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Synthesis and Antioxidant Activity of Hydroxycinnamic Acid Xylan Esters

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Naturally occurring hydroxycinnamic acids, such as ferulic and sinapic acids, are known to possess antioxidant activity. In this study, ferulic acid and sinapic acid were covalently attached to oat spelt arabinoxylan and birch wood glucuronoxylan by esterification in a two-step feasible synthesis to generate modified xylans with various degrees of substitution. The obtained derivatives were fully analyzed by FT-IR, NMR, and HPSEC experiments to confirm the esterification of xylans and the degree of substitution. The antioxidative potential of the conjugates was evaluated using the emulsion lipid oxidation test. The results demonstrate that the derivatized xylans inhibited lipid oxidation notably better than the native oat spelt and birch wood xylans. It was found that ferulic acid esters of glucuronoxylan were more efficient antioxidants than those of arabinoxylan and that sinapic acid xylan esters were more efficient than their ferulic acid counterparts.

KEYWORDS: Hemicellulose; xylan; degree of substitution; hydroxycinnamic acid; antioxidative activity

INTRODUCTION

In recent years, lignocellulosic materials have received considerable interest as a source of renewable materials based on wood biomass and agricultural residues. These naturally occurring biodegradable and recyclable materials are regarded as promising alternatives for the production of biofuels and replacement of the synthetic polymers, consequently reducing the global dependence on fossil hydrocarbons (*1*).

Hemicelluloses are a group of polymeric carbohydrates found in vegetable fibers and cell walls of woods and annual plants, comprising roughly 20−40% of the total mass of many plants (*2*). They are branched polymers of low molecular weight with a degree of polymerization (DP) of 80−200 (*3*) and are comprised of hexose or pentose sugar monomer units, such as galactose, glucose, mannose, arabinose, and xylose (*4*).

Xylans are the main hemicellulose components in hardwoods, grasses, and cereals representing up to 40% of the total mass of cell wall material. The xylan polymers consist of xylopyranosyl (Xyl*p*) units that are linked together by β-(1→4)-glycosidic bonds and are substituted in C-2 and/or C-3 positions by the side groups consisting of α-4-*O*-methylglucopyranosyluronic acid (meGlc*p*A), α-L-arabinofuranosyl (Ara*f*) or xylopyranosyl moieties, or acetyl units (*5*). The most common xylans in cereal grains are arabinoxylans, in which various amounts of Ara*f* residues are attached to the xylan backbone at position 2 or 3 or at both positions 2 and 3. In addition, arabinoxylans may carry small amounts of esterified phenolic acids, mainly hydroxycinnamic acids (*6*), linked to the O-5 position of some Ara*f* residues. These hydroxycinnamic acids, especially ferulic acid, have a significant role in establishing cross-linked networks in polysaccharides by oxidative coupling to form dihydrodimers between the arabinose chains (*7*).

In addition to ferulic acid, other hydroxycinnamic acids such as caffeic acid, *p*-coumaric acid, chlorogenic acid, and sinapic acid also are widely distributed in plant tissue (*8*, *9*). Their derivatives most commonly appear as esters of quinic acids, glucose, or plant cell wall polysaccharides and are present in wide variety of fruits, vegetables, and cereals (*9*). Such ester conjugates have recently drawn attention due to their anticarcinogenic, antiinflammatory, and antioxidant properties, linked to their ability to form stable radicals as well as their ability to chelate metals (*10*−*12*). The presence of the −CH=CH−COOR chain in hydroxycinnamic acids and in their derivatives facilitates the radical stabilization, initially formed by dehydrogenative oxidation of phenolic hydroxyl. The antioxidativity is further influenced by electron-donating substituents and the polarity of the solvent.

Interesting applications of the chemically modified xylan biopolymers include wound dressing materials, drug carrier, thermoplastics, or additives in papermaking (*13*). The necessary enhancement of chemical properties required for applications has been achieved mostly by means of esterification and etherification. The esterification reactions are usually carried out with methods such as reactions with anhydrides in alkaline conditions (*14*) or with acid chlorides with a proper catalyst such as triethylamine and 4-dimethylaminopyridine (*15*). The etherification reactions have been conducted by using epoxides (*16*) or by conventional Williamson etherification with alkyl halogens (*17*). Also, due to the insolubility of xylans in most common solvents, different solvent systems such as *N*,*N*-dimethylacetamide/LiCl (*18*) and *N*,*N*-dimethylformamide/LiCl (*15*) have been introduced.

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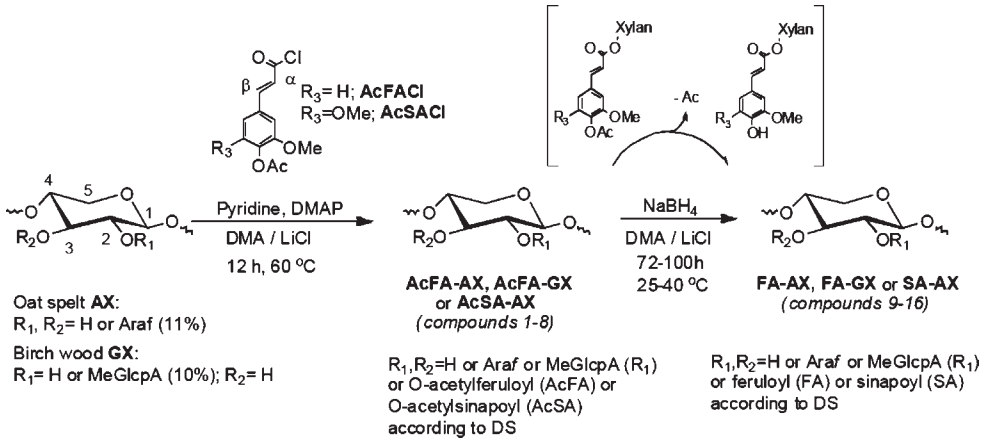

Figure 1. Esterification of oat spelt arabinoxylan and birch wood glucuronoxylan with 4-*O*-acetyl-protected hydroxycinnamic acids and their subsequent deprotection by sodium borohydride.

Table 1. Esterification of Oat Spelt Arabinoxylan and Birch Wood Glucuronoxylan by 4-*O*-Acetyl-Protected Hydroxycinnamic Acid Chlorides with Pyridine and DMAP in DMA/LiCl

reagent	reagent[a]	molar ratio/XU[b]	WPG (%)[c]	compd (no.)[d]
oat spelt (AX)	AcFACl	0.5	−15	AcFA-AX (**1**)
oat spelt (AX)	AcFACl	1	25	AcFA-AX (**2**)
oat spelt (AX)	AcFACl	1.5	43	AcFA-AX (**3**)
oat spelt (AX)	AcFACl	3	12	AcFA-AX (**4**)
oat spelt (AX)	AcSACl	1	3	AcSA-AX (**5**)
oat spelt (AX)	AcSACl	1.5	30	AcSA-AX (**6**)
birch wood (GX)	AcFACl	1	−19	AcFA-GX (**7**)
birch wood (GX)	AcFACl	1.5	35	AcFA-GX (**8**)

[a] AcFACl = 4-*O*-acetylferulic acid chloride; AcSACl = 4-*O*-acetylsinapic acid chloride. [b] XU = anhydroxylose units, i.e., the amount of hydroxyl functionality in xylan. [c] WPG = weight percent gain. [d] AcFA-AX = 4-*O*-acetylferulic acid oat spelt arabinoxylan ester; AcSA-AX = 4-*O*-acetylsinapic acid oat arabinoxylan ester; AcFA-GX = 4-*O*-acetylferulic acid birchwood glucuronoxylan ester.

In this study, we examined the derivatization of xylans by antioxidative hydroxycinnamic acids. The idea of construction of such polymers was to mimic the naturally occurring arabinoxylans by attaching ferulic and sinapic acid to oat spelt arabinoxylan by esterification to produce polymers with various degrees of substitution. Ferulic acid was also attached to birch wood glucuronoxylan, which contains methylglucopyranosidic acid (MeGlc*p*A) attached to C-2 in the xylan backbone. Based on the antioxidative activity of hydroxycinnamic acid derivatives of methyl α-D-glucopyranoside demonstrated recently (*11*), we hypothesized that these esterified xylan polymers would also show antioxidative properties.

MATERIALS AND METHODS

Materials. Commercial grade reagents and solvents were used unless otherwise mentioned. Oat spelt arabinoxylan and birch wood glucuronoxylan were purchased from Sigma-Aldrich. Oat spelt has a low degree of substitution with arabinose to xylose ratio 0.11 (*19*). The content of substituents in birchwood is similar as the ratio of 4-*O*-methylglucuronic acid to xylose is about 0.1 (*13*). 4-*O*-Acetylferulic acid and 4-*O*-acetylsinapic acid and their corresponding acid chlorides 4-*O*-acetylferulic acid chloride and 4-*O*-acetylsinapic acid chloride (**Figure 1**) were prepared following the procedures described by Kylli et al. (*11*).

General Methods. Fourier transform infrared spectra (FT-IR) were recorded on a Perkin-Elmer spectrometer using a diamond crystal. NMR spectra were recorded with a Varian Inova 500 spectrometer (^{1}H, 500 MHz, and ^{13}C, 125 MHz, respectively). Unless stated otherwise, DMSO-d_6 was used as a solvent in all NMR experiments, and the chemical shifts are expressed in δ (ppm).

Synthesis of Oat Spelt Arabinoxylan and Birch Wood Glucuronoxylan Hydroxycinnamic Acid Esters. For a typical synthesis of compounds **1**−**8** (**Figure 1**), 1.65 g of xylan (containing approximately 0.025 mol of free hydroxyl groups) was added into a solution of 60 mL of 4% lithium chloride in *N*,*N*-dimethylacetamide (LiCl/DMA) (w/w) under argon. The resulting mixture was stirred at 125 °C until homogeneous (approximately 2−3 h). After being cooled to room temperature, the required quantity of pyridine (1.5 mol equiv to acid chlorides) and 4-dimethylaminopyridine (DMAP, 5 mol % of acid chlorides) was added, and the temperature of the reaction bath was raised to 60 °C. To this solution was gradually added the required amount of acid chlorides (**Table 1**) in DMA using an automated syringe over 8 h. The mixture was further stirred for an additional 4 h, and after cooling to room temperature the product was precipitated by pouring the reaction solution into four volumes of 80% ethanol and stirring for 1 h. The mixture was centrifuged at 2500 rpm for 10 min, and the precipitate was filtered off and purified by washing two times with a saturated solution of sodium bicarbonate, five times with water, and three times with ethanol. The resulting product was air-dried for 15 h and further dried in an oven at 60 °C for another 15 h.

Deacetylation of Oat Spelt Arabinoxylan and Birch Wood Glucuronoxylan Esters. In a typical deacetylation procedure, 1.3 g of esterified xylan compounds **1**−**8** (**Figure 1**, **Table 1**) and 1 g (0.025 mol) of LiCl were added in 50 mL of DMA under argon atmosphere, and the mixture was stirred vigorously at 90 °C until it became homogeneous (approximately 30 min). After the solution was cooled to room temperature, 0.95 g (0.025 mol) of $NaBH_4$ was added, and the mixture was stirred at room temperature for 72 h ferulic acid arabinoxylan esters, at 40 °C for 72 h sinapic acid arabinoxylan esters, or at 40 °C for 100 h ferulic acid glucuronoxylan esters. Afterward, the solution was poured into 50 mL of ice water, and the product was precipitated by careful addition of 2 M H_2SO_4 until pH 2−3. The precipitate was filtered off and washed six times with 20 mL of water. The product was air-dried for 15 h and further dried in an oven at 60 °C for 12 h (products: FA-AX, SA-AX, and FA-GX, **Figure 1**, **Table 2**).

Weight Percent Gain (WPG). WPG values were determined in order to follow the esterification efficiency quantitatively (*20*). The WPG values reported in this study (**Tables 1** and **2**) were calculated according to the formula

$$\mathrm{WPG}\ (\%) = 100(W_{\mathrm{mod}} - W_{\mathrm{unmod}})/W_{\mathrm{unmod}}$$

where W_{unmod} is the initial oven-dried mass of the xylans (in the first reaction step) before esterification and W_{mod} represents the oven-dried mass of the products after the esterification. In the second reaction step W_{unmod} is the oven-dried mass of the esterified xylans (AcFA-AX, AcSA-AX, and AcFA-GX, **Figure 1**, **Table 1**) before deacetylation, and W_{mod} represents the oven-dried mass of the compounds after the liberation of phenolic hydroxyls (FA-AX, SA-AX, and FA-GX, **Figure 1**, **Table 2**).

Degree of Substitution (DS). The DS of the products (maximum DS of xylan backbone sugar monomer unit is 2.0) was determined from the

Table 2. Deacetylation of 4-*O*-Acetyl-Protected Hydroxycinnamic Acid Xylan Esters by $NaBH_4$ in DMA/LiCl

substrate (no.)	temp (°C)	time (h)	WPG (%)[a]	DS[b]	compd (no.)[c]
AcFA-AX (**1**)	25	72	−13	0.05	FA-AX (**9**)
AcFA-AX (**2**)	25	72	−18	0.36	FA-AX (**10**)
AcFA-AX (**3**)	25	72	−24	0.45	FA-AX (**11**)
AcFA-AX (**4**)	25	72	−27	0.89	FA-AX (**12**)
AcSA-AX (**5**)	40	72	−9	0.09	SA-AX (**13**)
AcSA-AX (**6**)	40	72	−21	0.26	SA-AX (**14**)
AcFA-GX (**7**)	40	100	−10	0.05	FA-GX (**15**)
AcFA-GX (**8**)	40	100	−19	0.39	FA-GX (**16**)

[a] WPG = weight percent gain (see Materials and Methods). [b] Determined with HPLC after alkali hydrolysis (1% NaOH at 25 °C in 12 h). [c] FA-AX = ferulic acid oat spelt arabinoxylan ester; SA-AX = sinapic acid oat spelt arabinoxylan ester; FA-GX = ferulic acid birch wood glucuronoxylan ester.

quantity of hydroxycinnamic acid released in basic hydrolysis of the hydroxycinnamic acid xylan esters (**Figure 1**, **Table 2**) using HPLC. Accordingly, the polymers (70 mg) were hydrolyzed in 3 mL of 1% NaOH at 25 °C in 12 h, followed by acidification to pH 2−3 by 1 M HCl and extraction with EtOAc. After workup the liberated ferulic and sinapic acid contents were determined by the HPLC system consisting of a Waters 2690 separation module, a PDA 996 diode array detector, and Empower 2 (build 2154) software. The column used was a 150 × 3.9 mm, 4 μm, Nova-Pak C18 with a C18 guard column (Waters, Millipore, Bedford, MA). Samples were dissolved in 50 mM ammonium dihydrogen phosphate, pH 2.6. For detection, the wavelength at 320 nm was used. The HPLC analysis was performed according to the method outlined by Kähkönen et al (*21*). Commercial ferulic and sinapic acids were used as standards. DS was also estimated by means of ^{1}H NMR spectroscopy by comparing the signals between 1-H xylan and α-H and β-H of the hydroxycinnamic acid moiety. The data were in accordance with the DS determined with HPLC by hydrolysis except in the case of compounds **4** and **12** (with DS of 0.89, **Tables 1** and **2**) since in these samples the peaks were too broad for accurate determination.

Molar Mass Analysis. The ferulic acid ester with DS of 0.36 (compound **10**, **Table 1**) was analyzed with high-performance size exclusion chromatography (HPSEC) using DMSO with 0.01 M LiBr as eluent (*22*). The HPSEC system consisted of an integrated autosampler and pump module (GPCmax, Viscotec Corp., Houston, TX), two linear columns, 8 × 300 mm, Shodex LF-804, and a guard column, 4.6 × 10 mm, Shodex LF-G (Showa Denko, Tokyo, Japan), a UV detector at λ_0 = 280 nm (Waters 486 tunable absorbance detector, Milford, MA), a combined light scattering and viscometric detector (270 dual detector, Viscotek Corp.), and a refractive index (RI) detector (VE 3580, Viscotek Corp.). The flow rate was 1 mL/min and injection volume 100 μL. The samples (4 mg/mL) were dissolved in eluent at room temperature for 4 days to ensure sufficient solubility. All of the samples were filtered before analysis with 0.45 μm syringe filters (GHP Acrodisc 13, Pall Corp., Ann Arbor, MI). OmniSEC 4.5 was used for data processing.

Antioxidative Activity. Antioxidant activities of xylan esters were tested by using the emulsion lipid oxidation test according to Kylli et al. (*11*). Emulsions were prepared from oil (10% o/o), water, and soybean lecithin emulgator (2% w/w). Tested samples were suspended to the flasks at a concentration of 500 μg of xylan esters/g of oil. Samples were incubated at 37 °C for 6 days. The inhibition against emulsion oxidation was calculated at day 6 by measuring the formation of hexanal (primary lipid oxidation product) by headspace gas chromatography and conjugated hydroperoxide dienes (secondary lipid oxidation product) by measuring the absorbance spectrometrically at 234 nm as described earlier (*11*). Analyses were made in triplicate, and α-tocopherol was used as a reference.

RESULTS AND DISCUSSION

Esterification of Oat Spelt Arabinoxylan (AX) and Birchwood Glucuronoxylan (GX). To prepare moderately esterified xylans with hydroxycinnamic acids (ferulic acid and sinapic acid), the route described in **Figure 1** for their synthesis was developed.

In our first experimental setup, we studied the reaction in heterogeneous conditions using pyridine as a solvent and DMAP as a catalyst under microwave heating; however, the reaction did not succeed. As it is well-known that cellulose and hemicelluloses can be dissolved in DMA/LiCl solution system (*18*, *23*), we changed the approach in which xylans were first dissolved in DMA/LiCl (4% w/w) solution, followed by the addition of pyridine, DMAP, and acetyl-protected acid chlorides at room temperature under argon and finally stirring the reaction mixture at 60 °C overnight for the esterification (**Figure 1**, **Table 1**). In the optimized conditions shown in **Table 1**, the quantities of pyridine and DMAP were 1.5 mol equiv to acid chlorides and 5 mol % of the xylans, respectively. The amounts of 4-*O*-acetylferulic acid chlorides and 4-*O*-acetylsinapic acid chlorides in the esterification of oat spelt arabinoxylan were 0.5, 1.0, 1.5, 3.0, 1.0, and 1.5 mol equiv to XU (anhydroxylose units, i.e., the amount of hydroxyl functionality in xylan, **Table 1**), respectively. In the case of birch wood glucuronoxylan, the derivatization was carried out with 1.0 and 1.5 mol equiv of 4-*O*-acetylferulic acid chlorides to XU.

Weight Percent Gain and Solubility of Ester Adducts. Since some loss of weight of xylans occurred during the esterification step, we employed weight percent gain (*20*) to describe the amount of substitution in the polymers instead of calculating yields to quantitatively follow the modification of xylans. The loss of weight is obviously due to the salts and minor sugar components present in commercially available oat spelt and birch wood xylans.

The WPG values presented in **Table 1** clearly show that an increase in the molar ratio of acid chlorides to anhydroxylose units in the esterification leads to an increase in the WPG values. The rising trend in WPG values is apparently linked to the increase in the degree of substitution to the xylan backbone (or to the Ara*f* or meGlc*p*A side chains). The only exception was found in the case of compound **4**, which gave a lower WPG value than expected. This could be due to its higher solubility in the solvents used for the precipitation.

In determination of the solubility properties of the polymers, it was found that all of the prepared oat spelt arabinoxylan esters (compounds **1**−**6**, **Figure 1**) were soluble in DMA/LiCl while the birch wood glucuronoxylan esters (compounds **7** and **8**), surprisingly, were only partially soluble in that solvent even if stirred at 130 °C for 6 h. In addition, all of the compounds were insoluble in common polar solvents (chloroform, ethanol, tetrahydrofuran, toluene, ethyl acetate, or water) and nonpolar solvents (hexane) except compound **4**, which was slightly soluble in THF and chloroform (10−15%). In the preparation of the NMR samples we also found that the solubility of the samples in DMSO increased with the amount of acid chlorides applied in the esterification. Thus, compounds **1**, **2**, **3**, **5**, **6**, **7**, and **8** were only partially soluble in DMSO at room temperature, while the ester compounds **2**, **3**, and **4** were completely soluble when heated up to 100 °C (NMR tube, 50 mg of compounds in 0.7 mL of DMSO-d_6). Compound **4** was completely soluble even at room temperature, which could further explain its low WPG value obtained.

Deacetylation of Xylan Derivatives. In our previous studies we found that pyrrolidine removes the acetyl group selectively from the 4-*O*-acetyl-protected feruloyl- and sinapoyl glucoside esters in homogeneous conditions (*11*). However, in the case of xylan esters, no reaction took place. When using 1% sodium hydroxide (with compound **3**, **Figure 1**), a complete deprotection took place in 45 min at 0 °C, but unfortunately, a significant amount of ferulic acid was also liberated. For example, the DS of compound **10** with DS of 0.36 (**Table 2**) was reduced to less than 0.05 according to ^{1}H NMR.

The selective deesterification of the xylan esters was ultimately achieved by using $NaBH_4$ in DMA/LiCl in large excess (over 10 mol equiv) (**Table 2**). Thus, for the deacetylation, xylan esters

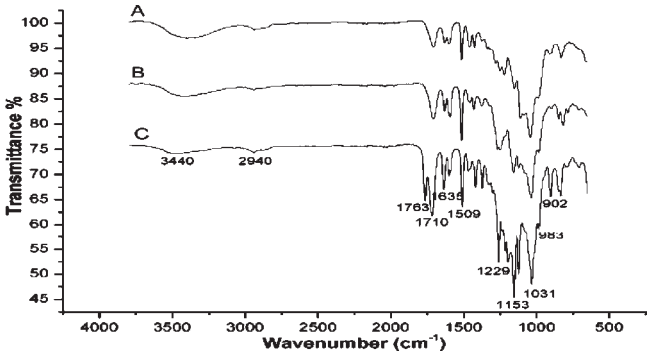

Figure 2. FT-IR spectra of sinapic acid oat spelt arabinoxylan ester with DS of 0.26 (**A**), ferulic acid oat spelt arabinoxylan ester with DS of 0.36 (**B**), and 4-*O*-acetylferulic acid oat spelt arabinoxylan ester with DS of 0.86 (**C**).

were dissolved in DMA/LiCl at 90 °C until the solution became homogeneous (approximately 30 min, except for compounds **15** and **16** since the compounds were not completely soluble), followed by the addition of $NaBH_4$. After the addition of the reducing agent, the mixture changed rapidly to a yellow color indicating the formation of free phenolic residues. As shown in **Table 2**, the complete deacetylation of oat spelt arabinoxylan ferulic acid esters (compounds **1**−**4**) was achieved at room temperature in 72 h, whereas sinapic acid esters (compounds **5** and **6**) required elevated temperature (72 h at 40 °C). With birch wood glucuronoxylan esters (compounds **7** and **8**) that showed poor solubility in DMA/LiCl, the deacetylation required 100 h at 40 °C. In all cases, no hydrolysis of the cinnamic acid ester was detected according to ^{1}H NMR and ^{13}C NMR spectra, and no free acids were recovered from the reaction mixture after the reaction.

Weight Percent Gain, DS, and Solubility of the Deacetylated Xylan Esters. The negative WPG values obtained from the deacetylation step (**Table 2**) indicate that material lost weight during the removal of the acetyl protection group. Since no hydrolysis of the cinnamic acid esters was detected in ^{1}H NMR and ^{13}C NMR spectra, and no free acids were recovered from the reaction mixture, the loss in weight of the materials is mostly due to the removal of acetyl protection. Additionally, the acetyl-protected cinnamic acid xylan esters always contained some water trapped inside the polymer (**Figure 3**), and this could explain the rest of the decreased WPG values.

The DS of compounds **9**−**16** (**Table 2**) was determined by HPLC after alkali hydrolysis. Since no deesterification of the hydroxycinnamic acid xylan esters occurred during the removal of the acetyl protection group by $NaBH_4$, it can be concluded that an increase in the molar ratio of acid chlorides to anhydroxylose units in xylans used in the first reaction step (esterification) leads to an increase in DS.

In determination of the solubility characteristics of the materials we found that the deacetylated products (compounds **9**−**16**, **Table 2**) exhibited lower solubilities in DMSO than their acetylated counterparts. All of the deacetylated products were only partially soluble in DMSO and nonsoluble in nonpolar and in polar solvents (chloroform, ethanol, tetrahydrofuran, toluene, ethyl acetate, or water) even if heated to the boiling point.

Spectroscopic Characterization of Xylan Derivatives. The esterification of xylans with acetyl-protected hydroxycinnamic acid chlorides and the subsequent removal of acetyl protection were both verified by FT-IR and NMR experiments. **Figure 2A**−**C** shows the FT-IR spectra of the sinapoylated, feruloylated, and 4-O-acetylferuloylated xylans, respectively. The adsorptions at 3440, 2940, 1465, 1418, 1258, 1153, 1031, 983, and 902 cm^{-1} are associated with the xylan backbone (*23*). A sharp band at 902 cm^{-1} is characteristic of the β-glycosidic linkages between the Xyl*p* residues indicating the sugar residues are linked by β-form bonds (*24*). A low intensity band at 983 cm^{-1} suggests the presence of Ara*f* units that are attached to position 3 of Xyl*p* constituents (*24*).

According to IR spectra the existence of an ester linkage between the cinnamic acid and the carbohydrate is evident. Spectrum C shows the presence of two ester groups at 1763 and 1710 cm^{-1}. The former is associated to the phenolic acetate carbonyl group, and the latter is characteristic of the conjugated carbonyl group resulting from the esterification of xylan. Moreover, the presence of feruloyl and sinapoyl groups is confirmed by the adsorption bands at 1635, 1600, and 1509 cm^{-1}, which contribute to the conjugated double bond and the phenyl ring, respectively. As expected, the intensity of the ester band at 1710 cm^{-1} increases with the increasing degree of substitution in the polymer. The cleavage of acetyl groups in treatment with $NaBH_4$ (**Figure 2A**,**B**) is also clearly indicated by the disappearance of the adsorption band at 1763 cm^{-1} arising from the acetyl carbonyl group.

In order to closer characterize the structural features of the esterified xylans, the isolated products and the native oat spelt arabinoxylan were analyzed by ^{1}H NMR and ^{13}C NMR. The ^{1}H NMR spectrum of the oat spelt xylan in DMSO-d_6 is shown in **Figure 3**. For comparative purposes, the ^{1}H NMR spectra of acetylated ferulic acid oat spelt arabinoxylan ester with DS of 0.36 (compound **2**, **Figure 1**) and its deacetylated derivative (compound **10**, **Figure 1**) and ferulic acid oat spelt arabinoxylan ester with DS of 0.89 (compound **12**, **Figure 1**) are also shown. The downfield signals at δ 6.3−9.6 ppm and signals at δ 3.77 ppm (methoxy protons) and at δ 2.25 ppm (acetate methyl protons of the AcFA-AX) are characteristic of the feruloyl groups, and those at δ 3.0−4.3 ppm correspond to the main (1→4)-linked β-D-Xyl*p* units (*25*). Two signals at δ 4.9−5.1 ppm originate from the hydroxyl protons of the xylan. The broadness of the characteristic signals of the feruloyl moiety that is due to the hindered rotation in the polymeric backbone indicates strongly their covalent attachment to xylan. In addition, the similarity among the spectra of acetylated ferulic acid ester and its deacetylated form (AcFA-AX and FA-AX) signifies that no deesterification, at least in appreciable amount, occurred during the removal of the acetyl protection group by $NaBH_4$. The disappearance of the acetate methyl signals at δ 2.25 ppm and the evolved phenolic hydroxyl signal at δ 9.6 ppm confirms the successful deacetylation. By comparing the spectra in **Figure 3**, it is obvious that the DS increases by increasing the amount of acid chlorides in the esterification step. This is confirmed by the increased signals of the feruloyl moiety in contrast to the signals of xylan backbone. The evidence is also supported by the reduced intensity of the free hydroxyl proton signals of xylan at δ 4.9−5.1 ppm with increasing DS.

The ^{13}C NMR characteristics of the unmodified oat spelt xylan and oat spelt ferulic acid and sinapic acid esters (compounds **10** and **14**, **Figure 1**) with various DS are illustrated in **Figure 4**, as an example. The spectrum of oat spelt xylan shows five major signals at δ 101.8, 75.5, 74.0, 72.7, and 63.3 ppm, which are assigned to C-1, C-2, C-3, C-4, and C-5 of the β-D-Xyl*p* units, respectively. The presence of arabinose is confirmed by the weak signals at δ 107.1, 86.3, 80.3, 77.9, and 62.0 ppm that are characteristic of C-1, C-2, C-3, C-4, and C-5 of α-L-arabinofuranosyl residues linked to β-D-xylans, respectively. From the spectra of ferulic acid and sinapic acid esters (FA-AX and SA-AX, **Figure 4**), the signals at δ 106.2−149.5 ppm are assigned to the sinapic and ferulic acid residues, and the formation of the ester bond is confirmed by the carbonyl signals at δ 165−166 ppm. In addition, **Figure 4** shows that in the case of esterified xylans (FA-AX and SA-AX) the typical signals of C-2, C-3, and C-1 of the β-D-Xyl*p* shift (C-2, C-3

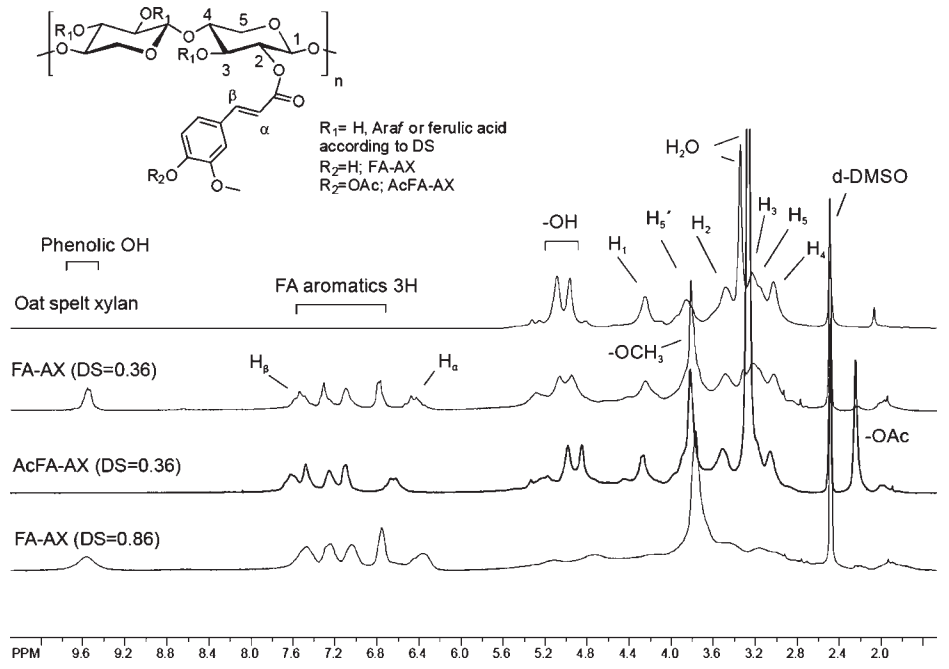

Figure 3. ^{1}H NMR spectra of various xylan derivatives in DMSO-d_6.

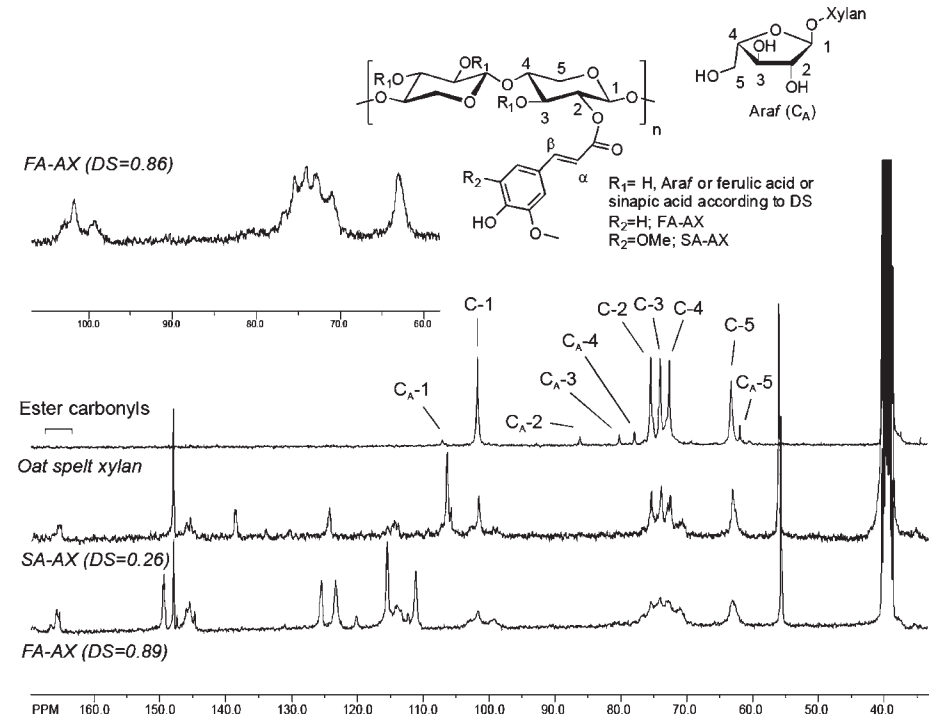

Figure 4. ^{13}C NMR spectra of oat spelt arabinoxylan, sinapic acid oat spelt arabinoxylan ester with DS of 0.26, and ferulic acid oat spelt arabinoxylan ester with DS of 0.89 in DMSO-d_6.

to 70−72 ppm and C-1 to 98 ppm), indicating that O-2 and/or O-3 of the β-D-Xyl*p* are partially substituted. However, disappearance of the signals arising from the arabinofuranose moiety in the spectra of sinapic and ferulic acid xylan esters suggests that some of its hydroxyl groups were also esterified.

HPSEC Analysis of Xylan Derivatives. Compounds **2** and **5** (**Figure 1**) were analyzed with HPSEC to ensure that the xylans were not degraded during the esterification reaction. In addition, the covalent binding of ferulic or sinapic acid to xylan can be confirmed with an increased UV signal of the polymeric xylan. As an example, the HPSEC chromatograms of oat spelt arabinoxylan and oat spelt arabinoxylan ferulic acid ester with DS of 0.36 in DMSO with 0.01 M LiBr are presented in **Figure 5**. RI signals indicate that the elution volumes of both samples are almost identical. Only a small peak in the UV signal was detected in the chromatogram of the oat spelt sample (data not shown). Strong peaks in both RI and UV signals at the same position in compound **2** clearly indicate the binding of ferulic acid to polymeric arabinoxylan (**Figure 5B**). The esterified compound was more soluble in DMSO compared to unmodified arabinoxylan sample, which can be seen as a larger area of RI peak in the chromatogram of esterified compound. HPSEC analysis thus

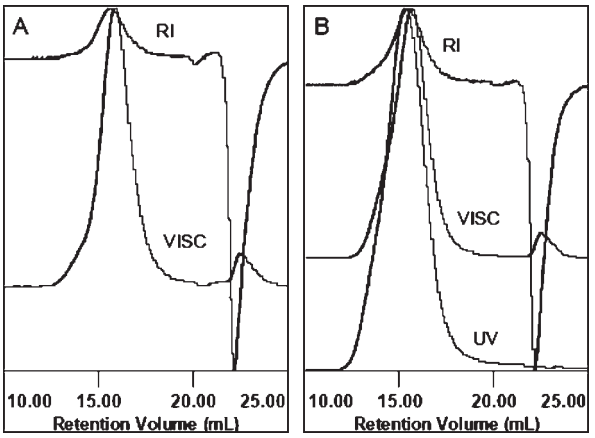

Figure 5. HPSEC chromatograms of oat spelt arabinoxylan (**A**) and 4-*O*-acetylferulic acid oat spelt arabinoxylan ester with DS of 0.36 (**B**).

proved that the esterification reactions succeeded without xylan degradation. Weight average molar masses (M_w) of samples are not presented due to the partial aggregation of the samples, seen with the light scattering detector (data not shown). In addition, the deacetylated compounds **9**−**16** (**Figure 2**) were not analyzed with HPSEC due to their poor solubility.

Antioxidative Activity. Previously, Katapodis et al. (*26*) prepared feruloylated arabinoxylan oligosaccharides having a molar ratio of arabinose and xylose of 1:3. They compared the inhibitory effect of ferulic acid and feruloylated oligosaccharides in the copper-mediated human low-density lipoprotein (LDL) oxidation and in the DPPH radical scavenging assays. Feruloylated oligosaccharides inhibited the LDL oxidation better than free ferulic acid, while the inhibitory effect in the DPPH test was reversed. In addition, formerly prepared ferulic acid and sinapic acid glycoside esters have shown good or even improved antioxidative activity than their free acid forms toward the oxidation of liposomes and emulsions (*11*). Ferulic acid has also been attached to microcrystalline cellulose, and the obtained polymers displayed good antioxidant activity in inhibiting lipid peroxidation in rat liver microsomal membranes (*27*).

In this study, the antioxidant potential of prepared hydroxycinnamic acid oat spelt arabinoxylan and birch wood glucuronoxylan esters was evaluated in the emulsion lipid oxidation model. Xylan esters were added to the emulsions in equal amounts (500 μg/g of lipid) based on the weight. **Table 3** shows that all ferulic acid oat spelt xylan esters tested had more or less equal antioxidative activities regardless of their degree of substitution (DS of 0.05 made in duplicate and DS of 0.36). They inhibited conjugated diene hydroperoxide formation and hexanal formation moderately. Furthermore, all ferulic acid oat spelt xylan esters showed clearly higher antioxidative efficiency than the nonderivatized oat spelt xylan (oat spelt control sample). This result is in agreement with the results reported previously in the cases of feruloylated arabinoxylan oligosaccharides and cellulose ferulates (*26*, *27*). However, in the latter case the antioxidant activity was reported to increase with higher DS (*27*).

Birch wood ferulic acid xylan esters were tested as well with two samples having a different degree of substitution. In contrast to oat spelt ferulic acid esters, birch wood ferulic acid ester containing more ferulic acid (DS of 0.39) was clearly a better antioxidant compared to the less substituted sample (DS of 0.05). Comparison of the control samples suggests that birch wood glucuronoxylan (control sample) itself is a better antioxidant than oat spelt arabinoxylan. Moreover, the glucuronoxylan derivative with similar substitution (compound **16**, DS of 0.39) exhibited much higher antioxidative efficiency in the lipid oxidation model than the corresponding arabinoxylan ester with DS of 0.36. These results may be due to the presence of glucuronic acid in birch wood xylan that has been proposed to influence the antioxidant activity of sulfated polysaccharides (*28*).

Table 3. Inhibition of Lipid Oxidation Determined by Formation of Conjugated Dienes and Hexanal in an Emulsion Oxidation Model System with 500 μg/g Xylan Derivatives[a]

		% ± SD[e]	
compd (no.)	DS	conjugated dienes[e]	hexanal
FA-AX (**9**)[b]	0.05	49.7 ± 16.2	71.8 ± 18.3
FA-AX (**9**)	0.05	49.5 ± 16.0	71.1 ± 20.2
FA-AX (**10**)	0.36	52.0 ± 12.5	70.8 ± 17.0
SA-AX (**13**)	0.09	72.8 ± 0.2	91.6 ± 5.60
AX control[c]		6.8 ± 2.3	31.0 ± 15.2
FA-GX (**15**)	0.05	25.6 ± 9.5	33.4 ± 22.4
FA-GX (**16**)	0.39	65.8 ± 6.7	90.0 ± 7.4
GX control[d]		24.3 ± 18.4	38.7 ± 24.2

[a] α-Tocopherols used as a reference. [b] Sample **9** was made in duplicate. [c] Oat spelt arabinoxylan (AX) control sample. [d] Birch wood glucuronoxylan (GX) control sample. [e] Percent inhibition, mean ± SD (standard deviation).

The strongest antioxidant in this study was the oat spelt arabinoxylan sinapic acid ester with DS of 0.09. Interestingly, it inhibited hexanal formation as effectively as birch wood glucuronoxylan ferulic acid ester with DS of 0.39 but with a much lower degree of substitution. We have noticed similar behavior in our previous studies where sinapic acid derivatives proved to be better antioxidants than ferulic acid derivatives toward the oxidation of liposomes and emulsions (*11*). This can be addressed to the significant antioxidant potency of sinapic acid compared to the other hydroxycinnamic acids (*29*).

In conclusion, we have synthesized ferulic acid and sinapic acid xylan esters with various degrees of substitution and fully characterized the obtained polymeric materials by spectroscopy. We have also demonstrated that no chain degradation occurred during the preparation of the xylan esters and that the xylan derivatives obtained exhibit more antioxidant activity toward the lipid oxidation than the native oat spelt and birch wood xylans. Unfortunately, further studies to reveal the antioxidant properties such as their radical scavenging activities (DPPH test) were prevented by the low solubility of the polymers. The applicability of these types of antioxidative xylan derivatives in various applications will be a subject of further studies.

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