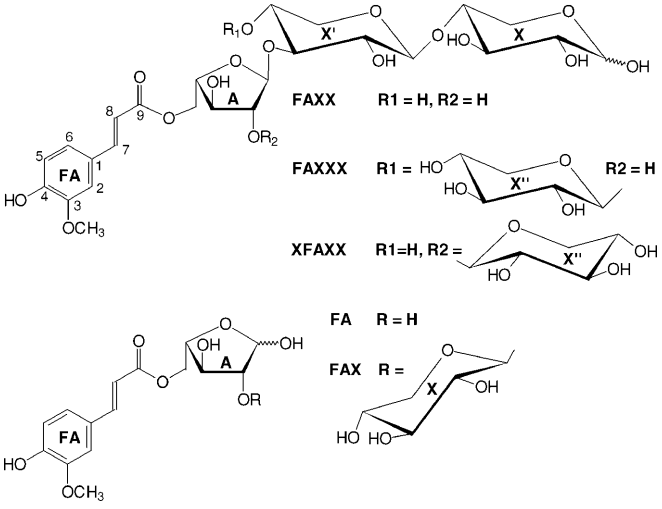

Fig. 2 Structures of feruloylated oligo-/monosaccharides isolated in this study from insoluble wild rice dietary fibre

Identification of oligosaccharide hydroxycinnamates from enzymatically hydrolysed fractions

FA was the only phenolic acid found in the purified fractions E5, E6 and E7. The ratio of *trans*-FA to *cis*-FA was between 23:1 and 15:1, but *cis*-FA might be an isomerisation product of *trans*-FA formed during the alkaline hydrolysis. In all fractions, the only detected carbohydrate compounds, determined as their alditol acetates after acidic hydrolysis, were xylose and arabinose.

E5 and E6 showed quasi-molecular ions with *m*/*z* 745 $(M+Na)^+$ and *m*/*z* 761 $(M+K)^+$, indicating a molecular weight of 722, which is consistent with a molecule consisting of FA and four pentoses. The molecular weight of E7 was 590 (quasi-molecular ions with *m*/*z* 613 $(M+Na)^+$ and *m*/*z* 629 $(M+K)^+$), corresponding to one FA and three pentoses. The structures of E5, E6 and E7 were obtained, using one- and two-dimensional NMR experiments. Anomeric protons in the carbohydrate region were used as starting points for the assignment of proton signals by the H,H-COSY and the TOCSY experiments. Carbon signals were assigned by the HMQC or HMBC experiment. The chemical shifts are reported in Tables 3 and 4. The inter-residue linkages and the differentiation between furanose and pyranose residues were also established by HMBC.

The ^{13}C NMR spectrum of E5 corresponded to those previously reported for *O*-β-D-xylopyranosyl-(1→4)-*O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXXX) (Fig. 2) [22, 23]. ^1H NMR data and the HMBC experiment confirmed this structure. FAXXX was previously identified from sugar cane bagasse [22] and from bamboo shoots [23]. Concerning material suitable for human nutrition, FAXXX was isolated from wheat bran [24].

E6 was identified by its NMR data as {[5-*O*-(*trans*-feruloyl)][*O*-β-D-xylopyranosyl-(1→2)]-*O*-α-L-arabinofuranosyl-(1→3)}-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (XFAXX) (Fig. 2). The HMBC experiment clearly showed the glycosidic (1→2)-bond between xylose and arabinose. This compound, which was previously isolated from Coastal Bermuda grass [25] but not from edible plants, shows that the arabinose linked to the xylan backbone may be further linked to another xylose or even a xylan chain.

NMR data of E7 were similar to those published for *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXX) (Fig. 2) [23]. Among the cereals usable for human nutrition, FAXX was previously identified in barley aleurone [26], wheat bran [24] and corn bran [27, 28].

Identification of oligosaccharide hydroxycinnamates from acid hydrolysed fractions

The only phenolic compounds identified after alkaline hydrolysis of A8 and A9 were *trans*-FA and *cis*-FA in a ratio of about 15:1. Carbohydrate compounds were

Table 3 ^{13}C NMR data of feruloylated oligosaccharides from insoluble wild rice dietary fibre (chemical shifts (δ) in ppm)

		C1	C2	C3	C4	C5	C6	C7	C8	C9	OMe
FAXXX	β-D-Xyl*p*	96.7[a]	74.7[a]	74.1[a]	76.9[a]	63.6[a]					
	α-D-Xyl*p*	92.3[a]	71.5[a]	71.5[a]	n.d.	59.4[a]					
	β-D-Xyl'*p*	101.9[a]	73.7[a]	78.3[a]	74.1[a]	63.1[a]					
	β-D-Xyl''*p*	101.9[a]	73.7[a]	76.0[a]	69.5[a]	65.4[a]					
	α-L-Ara*f*	107.5[a]	80.8[a]	78.2[a]	82.4[a]	64.4[a]					
	FA	126.6[b]	111.7[a]	147.7[b]	148.3[b]	116.1[a]	123.8[a]	147.0[a]	113.7[a]	169.8[b]	56.3[a]
XFAXX	β-D-Xyl*p*	96.5[a]	73.9[a]	74.3[a]	76.9[a]	63.3[a]					
	α-D-Xyl*p*	92.1[a]	72.0[a]	71.5[a]	n.d.	n.d.					
	β-D-Xyl'*p*	101.8[a]	73.2[a]	81.9[a]	68.1[a]	65.5[a]					
	β-D-Xyl''*p*	103.1[a]	73.2[a]	76.0[a]	69.4[a]	65.5[a]					
	α-L-Ara*f*	106.9[a]	89.4[a]	76.3[a]	81.5[a]	63.9[a]					
	FA	126.1[b]	111.5[a]	148.3[b]	150.1[b]	116.1[a]	123.8[b]	147.0[a]	113.7[a]	169.8[b]	56.5[a]
FAXX	β-D-Xyl*p*	97.11	74.52[c]	74.59[c]	77.09	63.5[a]					
	α-D-Xyl*p*	92.3[a]	71.9[a]	71.5[a]	77.2[a]	59.5[a]					
	β-D-Xyl'*p*	102.34	73.39	82.40	68.33	65.63					
	α-L-Ara*f*	108.80	81.73	77.44	82.04	64.45					
	FA	127.23	111.83	148.1[b]	149.1[b]	116.0[a]	123.7[a]	147.17	114.42	169.78	56.41
FAX	α-L-Ara*f*	100.4[a]	89.4[a]	n.d.	n.d.	n.d.					
	β-L-Ara*f*	95.3[a]	83.7[a]	73.5[a]	78.8[a]	65.4[a]					
	β-D-Xyl*p*	104.0[a,d], 103.1[a,d]	73.4[a]	n.d.	n.d.	n.d.					
	FA	126.5[b]	111.7[a]	148.3[b]	149.7[b]	116.4[a]	123.9[a]	147.1[a]	114.0[a]	169.7[b]	56.4[a]
FA	α-L-Ara*f*	101.91	81.93	76.69[c]	81.36	64.13					
	β-L-Ara*f*	95.99	76.59[c]	75.00	79.30	65.74					
	FA	127.23	111.86	148.31	149.2[b]	116.24	124.11	147.15	114.50[d], 114.37[d]	169.86[d], 169.80[d]	56.44

[a] δ taken from HMQC, [b] δ taken from HMBC, [c] assignment may be interchanged, peaks to close to reliably differentiate, [d] doublet peaks due to anomers, n.d. not determined

Table 4 ^{1}H NMR data of feruloylated oligosaccharides from insoluble wild rice dietary fibre[a]

		H1 ($^{3}J_{1,2}$)	H2	H3	H4	$H5_{eq}$	$H5_{ax}$	H5 ($^{3}J_{5,6}$)	H6	H7 ($^{3}J_{7,8}$)	H8	OMe
FAXXX	β-D-Xyl*p*	4.57 (7.9)	3.24	3.54	3.76	4.04	3.36					
	α-D-Xyl*p*	5.18 (3.8)	3.54	n.d.	n.d.	n.d.	n.d.					
	β-D-Xyl'*p*	4.49 (7.6)	3.44	3.72	3.81	4.11	3.38					
	β-D-Xyl''*p*	4.42 (7.9)	3.26	3.40	3.58	3.89	3.26					
	α-L-Ara*f*	5.39 (–)	4.20	3.98	4.54	4.51	4.33					
	FA		7.27					6.92 (8.5)	7.19	7.71 (15.4)	6.44	3.88
XFAXX	β-D-Xyl*p*	4.58 (7.9)	3.25	3.54	3.76	4.04	3.37					
	α-D-Xyl*p*	5.18 (3.7)	3.54	3.76	n.d.	n.d.	n.d.					
	β-D-Xyl'*p*	4.47 (7.9)	3.40	3.57	3.70	4.00	3.33					
	β-D-Xyl''*p*	4.57 (7.9)	3.26	3.44	3.56	3.96	3.32					
	α-L-Ara*f*	5.54 (–)	4.30	4.20	4.40	4.50	4.36					
	FA		7.25					6.90 (7.9)	7.17	7.67 (16.4)	6.40	3.89
FAXX	β-D-Xyl*p*	4.57 (8.2)	3.24	3.53	3.76	4.03	3.35					
	α-D-Xyl*p*	5.18 (3.8)	3.55	3.78	n.d.	n.d.	n.d.					
	β-D-Xyl'*p*	4.46 (7.9)	3.42	3.57	3.70	4.00	3.32					
	α-L-Ara*f*	5.35 (–)	4.23	4.06	4.42	4.51	4.33					
	FA		7.21					6.90 (8.5)	7.14	7.64 (15.9)	6.38	3.89
FAX	α-L-Ara*f*	5.43 (1.9)	4.17	n.d.	n.d.	n.d.	n.d.					
	β-L-Ara*f*	5.38 (4.7)	4.23	4.38	4.10	4.50	4.31					
	β-D-Xyl*p*	4.60[b] (7.9), 4.56[b] (7.9)	3.37[b], 3.29[b]	n.d.	n.d.	n.d.	nd					
	FA		7.26					6.90 (7.9)	7.17	7.69[b] (16.0)	6.41[b]	3.88
FA	α-L-Ara*f*	5.29 (–)	4.07	n.d.	n.d.	4.45	4.31					
	β-L-Ara*f*	5.32 (4.4)	4.12	n.d.	n.d.	4.46	4.27					
	FA		7.20					6.88 (8.2)	7.13	7.64[b] (16.0)	6.37[b]	3.74

[a] chemical shifts (δ) in ppm, coupling constants (*J*) in Hz, [b] doublet peaks due to anomers, n.d. not determined

xylose and arabinose in A8 and solely arabinose in A9. A8 showed quasi-molecular ions at *m*/*z* 481 $(M+Na)^+$ and *m*/*z* 497 $(M+K)^+$, indicating a molecular weight of 458, consistent with a molecule composed of one FA and two pentoses. The molecular weight of A9 was 326 (quasi-molecular ions at *m*/*z* 349 $(M+Na)^+$ and *m*/*z* 365 $(M+K)^+$), indicating that A9 consisted of FA and arabinose. The structures were obtained from NMR. ^{1}H NMR of A8 showed that this fraction was impure. The impurity was estimated to about 30% and therefore complete assignment of the ^{1}H NMR spectrum was not achieved (Table 4). From comparison of the ^{13}C NMR data of the main compound (Table 3) with published data [11] it was suspected that the main compound of A8 was *O*-β-D-xylopyranosyl-(1→2)-[5-*O*-(*trans*-feruloyl)-L-arabinofuranose] (FAX) (Fig. 2). Inter-residue bonds of FAX were also found in the HMBC spectrum of A8. Up to now, FAX was found in leaves of several grasses [29], but its only isolation from edible material (corn bran) is described in Saulnier et al. [11]. It was not possible to characterise the impurities in detail, but as described above (phenolic and carbohydrate composition, molecular weight) they had to be feruloylated disaccharides with xylose and/or arabinose as the carbohydrate compounds.

A9 was identified as 5-*O*-(*trans*-feruloyl)-L-arabinofuranose (FA) (Fig. 2), a relatively uncharacteristic fragment, previously isolated from cereals such as corn bran [11, 28] and wheat bran [24, 27].

Structures of arabinoxylans of wild rice insoluble dietary fibre

The isolation of FAXXX shows that wild rice arabinoxylans consist of a xylan backbone to which arabinose substituents are bound through a (1→3)-linkage. The detection of XFAXX and probably FAX shows that arabinose may be further linked to an additional xylose. This structural characteristic is very frequent and much more common than in arabinoxylans from insoluble rye dietary fibre (as will be detailed elsewhere). As previously shown for other cereal grains (corn, wheat), FA seems to be bound exclusively to the five-position of arabinose through an ester-linkage. As mentioned before, wild rice arabinoxylans contain structural features which may hinder xylanase from degrading the arabinoxylans to oligosaccharides of low dp. Wild rice arabinoxylans show two striking properties that may influence their enzymatic degradability: the high arabinose/xylose ratio (with the interpretive limitation) and the frequent existence of feruloylated arabinose with additional xylose links.

Indications of the attachment of SA to polysaccharides, e.g. arabinoxylans, were observed. Especially the detection of SA in soluble form after enzymatic degradation with carbohydrate hydrolases showed that SA is at least partially bound to polysaccharides. However, the isolation of SA-oligosaccharides, which would be proof for the attachment of SA to polysaccharides, was not successful.

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