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Studies on the oxidative cross-linking of feruloylated arabinoxylans from wheat flour and wheat bran

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Abstract

Feruloylated arabinoxylans isolated from wheat flour and wheat bran were compared in their cross-linking behaviour with respect to viscosity properties and cross-linking products formed when various oxidative agents were applied to dilute solutions. Optimal conditions for each oxidative agent were investigated. In case of hydrogen peroxide and peroxidase, similar conditions were found for both types of arabinoxylans but wheat bran arabinoxylans gave a larger viscosity increase upon cross-linking than those of wheat flour.

When glucose, glucoseoxidase and peroxidase or ammonium persulphate were used as oxidative agents, differences in the concentration of reagent needed to induce cross-linking and in viscosity increase were observed. The distribution of coupling products for both types of arabinoxylans and the different oxidative treatments was approximately 5 : 3 : 1 : 1 for 8-5, 8-O-4, 8-8 and 5-5, respectively. The low ferulate recovery after oxidative treatment was assumed to be caused by formation of unknown compounds, such as higher oligomers and lignin-linked products.

A 1 : 1 mixture of flour arabinoxylan and feruloylated pectin showed a maximum synergistic effect on viscosity upon oxidative treatment using hydrogen peroxide and peroxidase. Both polysaccharides were shown to participate in cross-linking.

Keywords: Wheat bran; Feruloylated arabinoxylans; Cross-linking

1. Introduction

Hydrogen peroxide/peroxidase mediated cross-linking of water-extractable arabinoxylans of rye, and especially wheat flour, has been investigated extensively for over 30 years (Fausch et al., 1963; Geissmann and Neukom, 1971; Hoseney and Faubion, 1981; Ciacco and D'Appolonia, 1982a; Izydorczyk et al., 1990; Moore et al., 1990; Vinkx et al., 1991; Girhammar and Nair, 1992). Ferulic acid, linked to arabinose branches of the xylan backbone, was quickly recognized to play a crucial role in cross-linking (Fausch et al., 1963; Geissmann and Neukom, 1971). However, contradictions about the coupling positions of ferulic acid moieties (Hoseney and Faubion, 1981; Moore et al., 1990; Vinkx et al., 1991), and about the possible participation of cysteine (Fausch et al., 1963; Morita et al., 1974; Hoseney and Faubion, 1981; Vinkx et al., 1991) and tyrosine (Neukom and Markwalder, 1978) residues of proteins in cross-linking await clarification. Only recently have ester linked dehydrodiferulic acids with various linkage positions (Fig. 1) been demonstrated in grass cell walls (Ralph et al., 1994). The 5-5 linked dimer, previously thought to be the only dimer produced during oxidative coupling of ferulate (Geissmann and Neukom, 1971; Markwalder and Neukom, 1976; Ishii, 1991), was present only in relatively low amounts compared to dimers formed by coupling at the 8-position of one or both ferulate moieties (Ralph et al., 1994; Grabber et al., 1995).

In the current study, we compared the diferulate coupling products and the viscosity properties of wheat bran glucuronoarabinoylans to wheat flour arabinoxylans, before and after treatment with several oxidative agents. Cross-linking

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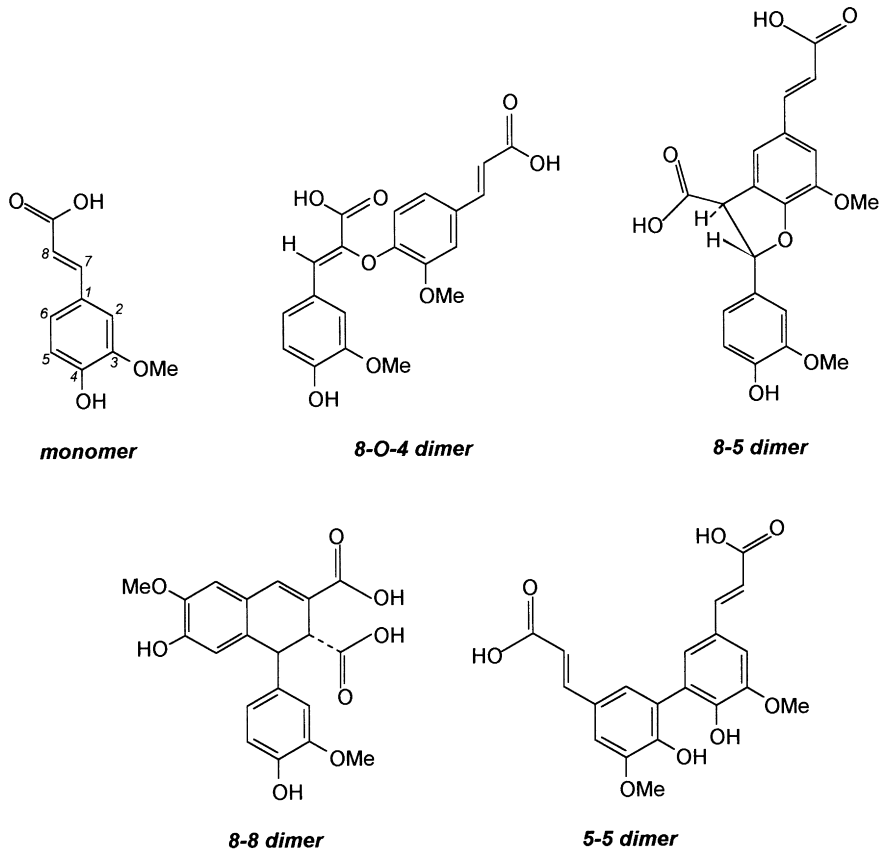

Fig. 1. Ferulic acid monomer and some of the more prevalent ferulate dehydrodimers found in grasses.

studies with these xylans were also performed with the addition of other phenolic or phenolic acid containing materials, such as tyrosine and feruloylated pectin.

2. Experimental

2.1. Materials

Feruloylated arabinoxylans were isolated from commercial wheat flour by cold water extraction according to a procedure previously described for wheat bran (Schooneveld-Bergmans et al., 1998). The extract was concentrated by vacuum evaporation to a dry matter concentration of 9.2 mg/ml, stored at 4°C with the addition of 0.01% sodium azide and designated WF.WE. Feruloylated glucuronoarabinoxylans were isolated from water-unextractable cell wall material (WUS) of industrial wheat bran by saturated calcium hydroxide extraction and acetone precipitation as described previously (Schooneveld-Bergmans et al., 1998). The extract of 23.4 mg/ml dry matter was stored as described before and designated WB.CE. Feruloylated beet pectin (Lot no. X-6938/61-735-0, Copenhagen Pectin Factory, Lille Skensved, Denmark) was dissolved in distilled water and dialysed before use.

2.2. Cross-linking

All coupling reactions were carried out in 0.1 M sodium phosphate buffer (pH 6) at 25°C and were monitored by viscometry for periods up to 24 h.

2.2.1. Hydrogen peroxide and peroxidase

Feruloylated arabinoxylan fractions (1–8 mg/ml) were incubated with 5 μg/ml of horse radish peroxidase (Type I, Art. P-8125, Sigma, St. Louis, CA, USA) and 1.6 μmoles/ml of hydrogen peroxide. Oxidative coupling with 50 μg/ml of horse radish peroxidase and 0.2–2 μmoles/ml of hydrogen peroxide were also evaluated. Oxidations of wheat flour arabinoxylan were also carried out with 1.6 or 3.2 μmoles/ml of tyrosine present in the solution. Additionally, both arabinoxylan fractions (4 or 7 mg/ml) were incubated with beet pectin solution of equal concentration. The arabinoxylan and pectin solutions were mixed at different ratios (1 : 0; 1 : 3; 1 : 1; 3 : 1; 0 : 1).

2.2.2. Glucose, glucoseoxidase and peroxidase

Feruloylated arabinoxylan fractions (4 mg/ml) were also cross-linked in the presence of glucose (20 μg/ml) with 5 μg/ml of glucoseoxidase (Type II from *Aspergillus niger*, Art. G-6125, Sigma, St. Louis, CA, USA) and 5 μg/ml of horse radish peroxidase. Oxidations in the

Table 1
Yield, composition, molecular weight and intrinsic viscosity of isolated feruloylated arabinoxylans from wheat flour (WF.WE) and wheat bran (WB.CE)

	WF·WE	WB.CE
Yield[a]	0.5	1.2
Composition[b]		
Total sugar	94.2	85.8
Protein	0.5	12.6
Ferulic acid	0.2	0.6
Molar sugar composition[c]		
Ara	36.3	40.1
Xyl	44.0	48.9
Man	1.9	1.8
Gal	16.0	2.7
Glc	1.1	2.7
UA	0.6	3.8
Molecular weight		
$M_{w;LS}$[d]	243.5	191.1
M_w/M_n[e]	2.5	1.8
Viscosity		
$[\eta]$[f]	2.3	1.6

[a] Expressed as weight percentage (dm) of original flour or bran.
[b] Expressed as weight percentage (dm) of each extract.
[c] Expressed as percentage (mole per 100 mole).
[d] weight average molecular weight as calculated from light-scattering data; expressed in kDa.
[e] number average molecular weight.
[f] $[\eta]$ intrinsic viscosity (dl/g).

presence of 5 or 50 μg/ml of glucose and 50 μg/ml of glucoseoxidase and peroxidase were also evaluated.

2.2.3. Ammonium persulphate

Feruloylated arabinoxylan fractions (4 mg/ml) were cross-linked with ammonium persulphate at a concentration of 0.01 M or 0.03 M. Similar oxidations were also performed in distilled water or distilled water containing 0.01% sodium azide.

2.2.4. Viscometry

Viscosities of all arabinoxylan solutions were measured before and after the addition of reagents with an Ubbelohde capillary viscometer (Schott, Mainz, Germany) submerged in a thermostatically controlled waterbath at 25°C. Capillary diameters ranged from 0.53 to 0.95 mm.

2.3. Analytical methods

Neutral sugar composition, uronic acid content and protein content of the extracts and fractions were determined as described previously (Bergmans et al., 1996).

2.3.1. Ferulic monomer and dehydrodimer contents

Untreated and dialysed oxidatively treated samples were hydrolysed with 2 M sodium hydroxide (4 ml) for 20 h at room temperature in nitrogen atmosphere. 2-Hydroxycinnamic acid was added as internal standard. The samples were acidified to pH 1 with 0.7 ml of 12 M hydrochloric acid and extracted twice with 3 ml diethyl ether. After drying, the extracts were silylated with pyridine (5 μl) and *N*,O-bis(trimethylsilyl)-trifluoroacetamide (30 μl) for 20 min at 60°C. Trimethylsilylated phenolic acid derivatives were separated on a 30 m × 0.25 mm i.d. DB-1 column in a Perkin Elmer 8500 gas chromatograph. The column was held at 210°C for 1 min, ramped from 210 to 230°C at 3°C/min, then ramped from 230 to 310°C at 10°C/min and held at 310°C for 9.5 min. The injector and detector were set at 310°C and helium was used as carrier gas (1 ml/min). Amounts of individual esterified phenolic acids were calculated using response factors as described by Ralph et al. (1994).

2.3.2. Size-exclusion chromatography

Aliquots (7 ml) of untreated and hydrogen peroxide/peroxidase treated mixtures of beet pectin and wheat flour arabinoxylan (1 : 1 ratio; 7 mg/ml) were applied to a Sephacryl S500 column (95 × 2.5 cm; fractionation range for dextrans: M_w $4\cdot10^4$–$2\cdot10^7$; Pharmacia, Uppsala, Sweden). The column was eluted with 0.05 M sodium acetate buffer of pH 5 at a flow rate of 2.5 ml/min. Fractions (2.5 ml) were collected and assayed by automated methods for total neutral sugars (Tollier and Robin, 1979) and uronic acids content (Thibault, 1979) using arabinose and galacturonic acid as standards.

2.3.3. High-performance size-exclusion chromatography (HPSEC)

Molecular weight distributions of the extracts and cross-linked fractions were determined by HPSEC using three Bio-Gel TSK columns in series as described elsewhere (Bergmans et al., 1996). The weight average molecular weight and intrinsic viscosity were determined using a triple detection system composed of a dual refractometer/viscometer detector model 250 in combination with a right angle laser light scattering detector LD600 (Viscotek, Houston, TX, USA) as described previously (Schooneveld-Bergmans, in press).

3. Results and discussion

3.1. Characterization of extracts

The yield, composition, and some physicochemical characteristics of feruloylated arabinoxylan extracts of wheat flour and wheat bran are presented in Table 1. The yield of the bran extract, on a dry matter basis, was over two times higher than that of flour but the total sugar content of the flour extract was slightly higher than that of wheat bran. The molar sugar composition of the xylan fractions suggests that the wheat bran extract was of higher purity, because it contained only minor amounts of mannose, galactose and

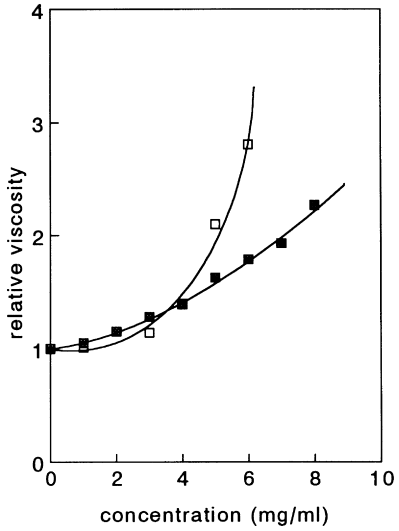

Fig. 2. Viscosities of wheat flour (■) and wheat bran (□) arabinoxylans, at varying dry matter concentrations, 4 min after addition of hydrogen peroxide and peroxidase, expressed relative to the viscosity before addition of reagents.

glucose. In the flour extract, the galactose content was relatively high because of coextraction of arabinogalactans (Fincher and Stone, 1974). The yield calculated as the amount of arabinoxylan recovered from total dry matter is therefore more indicative for the efficiency of the extractions. The total feruloylated glucuronoarabinoxylan content of the bran extract, calculated as the sum of arabinose, xylose, uronic acid and ferulic acid, amounted to 79.0% (w/w). For wheat flour arabinoxylan a correction of the arabinose content was necessary, because of the coextracted arabinogalactan. Arabinogalactans have an Ara/Gal-ratio of 0.67 when obtained from wheat flour by the same procedure as was used here (Fincher and Stone, 1974). As the composition of our extract was similar to their crude extract, the content of feruloylated arabinoxylan can be estimated as the sum of arabinose, xylose, uronic acid and ferulic acid minus 0.67 × galactose to give an arabinoxylan content of 61.4% (w/w). Based on these calculations, arabinoxylan yields, based on total dry matter, were 0.3% and 0.9% for flour and bran extract, respectively. For both extracts the Ara/Xyl-ratio was 0.82. However, after correction for the presence of arabinogalactan, the Ara/Xyl-ratio of the flour extract was 0.53, which was in good agreement with previous results (Izydorczyk et al., 1990; Hoffmann et al., 1991; Gruppen et al., 1992).

The protein content of our extract was low compared with extracts obtained without hot 80% ethanol treatment (Ciacco and D'Appolonia, 1982a; Izydorczyk et al., 1990; Girhammar and Nair, 1992; Cleemput et al., 1993). The protein and ferulic acid content of the bran extract was higher than for the flour extract. The latter observation corresponds with the higher ferulic acid content of bran compared with flour (Pussayanawin and Wetzel, 1987).

The weight average molecular weight of the flour extract was higher than that of bran. This was in agreement with their molecular weight distributions observed by HPSEC-analysis (results not shown) and those observed for the alkali-extractable arabinoxylans from flour and bran (Gruppen et al., 1991). The higher polydispersity index of the flour extract was probably caused by the coextracted arabinogalactans, which are of low molecular weight (Fincher and Stone, 1974). As these arabinogalactans have very low intrinsic viscosities (Izydorczyk et al., 1991) and they do not interfere with oxidative cross-linking of arabinoxylans (Izydorczyk et al., 1990), the wheat flour extract was used in oxidative coupling studies without further purification. The intrinsic viscosity of the wheat flour extract corresponded well to that determined by Izydorczyk et al., (1991), and it was higher than of the bran extract, which was in agreement with the higher molecular weight.

3.2. Cross-linking with different oxidative agents

3.2.1. Hydrogen peroxide and peroxidase

The most extensively investigated cross-linking agent is hydrogen peroxide in combination with peroxidase but the amounts of enzyme and peroxide used vary widely. In early reports, 20 μg/ml of peroxidase and 10 μmoles/ml of hydrogen peroxide were used for solutions containing approximately 3 mg/ml of arabinoxylan. Even larger quantities of reagents have been reported but then degradation losses, as observed by large decreases of the viscosity upon prolonged incubation, can be high (Geissmann and Neukom, 1973; Hoseney and Faubion, 1981; Ciacco and D'Appolonia, 1982a; Ciacco and D'Appolonia, 1982b). In more recent reports approximately 0.5 μg/ml of peroxidase and 0.05–0.2 μmoles/ml of hydrogen peroxide have been used in solutions of 3–20 mg/ml of arabinoxylan (Izydorczyk et al., 1990; Izydorczyk et al., 1991; Vinkx et al., 1991). Lower concentrations of reagents give a slower increase of viscosity but apparent degradative losses are eliminated. In the present study, different amounts of peroxide and peroxidase were investigated to identify optimal conditions for maximizing viscosity.

A maximum viscosity increase of 40% was observed for both the flour and bran extract, when 5 μg/ml of peroxidase and 1.6 μmole/ml of hydrogen peroxide were added to a 4 mg/ml arabinoxylan solution. Maximum viscosity was reached after 2 min and it lasted for at least 10 h. The effect of the concentration on the increase of viscosity, 4 min after addition of peroxidase and hydrogen peroxide, is shown in Fig. 2. It is clear from this figure that the relative viscosity increase for the bran extract was larger than for the flour extract, at extract concentrations of above 4 mg/ml. When the difference in arabinoxylan contents of the extracts are taken into account, we estimate that the viscosity increase for bran became greater than for flour starting at an arabinoxylan concentration of 3.5 mg/ml. The smaller viscosity increase for bran at lower arabinoxylan concentrations is most probably caused by its lower molecular weight

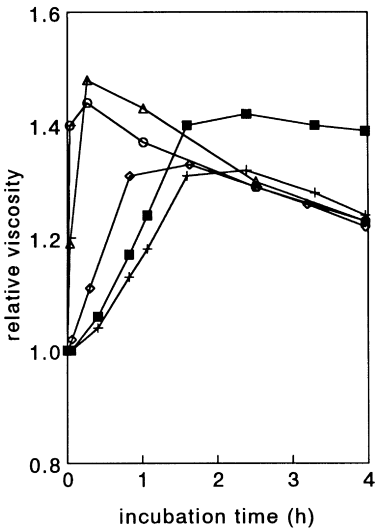

Fig. 3. Viscosities of wheat flour arabinoxylans after addition of varying amounts (μg/ml) of glucose, glucoseoxidase and peroxidase (○) 50-50-50; (△) 20-50-50; (◇) 5-50-50; (+) 20-5-50; (■) 20-5-5; the numbers give the amounts of glucose - glucoseoxidase - peroxidase, respectively, expressed as μg per ml of extract; viscosities are expressed relative to the viscosity of the extract before addition of reagents.

compared with flour arabinoxylan. Vinkx et al. (1991) used similar reasoning to explain viscosity differences between cross-linked wheat and rye flour arabinoxylans. However, at higher arabinoxylan concentrations the higher ferulic acid content of the bran arabinoxylans appears to compensate

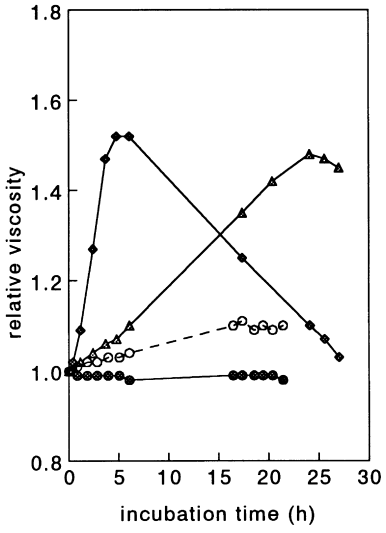

Fig. 4. Viscosities of wheat flour or wheat bran arabinoxylans during incubation with various ammonium persulphate solutions. (●) wheat flour arabinoxylan in water or phosphate buffer containing 0.01 M ammonium persulphate; (▲) wheat flour arabinoxylan in water containing 0.01% sodium azide and 0.01 M ammonium persulphate; (◆) wheat flour arabinoxylan in water containing 0.01% sodium azide and 0.03 M ammonium persulphate; (○) wheat bran glucuronoarabinoxylan in phosphate buffer containing 0.01 M ammonium persulphate. Viscosities are expressed relative to the viscosity of the extract before addition of reagent.

their lower molecular weight when compared to the flour arabinoxylans (Table 1). As a result of the higher ferulic acid content a higher cross-link density in the bran arabinoxylans can be anticipated, which results in a higher viscosity increase. Other differences in the arabinoxylans, such as the degree and distribution of substitution, are not expected to have effects in the viscosity increase upon oxidative coupling.

3.2.2. Glucose, glucoseoxidase and peroxidase

An oxidative system, not examined in previous studies on the gelation of feruloylated arabinoxylans, consists of glucose, glucoseoxidase and peroxidase. The former two reagents generate hydrogen peroxide, which is required by peroxidase to form ferulate radicals that undergo coupling to form diferulates. In Fig. 3, the relative viscosity of flour arabinoxylan, at an extract concentration of 4 mg/ml, after the addition of different amounts of reagents is shown. Maximum viscosity was obtained with 20 or 50 μg/ml of glucose in combination with 50 μg/ml of glucoseoxidase and peroxidase. With 5 μg/ml of glucose viscosity was less. The high reagent concentrations gave rapid viscosity increase but viscosity subsequently declined with continued incubation. These declines in viscosity were also observed for flour arabinoxylans when high concentrations of hydrogen peroxide and peroxidase were used, as mentioned earlier. These results suggest that the polysaccharides are degraded. This may have been caused by formation of hydroxyl radicals from hydrogen peroxide, which takes place competitively with cross-linking of ferulates (Ciacco and D'Appolonia, 1982a). Slow and minimal viscosity increase with subsequent decline was observed at 20 μg/ml of glucose, 5 μg/ml of glucoseoxidase and 50 μg/ml of peroxidase. The best conditions for increasing and maintaining arabinoxylan viscosity occurred with 20 μg/ml of glucose and 5 μg/ml of each enzyme. The maximum viscosity increase observed under these conditions was 40%, which corresponded to that of the peroxide/peroxidase-mediated cross-linking.

The conditions that were shown to be optimal for flour arabinoxylans gave almost no viscosity increase with bran glucuronoarabinoxylan. Similar viscosity effects as for flour arabinoxylans were observed only when higher concentrations of glucose and glucoseoxidase were applied (results not shown). This difference may be attributed to the presence of inhibitors of glucoseoxidase or radical scavengers in the bran extract. No further research was directed towards the nature of these compounds, although it is suspected that they may be lignin-like compounds (Bergmans et al., 1996; Schooneveld-Bergmans et al., 1998), which are not present in flour extracts.

3.2.3. Ammonium persulphate

Ammonium persulphate has been used as cross-linking agent for pectin (Thibault and Rombouts, 1986) and wheat flour arabinoxylan (Izydorczyk et al., 1990). Incubation of

Table 2
Ferulate monomer and dehydrodimer contents of untreated and oxidatively treated wheat flour (WF.WE) and wheat bran (WB.CE) extracts, expressed as mg/g of sample.

	Ferulate monomer	Ferulate dehydrodimer[a]				Total
		8-8	8-5	8-O-4	5-5	
WF.WE						
Untreated	1.75	0	0	0	0	1.75
Peroxide + HRP[b]	0.28	tr	0.39	0.30	0.09	1.07
Glc + GOX + HRP[c]	0.16	tr	0.45	0.25	0.05	0.91
WB.CE						
Untreated	5.77	1.06	0.34	0.28	0.77	8.22
Peroxide + HRP[b]	1.71	0.38	1.34	0.80	0.26	4.49
Ammonium persulphate	1.12	0.17	0.84	0.51	0.13	2.77

[a] As indicated by the linkage position.
[b] Hydrogen peroxide + horse radish peroxidase.
[c] Glucose + glucoseoxidase + horse radish peroxidase.

flour arabinoxylan (4 mg/ml) with 0.01 M ammonium persulphate in phosphate buffer or water gave no increase of viscosity (Fig. 4). Phosphate has been shown to inhibit the oxidative cross-linking of pectins by ammonium persulphate (Thibault and Rombouts, 1986). However, the absence of a viscosity increase in water was unexpected in view of results of Izydorczyk et al. (1990). The lack of response was probably caused by the low concentrations of arabinoxylan and ammonium persulphate used in the present study. When 0.01% (w/v) sodium azide was added to the solution as a preservative, slow development of a network was observed. This is most likely caused by propagation of the radical reaction by the azide ion. It has been shown that azide ions react very rapidly with hydroxyl radicals to produce an azide radical, which then oxidize phenols and phenolate ions (Alfassi and Schuler, 1985). Viscosity rapidly increased and then declined when an aqueous 0.03 M ammonium persulphate and 0.01% sodium azide solution was used. A similar effect of ammonium persulphate concentration on the cross-linking behaviour of pectins was shown by Thibault and Rombouts (1986). Sodium azide was not, however, added to their pectin solutions. Work by Izydorczyk et al. (1990) suggests that azide is not essential for increasing viscosity of flour extracts. It is likely that cross-linking of our flour extract could be induced without sodium azide if higher concentrations of ammonium persulphate were applied.

The wheat bran glucuronoarabinoxylans gave a slight viscosity increase with 0.01 M ammonium persulphate in phosphate buffer (Fig. 4), and gels formed in 16 h when aqueous 0.01 M ammonium persulphate with or without azide was used (results not shown). The higher ferulic acid content or the presence of other phenolic compounds may have enhanced the viscosity of this extract compared with the flour extract. Data in the following section support the latter possibility.

3.3. Ferulate monomer and dehydrodimer contents

In Table 2 ferulate monomer and dehydrodimer contents of various samples are given. The wheat flour arabinoxylan only contained 1.75 mg/g of ferulate monomer, whereas the wheat bran glucuronoarabinoxylans contained 8.22 mg/g of ferulate, 30% of which were dehydrodimers. Oxidative treatment of the wheat flour extract increased the proportion of dehydrodimers to total ferulate to 73% and 82% for the peroxide/peroxidase- and glucose/glucoseoxidase/peroxidase-mediated cross-linking, respectively. The predominant dimers were coupled by 8-5 (50%) and 8-O-4 linkages (38%). The 5-5 linked dehydrodimer, which has long been assumed to be the only coupling product, accounted for only 12% to the total dimer content. The 8-8 linked dimer was present in only trace quantities. The predominance of 8-5 and to a lesser extent of 8-O-4 linked dimers was also observed with isolated beet pectins cross-linked in vitro using hydrogen peroxide and peroxidase (Oosterveld et al., 1997) and with hydrogen peroxide treated maize cell walls (Grabber et al., 1995). The distribution of the dimers in the present study was not affected by the method of supplying hydrogen peroxide.

For the untreated wheat bran glucuronoarabinoxylans, the proportions of the various dehydrodimers were 45%, 15%, 10% and 30% for 8-8, 8-5, 8-O-4 and 5-5 linked dimers, respectively. After oxidative treatment with hydrogen peroxide/peroxidase or ammonium persulphate the proportion of dehydrodimers to total ferulates increased from 30% to 60%. The proportions of dimers after both oxidative treatments were 50% for the 8-5, 30% for the 8-O-4 and 10% for both 8-8 and 5-5 coupled dimers, quite similar to that observed with wheat flour extracts. Overall it appears that the type of oxidative agent and the source of arabinoxylans did not influence the distribution of the dimers formed. However, the type of oxidative treatment did effect the recovery of ferulates. After peroxide/peroxidase- or glucose/glucoseoxidase/peroxidase treatment ferulate recovery was 50%–60% based on the untreated extracts.

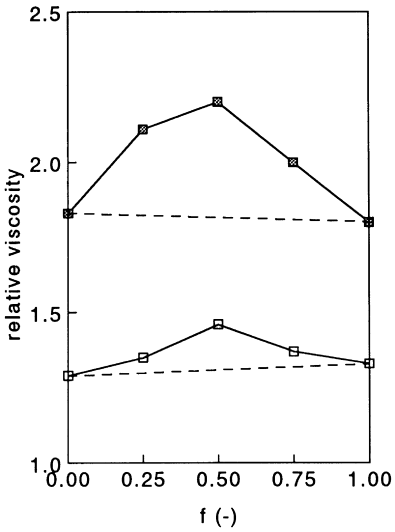

Fig. 5. Viscosities of feruloylated flour arabinoxylan and beet pectin with different proportions of arabinoxylan (*f*) at concentrations of 4 mg/ml (□) and 7 mg/ml (■), 4 min after addition of hydrogen peroxide and peroxidase, expressed relative to the viscosity of the mixtures before addition of reagent. (–) observed viscosity; (- - -) predicted viscosity.

When bran glucuronoarabinoxylans were treated with ammonium persulphate, ferulate recovery was only 35% compared to untreated controls. In all chromatograms a few unknown peaks were observed, several of which appeared to be diferulate isomers based on GC-MS (Grabber, unpublished).

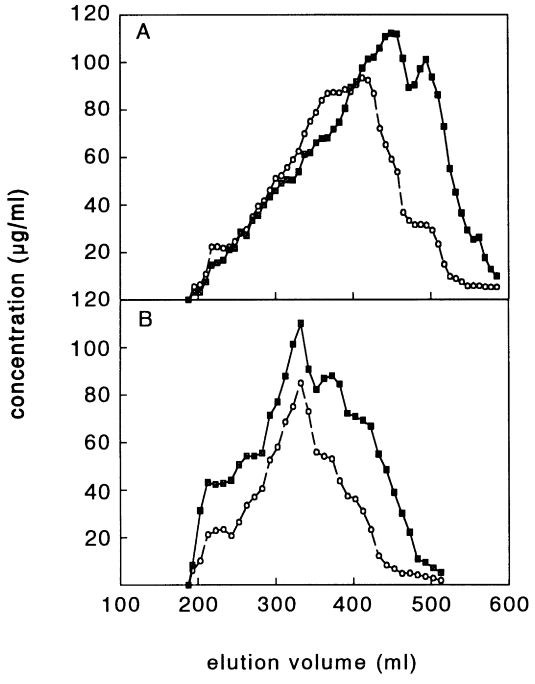

Fig. 6. Gel permeation elution patterns of mixtures of equal amounts of feruloylated beet pectin and wheat flour arabinoxylan (7 mg/ml total concentration) before (A) and after (B) incubation with hydrogen peroxide/peroxidase. (■) neutral sugar concentration; (○) uronic acid concentration.

The quantities of the unknown peaks could not be calculated as their GC-FID response factors are unknown. It is anticipated, however, that these unknowns do not account for all of the ferulate not recovered in the analyses.

Part of the ferulate may have been lost as a result of decomposition, as oxidative degradation in side chains or the aromatic ring of phenolics can occur at high oxidant concentrations (Taylor and Battersby, 1967). As the viscosity was stable when using standard hydrogen peroxide/peroxidase concentrations, the oxidant concentration was not expected to be too high. Ammonium persulphate treatment of wheat bran glucuronoarabinoxylans resulted in the formation of a strong gel, whereas only 35% of ferulate was recovered. Therefore it is suspected that ferulate monomer and dehydrodimers have been linked to other phenolic compounds, such as lignin fragments or tyrosine. The linkage of ferulate to lignin by C–C or ether bonds prevents release of ferulates during alkaline hydrolysis (Ralph et al., 1992; Grabber et al., 1995). Additionally, ferulate trimers or oligomers may also have been formed and thus escaped analysis by GC.

From these results it can be concluded that in vitro cross-linking of arabinoxylans results in production of dimers, the proportions of which are not affected by the type of cross-linking agent or the original proportions of dimers in the sample. The low ferulate recovery after oxidative treatment may have been caused by formation of other diferulate isomers, higher oligomers and ferulate-lignin cross-products, which could not be identified by GC and GC-MS.

3.4. Cross-linking with addition of other phenolic materials

3.4.1. Tyrosine

In order to obtain deeper insight into the potential involvement of protein in arabinoxylan cross-linking, particularly through tyrosine, the viscosity behaviour of flour arabinoxylan was studied when it was incubated with hydrogen peroxide/peroxidase in the presence of added tyrosine. The addition of tyrosine, in equal molarity as hydrogen peroxide, gave a quick viscosity increase with a maximum that was slightly less than a similar solution without added tyrosine. After reaching this maximum, a rapid decrease in viscosity was observed (results not shown). Analysis of cross-linking products formed showed that only a trace amount of ferulate monomer was left in the oxidatively treated material and that no dimers could be identified. The combination of viscosity increase and absence of dimeric cross-linking products suggests that cross-linking of arabinoxylan chains occurred, but in a different way then observed without added tyrosine. This may indicate that tyrosine or even tyrosine oligomers may have connected the arabinoxylans through their ferulic acid residues. As it has been shown that tyrosine can give di-, tri-, and even polymeric products of linked tyrosine residues after addition of hydrogen peroxide/peroxidase to solutions of pH 6 (Fry, 1987), as was used in this study, this seems a

very plausible explanation of the observations. The complete absence of ferulate dehydrodimers is most probably caused by the approximately 40 times higher concentration of tyrosine when compared to ferulate. As a consequence of the inability to analyse trimers and higher oligomers of cross-linking products, there is still no direct proof of involvement of tyrosine or protein in arabinoxylan cross-linking.

3.4.2. Feruloylated pectin

Mixtures of beet pectin with arabinoxylans from flour or bran were incubated with hydrogen peroxide and peroxidase and viscosities were measured before and 4 min after addition of reagents. For the flour extract a synergistic effect was observed, particularly when arabinoxylan and pectin were present in a 1 : 1 ratio (Fig. 5). This effect was less pronounced in case of the bran extract and pectin mixtures, investigated at a concentration of 4 mg/ml. Fig. 6 shows the elution pattern of an untreated and cross-linked sample of wheat flour and beet pectin in a 1 : 1 ratio. As beet pectin consisted predominantly of galacturonic acid, being 75% of its total sugar content of 70% (w/w), it can be observed from the neutral sugar and uronic acid distributions in Fig. 6A that its hydrodynamic volume is larger than that of the flour arabinoxylan. Upon cross-linking, an increase in hydrodynamic volume occurred, which was evident from the lower elution volume of a large part of the material. As the major peaks of neutral sugars and uronic acids coincided and a synergistic effect in viscosity was observed after oxidative treatment, it was anticipated that both polysaccharides participated in the cross-linking and were cross-linked to one another. These results indicate that cross-linking of different feruloylated polysaccharides might give rise to interesting cross-linking behaviour and need further research to explore new uses.

4. Conclusions

Wheat flour and bran arabinoxylans behaved differently upon cross-linking with various oxidative agents. When hydrogen peroxide and peroxidase were used, the relative viscosity increase was larger for the bran extract at arabinoxylan concentrations above 3.5 mg/ml, whereas below this concentration the opposite was observed. The distribution of coupling products for both extracts was approximately 5 : 3: 1 : 1 for 8-5, 8-O-4, 8-8 and 5-5 dimers, respectively. The 5-5 dimer was only of minor importance in in vitro cross-linking of isolated arabinoxylans. The distribution was similar when glucose, glucoseoxidase and peroxidase or ammonium persulphate were used as oxidative agents. The concentrations of reagents needed to induce cross-linking and the increase in viscosity were very different for flour and bran arabinoxylans when glucose, glucoseoxidase and peroxidase or ammonium persulphate were used. It was assumed that the presence of lignin-fragments in the bran extract interfered with the cross-linking of ferulates, resulting in higher concentrations of reagents required and increased coupling of ferulates to products which could not be analysed by GC.

Cross-linking of the flour extract in the presence of tyrosine resulted in viscosity increase but dimeric coupling products were not recovered, perhaps as a result of the formation of higher molecular weight products. In case of cross-linking of mixtures of feruloylated arabinoxylan and pectin with hydrogen peroxide and peroxidase it was shown that both polysaccharides participate in the cross-linking and that it results in a synergistic effect on viscosity.

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