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Diferulates as structural components in soluble and insoluble cereal dietary fibre

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Abstract: Cell wall cross-linking can have a substantial effect on the properties of the wall. To estimate cross-linking (between arabinoxylans) in cereal fibres, dehydrodiferulate levels were measured in soluble and insoluble dietary fibre (SDF and IDF) isolated from whole grains of maize (*Zea mays* L), wheat (*Triticum aestivum* L), spelt (*Triticum spelta* L), rice (*Oryza sativa* L), wild rice (*Zizania aquatica* L), barley (*Hordeum vulgare* L), rye (*Secale cereale* L), oat (*Avena sativa* L) and millet (*Panicum miliaceum* L). After saponification of the cereal fibres the extracts were investigated for dehydrodimers of ferulic acid using GLC–MS and GLC–FID. From most cereal IDF the whole spectrum of dehydrodiferulic acids (DFAs) (8-5′-, 8-8′-, 5-5′-, 8-O-4′- and 4-O-5′-coupled) could be identified. The absolute contents of total DFAs ranged between 2.4 and 12.6 mg g^{-1}. With the exception of 4-O-5′-coupled DFA, the whole range of DFAs was also detected from cereal SDF but only in amounts of 40–230 μg g^{-1}. It was estimated that arabinoxylans of cereal IDF contain 8–39 times more diferulates than arabinoxylans of cereal SDF (where measurement of DFA levels in SDF was possible). In cereal IDF, 8-5′-coupled dimers dominated, whereas in cereal SDF, 8-8′-coupled dimers were relatively enhanced and often became the major dimers.

Keywords: ferulic acid; ferulate; diferulic acids; diferulates; dietary fibre; cereals; arabinoxylans; cell wall cross-linking

INTRODUCTION

The term dietary fibre was first used about 50 years ago to describe plant cell walls in the diet.[1] For a long time the definition of dietary fibre has caused a great deal of confusion. Since the late 1970s there has been consensus that dietary fibre consists of the remnants of edible plant cells, polysaccharides, lignin and associated substances resistant to hydrolysis by the digestive enzymes of humans.[2]

Cereals form the quantitatively most important source of dietary fibre in many industrialised countries. In most European countries the consumption of dietary fibre has been estimated to be around 20 g day^{-1}.[3] An increased daily intake to approximately 30 g is encouraged to promote health benefits associated with fibre components.[4] Dietary fibre exerts physiological effects as it passes through the gastrointestinal tract. Fibre can interact with other nutrients and influence their uptake. It can also be fermented in the large bowel by a mixed flora of anaerobic bacteria. Most physiological effects of dietary fibre, such as regulation of blood sugar, lowering of blood cholesterol and prevention of bowel cancer, are thought to be based on these properties. However, prevention of constipation and regulation of transit time are mainly caused by the bulking effect of dietary fibre. The characteristics of different dietary fibres (water-holding capacity, viscosity, binding and adsorptive capacity, bulk and fermentability) are dependent on the chemical nature of the fibre components.[5]

Normally, plant cell walls constitute the major part of dietary fibre. Cell wall polymers mainly found in monocotyledonous grains are cellulose, hemicelluloses, pectins and lignin. Cereal dietary fibre arabinoxylans and mixed β-glucans are the main hemicellulose components. It is well known that arabinoxylans of monocotyledons are associated with ferulic acid via an ester bond.[6] The presence of ferulates attached to polysaccharides provides a convenient mechanism to cross-link polysaccharides via diferulates.[7] Dimerisation of ferulates is possible by photochemical coupling reactions[8] or radical coupling

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reactions. For over 20 years, 5-5′-coupled diferulic acid was the sole ferulic acid dehydrodimer known.[9] More recently, it was shown that free radical coupling of ferulates via the action of peroxidases produces several regio-isomeric diferulates that can be synthesised chemically.[10] Isolation and identification of 5-5′-diferuloyl oligosaccharides from the enzymatic hydrolysate of bamboo shoot[11] and from the acidic hydrolysate of maize bran[12] suggest that ferulate dimerisation products act as cross-links between plant cell wall polysaccharides. Furthermore, ferulates and dehydrodiferulates have a significant role in cross-linking polysaccharides to lignin.[7,13–15] Consequently, dehydrodiferulates may play an important role in dietary fibre by influencing the chemical structure of dietary fibre components.

In a previous study it was shown that dehydrodiferulates are components of insoluble cereal dietary fibre.[16] Owing to lack of standard substances, individual dehydrodiferulic acids were not identified and quantification was not possible. In this paper we describe the identification and quantification of the full range of dehydrodiferulates in insoluble cereal dietary fibre; soluble cereal dietary fibre was also investigated for dehydrodiferulates.

MATERIALS AND METHODS

Internal standard (IS) (*E,E*)-4-hydroxy-4′,5,5′-trimethoxy-3,3′-bicinnamic acid

Since the currently used internal standards (tetracosane and 2-hydroxycinnamic acid) have non-ideal retention times or response factors that are too large, an internal standard more like the dimers being analysed was sought. In this study we used monomethylated 5-5′-DFA produced by methylation of diethyl 5-5′-diferulate using dimethyl sulphate, followed by column purification on silica gel and saponification. Although it worked well for this study, the standard cannot be recommended at this time for the following reasons. It was not discovered until well into this study that the standard was contaminated by its dimethylated analogue (peak IS* in Fig 2). Subsequent attempts to purify the compound failed. Since response factors were derived for IS against the authentic diferulates, the quantitative aspects of this study are sound, but in future studies it will be necessary to find or prepare a pure internal standard and determine response factors to the pure standard. Although we sought the monomethylated diacid as an IS to mimic the properties of the DFA dimers most closely, the dimethylated product IS* may also be satisfactory. Certainly its relative retention time is appropriate and exhaustive methylation should produce it more cleanly.

Synthesis of dehydrodiferulic acids

Dehydrodiferulic acids were synthesised as described by Ralph *et al.*[10] Synthesis of 8-5′-coupled diferulate was carried out according to a simplified method.[17]

General

All samples were analysed in triplicate. Determinations of carbohydrate compounds were performed in duplicate. Dioxane and diethyl ether were purchased from Aldrich (Milwaukee, WI, USA). NaOH and HCl were from Fischer Scientific (Fair Lawn, NJ, USA). Pyridine was from Mallinckrodt (Paris, KY, USA). Bis (trimethylsilyl)trifluoroacetamide (BSTFA), Coomassie protein assay reagent and albumin standard solutions were from Pierce (Rockford, IL, USA). DMSO and $NaBH_4$ were purchased from Fluka (Buchs, Switzerland). All other chemicals were from Merck (Darmstadt, Germany). The heat-stable α-amylase Termamyl 120L (EC 3.2.1.1, from *Bacillus licheniformis*, 120 KNU g^{-1}), the protease Alcalase 2.4L (EC 3.4.21.62, from *Bacillus licheniformis*, 2.4 AU g^{-1}) and the amyloglucosidase AMG 300L (EC 3.2.1.3, from *Aspergillus niger*, 300 AGU g^{-1}) were from Novo Nordisk (Bagsvaerd, Denmark).

Plant material

Whole grains of maize (*Zea mays* L), wheat (*Triticum aestivum* L), spelt (*Triticum spelta* L), rice (*Oryza sativa* L), wild rice (*Zizania aquatica* L), barley (*Hordeum vulgare* L), rye (*Secale cereale* L), oat (*Avena sativa* L) and millet (*Panicum miliaceum* L) were obtained from a local German supplier.

Preparation of insoluble dietary fibre

Samples were milled to a particle size smaller than 0.5 mm. The sample material (10 g) was suspended in sodium phosphate buffer (0.08 M, pH 6.0, 300 ml), and 750 µl of α-amylase was added. Beakers were placed in a boiling water bath for 20 min and shaken gently every 5 min. The pH was adjusted with about 60 ml of 0.275 M NaOH to 7.5, and samples were incubated with 300 µl of protease at 60 °C for 30 min with continuous agitation. After adjusting the pH with about 60 ml of 0.325 M HCl to 4.5, 350 µl of amyloglucosidase was added and the mixture was incubated at 60 °C for 30 min with continuous agitation. The suspension was centrifuged. The supernatant was kept for isolation of soluble dietary fibre. The residue was washed two times with hot water, 95% (v/v) ethanol and acetone and finally dried at 60 °C overnight in a vacuum oven. Water washings were combined with the first supernatant for preparation of SDF. IDF was corrected for residual protein and ash contents.

Preparation of soluble dietary fibre

The volume of the combined supernatant solution and water washings was determined. Ethanol (95% (v/v), preheated to 60 °C, four volumes) was added and the precipitate was allowed to form overnight at room temperature. After centrifugation the residue was washed two times with 78% (v/v) ethanol, 95% (v/v) ethanol and acetone and finally dried at 60 °C in a vacuum oven. SDF was corrected for residual protein and ash contents.

Determination of residual protein and ash contents

The nitrogen content of IDF was determined by the Kjeldahl method.[18] Protein was calculated as $N \times 6.25$.

Owing to the small sample amounts and low residual protein content, the protein content of SDF was determined according to Bradford.[19] To facilitate resolution, aliquots of SDF used for the protein determination were sonicated in 0.1 M NaOH. Albumin standard solution was used for calibration.

Ash content was determined gravimetrically by incineration of IDF and SDF at 525 °C for 8 h.

Saponification of ester-linked phenolics from insoluble and soluble dietary fibre

IDF (40–90 mg) or SDF (60–120 mg) was weighed into a screw-capped tube, internal standard (5–50 μg) dissolved in dioxane 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 with 0.95 ml of concentrated HCl (resulting pH < 2) and extracted into diethyl ether (4 ml, three times). Extracts were combined and evaporated under a stream of filtered air. Finally, samples were dried under vacuum.

GLC–FID and GLC–MS analysis of dehydrodiferulic acids

Dried extracts were silylated by adding 10 μl of pyridine and 40 μl of BSTFA and heating for 30 min at 60 °C in sealed vials. Trimethylsilylated derivatives of phenolic acids were separated by GLC (Hewlett-Packard 5980, Palo Alto, CA, USA) using a 0.2 mm × 25 m DB-1 capillary column (0.33 μm film thickness) (J&W Scientific, Folsom, CA, USA) and identified by their electron impact (70 eV) mass data collected on a Hewlett-Packard 5970 mass-selective detector. He (0.54 ml min^{-1}) was used as carrier gas. GLC conditions were as follows: initial column temperature 220 °C, held for 1 min, ramped at 4 °C min^{-1} to 248 °C, ramped at 30 °C min^{-1} to 300 °C and held for 40 min; injector temperature 300 °C, split 1/50 or 1/15 (determination of IDF or SDF respectively). DFAs were authenticated by comparison of their relative retention times and mass spectra with synthesised standards (Fig 1). Mass spectra of DFAs for the library (Fig 1) were also recorded using another GLC–MS system consisting of a Thermoquest Trace 2000 GC (Austin, TX, USA) and a Thermoquest QSC ion trap MS under the conditions already described above. The small amounts of dehydrodiferulic acids from SDF required mass spectral identification to be carried out in selected ion-monitoring mode (SIM). Three groups were formed: 15–17.5 min, *m/z* 674, 602, 556, 512, 467, 389; 17.5–26 min, *m/z* 674, 602, 512; 26–30 min, *m/z* 674, 616, 602, 558. Quantitative determination of dehydrodiferulic acids was carried out by GLC (Hewlett-Packard 5980) using the same column and GLC conditions as above and a flame ionisation detector (detector temperature 300 °C). He (0.4 ml min^{-1}) was used as carrier gas. Owing to tiny amounts of 4-O-5′-coupled dehydrodiferulic acid (4-O-5′-DFA) in the samples and the limited availability of its standard, 4-O-5′-DFA was determined semi-quantitatively as described previously.[20]

Carbohydrate analysis

Analysis of carbohydrate components was performed as described previously.[21] Briefly, IDF and SDF were pre-treated with 12 M H_2SO_4 at 35 °C for 30 and 5 min respectively. Hydrolysis was performed with 2 M H_2SO_4 for 1 h at 100 °C. Neutral monosaccharides were reduced with $NaBH_4$. Resulting alditols were acetylated with acetic anhydride using 1-methylimidazole as catalyst. Myo-inositol was used as internal standard. 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). 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.

RESULTS AND DISCUSSION

Absolute contents of dehydrodiferulic acids in cereal dietary fibre

Ferulate esters dimerise via their phenoxy radicals *in planta* to form dehydrodiferulate esters.[10] Electron-delocalised phenoxy radicals couple at their 4-O-, C5- or C8-positions to give rise to 8-5′-, 8-O-4′-, 5-5′-, 8-8′- and 4-O-5′-coupled dehydrodiferulate esters. Saponification releases the eight dehydrodiferulic acids shown in Fig 1. The 8-8′-diferulates in the plant cell wall produce two isomeric 8-8′-diferulic acids (8-8′-DFA (aryltetralin), 8-8′-DFA); the structure(s) of the 8-8′-diferulates existing in the walls remains unknown.[10] Benzofuran 8-5′-dehydrodiferulates in the wall produce two diferulic acids as well as some decarboxylated product (8-5′-DFA (benzofuran), 8-5′-DFA, 8-5′-DFA (decarboxylated)). Since the ratios of the different 8-8′- or 8-5′-DFAs depend on the exact conditions of release, it is only the total 8-8′- or 8-5′-DFA level that is important (reflecting the coupling reaction occurring in the cell wall).

In IDF of maize, wheat, spelt, rye, rice, wild rice and barley the whole range of expected DFAs was identified after alkaline hydrolysis by their relative retention times (GLC–MS and GLC–FID) and their mass spectra (Fig 1). Fig 2 shows the GLC–FID chromatogram of maize IDF. Owing to the small amounts of 4-O-5′-DFA, its identification was by its relative retention time and detection of its molecular ion peak (*m/z* 602) in selected ion chromatograms. In

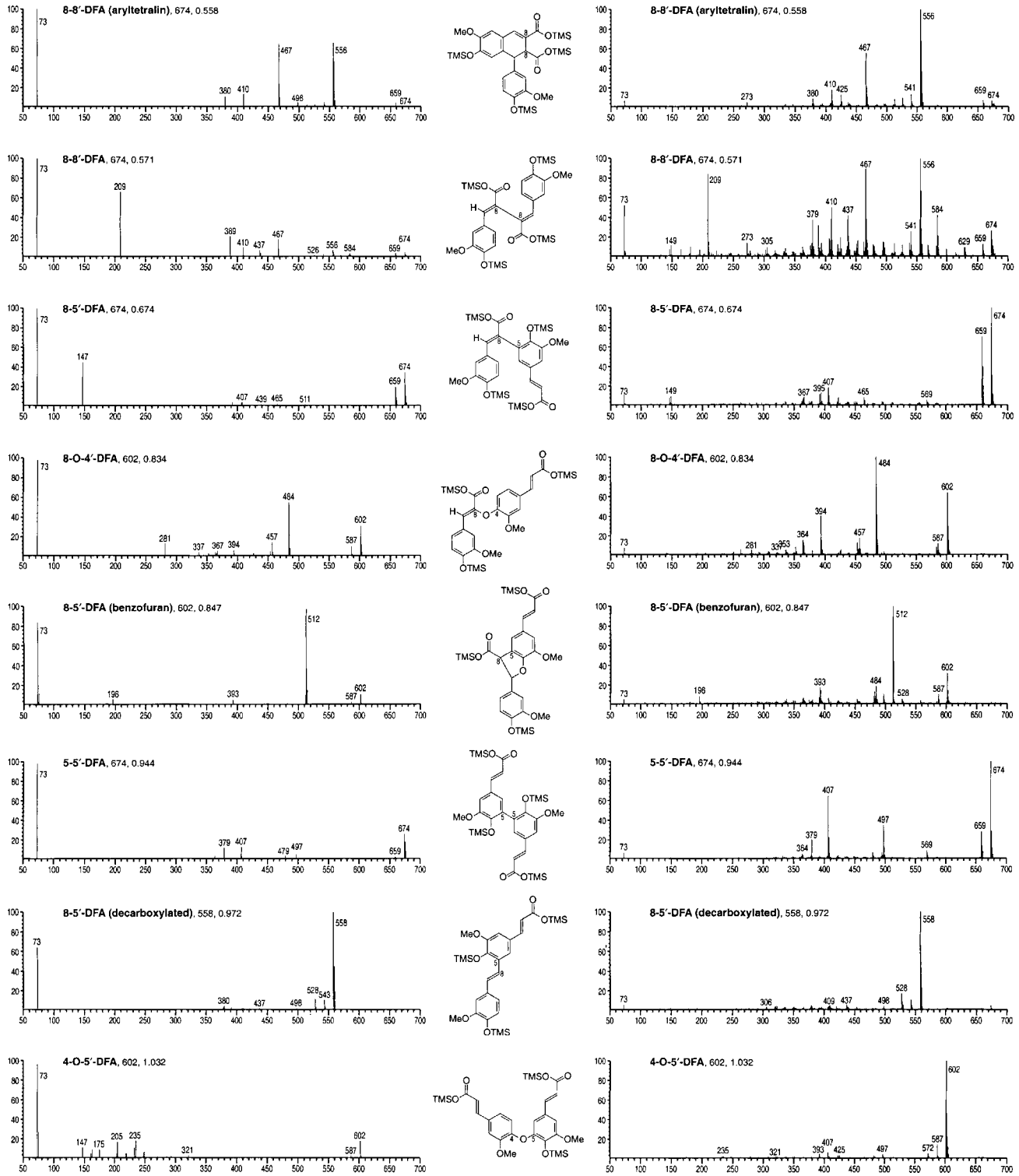

Figure 1. Electron impact mass spectra library from quadrupole (left, major peaks selected) and ion trap (right, full spectra) mass-selective detectors for silylated diferulic acids (title of each gives the abbreviated name, the nominal molecular weight and the retention time relative to the internal standard).

IDF of millet and oat, all expected DFAs with the exception of 4-O-5′-DFA were detected.

In the extracts of saponified SDF of all cereals investigated, the whole range of DFAs with the exception of 4-O-5′-DFA was identified.

Quantitative determination was carried out using a new internal standard. Response factors for all the DFAs against (*E*,*E*)-4-hydroxy-4′,5,5′-trimethoxy-3,3′-bicinnamic acid were close to unity (0.91–1.18, with the exception of the response factor for 8,5-DFA (benzofuran), which is inexplicably 2.20). Despite the simple extraction procedure without further clean-up, all compounds separated well and peaks could be integrated without difficulties. Under the chromatographic conditions described, no other substances co-eluted with the DFAs as determined by mass spectrometry. Although some clean-up procedures such as solid phase extraction[16] or liquid–liquid extraction

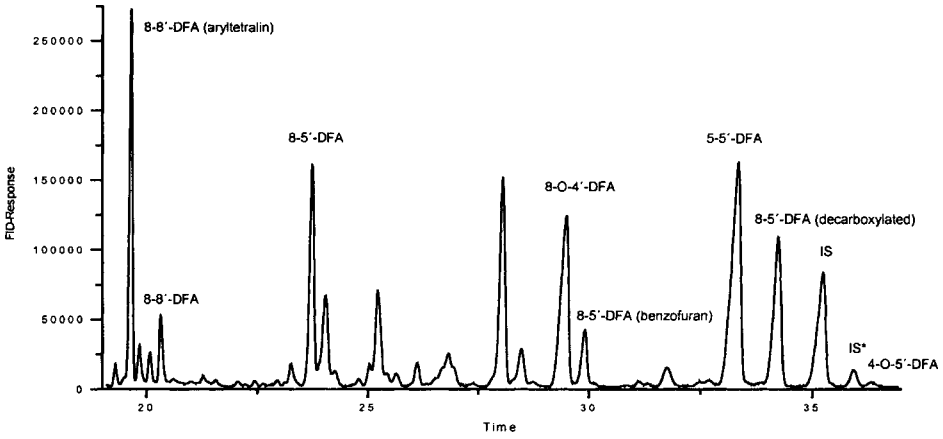

Figure 2. GLC–FID chromatogram of the extract of saponified maize insoluble dietary fibre.

with $NaHCO_3$ solution[22] have been described, there is no need for their application when using the GLC methods described here (and previously[10]).

The absolute contents of total DFAs in cereal IDF varied considerably among different cereal species investigated (Table 1). The contents ranged from 12.6 mg g^{-1} in maize to 2.4 mg g^{-1} in wheat. Even if the extreme high value for maize is not considered, the contents differ substantially. For example, millet contains twice as much total DFAs as wheat, spelt and wild rice.

In most cases, quantification of absolute contents of total DFAs in SDF was possible. In SDF of rice and wild rice, DFAs could be identified unambiguously, but quantification was not attempted as the levels were too low. The absolute contents of total DFAs from the remaining cereal SDFs varied between about 230 μg g^{-1} in spelt and 40 μg g^{-1} in oat (Table 1). There is no correlation between high absolute DFA contents in IDF and SDF. For example, SDF of maize contained only about 60 μg g^{-1}, whereas IDF of maize contained by far the highest absolute DFA content of the cereals investigated. However, the absolute total content of DFAs of spelt SDF is about 50 times lower than that of maize IDF and still 10 times lower than the content of wheat IDF.

Until now, no detailed investigations of the DFA contents in SDF and IDF have been reported. Renger and Steinhart[16] investigated IDF of maize (*Z mays* L), wheat (*T aestivum* L), rice (*O sativa* L), barley (*H vulgare* L), rye (*S cereale* L), oat (*A sativa* L) and millet (*P miliaceum* L) for DFAs and carried out a semi-quantitative determination of ester-bound DFAs by setting the response factors of DFAs against the internal standard *o*-coumaric acid as 1.0. The contents of absolute total DFAs ranged between about 150 μg g^{-1} in oat and 950 μg g^{-1} in millet. Ralph *et al*[10] determined response factors of DFAs against *o*-coumaric acid. They varied between 0.10 and 0.37. Setting the response factors as unity leads to considerable underestimation. Losses of DFAs could also occur owing to clean-up of the hydrolysate by use of SPE. Missing DFAs, 8-5-DFA (decarboxylated) and 8-5′-DFA (benzofuran), may also arise from the clean-up step and contribute to underestimation. Other studies on DFAs in cereal grains or parts of cereal grains are rare. Saulnier *et al*[12] investigated destarched and deproteinised maize bran for DFAs. They found an absolute total DFA content of 13.3 mg g^{-1}. This content is comparable to our content in maize IDF, but it has to be considered that we used the whole grain and that the ash content and residual protein content were not taken into account. Moreover, 8-8′-DFAs could not be detected owing to the lower

Table 1. Absolute contents of total dehydrodiferulic acids (DFAs) with corresponding standard deviations (SD) and DFA/xylose quotients (DFA/Xyl) from insoluble (IDF) and soluble (SDF) dietary fibre of different cereal grains

	IDF			*SDF*		
	Total DFAs[a]	*SD*[a]	*DFA/Xyl*[b]	*Total DFAs*[a]	*SD*[a]	*DFA/Xyl*[b]
Maize	12596	184	24.5	59	3	1.3
Wheat	2372	36	4.6	184	14	0.4
Spelt	2601	59	5.0	233	43	0.6
Rice	4042	59	18.8	Tr[c]	—	—
Wild rice	2840	130	14.6	Tr[c]	—	—
Barley	3658	170	7.7	69	10	0.9
Rye	3647	132	6.2	83	8	0.2
Oat	3599	69	9.5	38	5	0.8
Millet	5693	231	31.4	46	9	3.6

[a] μg g^{-1} IDF/SDF.

[b] [(mol total DFAs g^{-1} IDF/SDF)/(mol xylose g^{-1} IDF/SDF)] × 1000.

[c] Traces; some diferulic acids were below the determination limit of about 3 μg g^{-1}.

resolution of the HPLC method used, and the possibility of 8-5′-DFA (decarboxylated) was not considered. Differences in the DFA composition could also be a result of different saponification conditions. Bartolomé *et al*[23] determined a total DFA content of about 1.4 mg g^{-1} in barley spent grain (*H vulgare* L) and 1.8 mg g^{-1} in destarched wheat bran (*T aestivum* L). Owing to reasons discussed above, the results are only minimally comparable. Garcia-Conesa *et al*[24] found about 1.3 mg total DFAs g^{-1} destarched wheat bran (*T aestivum* L). Under their conditions they could not detect or separate 8-8′-DFAs as well as 8-5′-DFA and 8-5′-DFA (decarboxylated). Recently, Andreasen *et al*[25] investigated whole grains of rye (*S cereale* L) for DFAs using an HPLC method. They identified 8-5′-DFA (benzofuran), 8-5′-DFA, 8-O-4′-DFA as well as 5-5′-DFA and determined a total DFA content of 307 μg g^{-1} (dry matter, not destarched and not deproteinised). Figueroa-Espinoza and Rouau[26] as well as Dervilly *et al*[27] detected small amounts of DFAs (up to 350 μg g^{-1} total DFAs) in soluble arabinoxylans of wheat (*T aestivum* L). Lempereur *et al*[28] identified 8-5′-DFA (benzofuran), 8-5′-DFA, 8-O-4′-DFA as well as 5-5′-DFA in whole grains as well as in milling fractions of durum wheat (*T durum* Desf), a cereal not considered in this study.

Contents of total DFAs in relation to arabinoxylan contents

In grasses, ferulic acid is attached to heteroxylans via acylation of the primary hydroxyl at the C5-position of α-L-arabinofuranosyl residues.[6] Until now, the sole known exception are plant cell walls of bamboo shoots, from which a feruloylated xyloglucan disaccharide was isolated and identified.[29]

Absolute contents of DFAs do not give any information about the extent of arabinoxylan cross-linking. Therefore the carbohydrate composition of SDF and IDF was determined for each of the samples.

The composition of polysaccharides varied considerably among the different cereal IDFs and SDFs. For example, polysaccharides in SDF of rye, wheat and spelt were dominated by arabinoxylans, whereas SDF of oat and barley contained mainly mixed-linked β-glucans. Differences in carbohydrate composition of IDFs were smaller but still marked. The xylose content of rye IDF was three times as high as in millet, for example. Owing to different polysaccharide compositions, the xylose contents of different cereal IDFs and SDFs have to be taken into consideration to estimate the extent of cross-linking of arabinoxylans by DFAs. Formation of the DFA/xylose quotient ([(mol total DFAs g^{-1})/(mol xylose g^{-1})] × 1000) should be a useful way of reflecting the different extent of arabinoxylan cross-linking in different cereal IDFs and SDFs (Table 1).

The extent of arabinoxylan cross-linking was by far the highest in millet and maize IDF. Arabinoxylans in IDF of spelt and wheat were the least cross-linked. Owing to the low arabinoxylan content of millet SDF, the extent of cross-linking was the highest among the cereal SDFs, whereas arabinoxylans of rye SDF were the least cross-linked. Arabinoxylans of IDFs are 8 times (spelt) to 39 times (rye) more cross-linked than arabinoxylans of cereal SDFs.

Cross-linking is known to induce gelation of feruloylated arabinoxylans.[30–32] In the same way, diferulates may be partly responsible for the insolubility of arabinoxylan components in IDF.[33] Even if it is generally believed that differences in solubility of arabinoxylans are dependent on their substitution degree, these results may show that the physicochemical behaviour of arabinoxylans is also dependent on their degree of cross-linking via DFAs. This assumption seems to be reasonable if it is considered that the (apparent) molecular weights of arabinoxylans will be dramatically increased by cross-linking them through DFAs.

Evidence for covalent associations between polysaccharides and lignin in plant cell walls has accumulated over the last few years. Iiyama *et al*[13] demonstrated that monomeric ferulic acid that was etherified to lignin was also esterified to arabinoxylans, thus proving that ferulates cross-link lignin and polysaccharides. More recently, it was demonstrated that diferulates, which already have tied two polysaccharide chains together, can become involved in the lignification process to produce a highly cross-linked lignin–polysaccharide network.[7,14] Owing to the hydrophobicity of lignin, any arabinoxylans which are coupled to lignin would be part of IDF. Extensive cross-linking of cell wall polysaccharides may influence the physiological properties of cereal dietary fibre. After passing the small intestine, carbohydrates are partially fermented to short-chain fatty acids by anaerobic bacteria in the colon. Effects of short-chain fatty acids in the colon are manifold and they seem to play a key role in maintaining the health of the large intestine mucosa.[34] On the other hand, faecal bulking capacity of dietary fibre is inversely proportional to its fermentation. Fermentation of plant cell walls containing ferulates has almost exclusively been studied in ruminants.[35] More recently, the effect of diferulate cross-linking on the degradation of cell walls by fungal enzyme mixtures was investigated.[36] It was shown that diferulates have a significant impact on both the rate and extent of polysaccharide degradation. Cross-linking through diferulates may inhibit the binding of endoxylanases, thus limiting the extent of arabinoxylan degradation. Furthermore, cross-linking may prevent localised areas from swelling, excluding key enzymes for dietary fibre degradation.[37] Our investigations showed that polysaccharides of IDF are significantly more cross-linked than those of SDF. Among other things, this can be one factor to explain the observation that fibre sources relatively high in insoluble polysaccharides are effective in increasing stool weight owing to their bulking capacity, whereas polysaccharides that are dispersible in water are extensively degraded by the microflora.[5]

DFA patterns

The diferulate distribution patterns of different cereal IDFs were similar (Table 2). In all cereal IDF the 8-5′-coupled DFA dominated, accounting for up to 54% of the total DFAs from rye IDF. Next followed 8-O-4′-DFA with up to 21% from oat IDF or 8-8′-DFAs with up to 26% from wild rice IDF. The previously solely quantified diferulic acid, 5-5′-DFA, only contributed up to 16% of the total DFAs from wheat IDF. Maize IDF showed a slightly different pattern with 8-5′-DFAs dominating, but 5-5′-DFA contributed 25% of the total.

DFA patterns from different cereal SDFs were similar too (Table 2), but they differed considerably from IDF patterns. The relative amounts of 8-8′-DFAs were greater, and often major, with up to 46% from spelt SDF. Amounts of 8-5′-DFAs reached up to 45% in oat, whereas 5-5′-DFA and 8-O-4′-DFA only contributed up to 17% (barley SDF) or 12% (maize SDF) to total DFAs. 4-O-5′-DFA could not be detected in cereal SDF and only contributed up to 0.5% to spelt and wheat IDF DFAs.

The increased amounts of 8-8′-DFAs in SDF are interesting with regard to the structure of 8-8′-coupled diferulate esters *in planta*. Up to now, the structure(s) of the 8-8′-coupled diferulate esters in the cell wall are unknown. Ralph *et al*[10] described three possible 8-8′-coupled structures following the oxidative coupling pathway. Two of them are potential polysaccharide cross-linking structures, whereas the third does not represent cross-linked polysaccharides. This may be a reason for the increased contribution of 8-8′-DFAs in SDF, where a low degree of cross-linking is presumed.

Table 2. Dehydrodiferulic acid patterns from insoluble (IDF) and soluble (SDF) dietary fibre of different cereal grains

		8-5′ [a]	*8-8′* [a]	*5-5′* [a]	*8-O-4′* [a]	*4-O-5′* [a]
IDF	Maize	37.7	16.5	24.9	20.6	0.3
	Wheat	46.8	16.8	16.1	19.8	0.5
	Spelt	46.9	16.7	15.8	20.0	0.5
	Rice	45.5	22.2	13.3	18.6	0.4
	Wild rice	46.9	26.0	13.5	13.6	D
	Barley	50.7	16.1	14.9	18.4	D
	Rye	54.0	18.4	13.4	14.2	D
	Oat	47.1	18.8	12.9	21.2	ND
	Millet	46.9	25.0	12.9	15.2	ND
SDF	Maize	36.5	39.8	11.9	11.8	ND
	Wheat	37.4	36.4	16.0	10.2	ND
	Spelt	40.4	46.0	7.1	6.5	ND
	Rice	D	D	D	D	ND
	Wild rice	D	D	D	D	ND
	Barley	38.8	34.7	16.7	9.8	ND
	Rye	33.1	43.5	15.5	7.9	ND
	Oat	44.7	32.8	11.2	11.4	ND
	Millet	44.0	41.9	8.5	5.7	ND

[a] [(mol 8-5′-/8-8′-/5-5′-/8-O-4′-/4-O-5′-coupled dehydrodiferulic acid(s))/(mol total DFAs)] × 100.
D, dehydrodiferulic acid(s) detected but below determination limit of about 3 μg g^{-1}.
ND, not detected.

Consequently, it will be important to elucidate the structure(s) of 8-8′-diferulate(s) *in planta*.

CONCLUSIONS

All diferulates expected from ferulate free radical coupling were detected in cereal grains. Cross-linking is likely to change the physiological properties of dietary fibre components. Owing to their role in cross-linking arabinoxylans, diferulates may be partly responsible for producing IDF by increasing the arabinoxylan molecular weight and possibly cross-linking arabinoxylans to hydrophobic lignin. Cereal SDF contains only tiny amounts of diferulates. Other possible health benefits of diferulates and ferulates due to their antioxidant capacities[38–40] should also be taken into consideration. The observation that 8-8′-coupled diferulates dominate in SDF renews the interest in and the need to confirm the nature of 8-8′-diferulate(s) *in planta*.

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