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Extraction and partial characterization of feruloylated glucuronoarabinoxylans from wheat bran

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Feruloylated glucuronoarabinoxylans were extracted from wheat bran by means of cold water, steam and dilute alkali, yielding approximately 1, 20 and 3%, respectively, of the glucuronoarabinoxylans originally present in the bran. The extracts had ferulic acid contents ranging from 0.1 to 0.6% (w/w). Both the cold water and steam extract had a low total sugar content and a relatively low content of high molecular weight material. Moreover, the ferulic acid content of the water extract was low (0.1%) and the amount of degradation products in the steam extract was relatively large. Both extracts appeared to give no increase in viscosity upon addition of oxidative reagents, such as hydrogen peroxide plus peroxidase or ammonium persulphate. The alkaline extracts obtained with saturated calcium hydroxide or 0.05 M barium hydroxide had high total sugar contents, the majority of which was accounted for by glucuronoarabinoxylan (>90%). These extracts had moderate ferulic acid contents of 0.3% and consisted predominantly of high molecular weight material. Addition of oxidative reagents resulted in an increase of viscosity. The extent of this increase in viscosity was shown to be influenced by the oxidative reagent used, the purity of the extract, its molecular weight distribution and the degree of substitution of the glucuronoarabinoxylan.

Abbreviations: WUS = water unextractable cell wall material, HPLC = high-performance liquid chromatography, HPSEC = high-performance size-exclusion chromatography, dm = dry matter, GAX = glucuronoarabinoxylan, FA = ferulic acid.

INTRODUCTION

For several decades it has been known that ferulic acid plays a significant role in the increase of viscosity of water soluble extracts from wheat flour by the addition of hydrogen peroxide and peroxidase (Fausch *et al.*, 1963; Geissmann & Neukom, 1971 and 1973; Neukom & Markwalder, 1978). Because the water-binding capacity of wheat flours is of importance in dough preparation and baking performance (Meuser & Suckow, 1986), the components involved and the mechanism of the reaction have been of interest ever since.

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The increase in viscosity of water-extractable arabinoxylans, to which ferulic acid is esterified, has been shown to be the result of oxidative coupling of two ferulic acid residues after addition of hydrogen peroxide and peroxidase (Geissmann & Neukom, 1971 and 1973). Involvement of protein in this reaction was suggested also by these authors, but protein appeared not to be essential (Morita *et al.*, 1974). However, the amino acids tyrosine (Neukom & Markwalder, 1978) and cysteine (Hoseney & Faubion, 1981; Jackson & Hoseney, 1986) were presumed to participate in the cross-linking. For rye flour water soluble arabinoxylans it was shown that the cysteine residues in the coextracted protein in the extract seemed not to be involved in the gelation upon oxidative cross-linking (Vinkx *et al.*,

1991). Whether protein has any role in the cross-linking or not, is still not clear. Nevertheless, factors that have been shown to influence the extent of cross-linking are the wheat variety the extract originates from (Ciacco & d'Appolonia, 1982a; Izydorczyk *et al.*, 1991), the ash content of the flour (Neukom & Markwalder, 1978; Ciacco & d'Appolonia, 1982b), the intrinsic viscosity of the extract (Ciacco & d'Appolonia, 1982a,b; Izydorczyk & Biliaderis, 1992a,b) and the structure of the arabinoxylans, more precisely the degree of substitution (Izydorczyk & Biliaderis, 1992a).

In this study it was investigated whether feruloylated arabinoxylans can be extracted from wheat bran. Wheat bran is known to contain 0.4% to 0.7% (w/w) alkali extractable ferulic acid (Smith & Hartley, 1983), which is predominantly located in the aleurone layer and to a lesser extent in the pericarp (Fulcher *et al.*, 1972; Pussayanawin & Wetzel, 1987). Isolation of feruloylated arabinoxylans from wheat bran, which can form gels upon oxidative cross-linking, can open up possibilities for new uses of this by-product. Different extraction methods were, therefore, investigated for their ability to give extracts that can be cross-linked upon addition of oxidative reagents. In order to induce cross-linking not only hydrogen peroxide and peroxidase, but also ammonium persulphate was used, because both were reported to form gels with wheat flour arabinoxylans (Izydorczyk *et al.*, 1990).

EXPERIMENTAL

Materials

Industrial wheat bran was obtained from Presco International (Weert, The Netherlands). Wheat bran was destarched using a heat-stable α-amylase (Termamyl 120L, Novo Industri A/S, Copenhagen, Denmark). A wheat bran suspension with a solid to liquid ratio of 1:9 (w/v), containing 0.05 ml Termamyl per 100 g of bran and 30 ppm calcium was incubated for 2 h at 95°C with continuous stirring after adjustment to pH 6. Finally, the suspension was autoclaved for 1 h at 121°C. The residual solids were collected by centrifugation (17 700 ***g***; 30 min) and subsequent washing with distilled water. Water-unextractable cell wall material (WUS) from wheat bran was prepared as described previously (Bergmans *et al.*, 1996).

Cold water extraction

Water-extractable material was isolated from wheat bran according to a slightly modified method of Fincher & Stone (1974). The milled bran was boiled in 80% ethanol and filtered, which was repeated twice. The alcohol insoluble residue was suspended in distilled water with a solid to liquid ratio of 1:5, and stirred continuously at 4°C for 64 h. After removal of the solids by centrifugation (17 700 ***g***; 30 min) the liquid was adjusted to 0.01 M citric acid, 0.01 M sodium chloride, 0.001 M calcium chloride, pH 4.6 and 0.05% sodium azide. After incubation of this solution with amyloglucosidase (Boehringer, Mannheim, Germany) at 30°C for 40 h it was heated to 100 °C for 15 min, cooled, centrifuged, dialysed and freeze-dried (WE).

Steam explosion

Destarched wheat bran was treated in a laboratory defibrator with saturated steam (Institute of Wood Chemistry, Hamburg, Germany). Steam treatments were performed at temperatures of 180°C to 200°C for periods of 5–15 min. At the end of the treatment the defibrator was operated for 15 s, the steam was released and the humid material was removed with water. The solids were collected by centrifugation (17 700 ***g***; 30 min) and washed three times with distilled water. Solids and combined liquids were freeze-dried. The soluble fractions were designated $180SE^{5}$; $190SE^{5}$; $190SE^{10}$; $190SE^{15}$; $200SE^{5}$. The first three digits refer to the steam temperature (°C) and the superscript to the time of steaming (min).

Dilute alkali extraction

WUS was extracted with bivalent hydroxide solutions with a solid to liquid ratio of 1:100 (w/v). Calcium hydroxide was used as saturated solution as described previously (Bergmans *et al.*, 1996) and barium hydroxide at a concentration of 0.05 M. Addition of 0.26 M sodium borohydride as used before (Bergmans *et al.*, 1996) was omitted. Extraction lasted for 1–16 h and was performed at 20°C or 70°C. The extracted material was recovered by centrifugation, neutralization and dialysis as described before (Bergmans *et al.*, 1996). The extracts were either freeze-dried or concentrated by vacuum evaporation. The concentrated solutions were stored at 4°C with the addition of 0.05% sodium azide. Large scale extracts were designated $0.02CE_4$ and $0.05BE_2$, for calcium hydroxide and barium hydroxide, respectively. The first numbers refer to the concentration of alkali (M) and the subscript to the extraction time (h).

Purification of extracts

Solubilized extracts (2.0 mg/ml) were mixed with cold acetone giving mixtures with 70% or 80% (v/v) of organic solvent. These mixtures were stirred for 1 h at 4°C and then left at the same temperature for 16 h. Precipitate and supernatant were recovered by centrifugation (25 000 ***g***; 20 min). The precipitate was redissolved in distilled water and used for cross-linking studies. In preliminary small scale studies using methanol, ethanol and acetone the supernatants and redissolved precipitates were analysed for total neutral sugar content by an automated calorimetric method using arabinose as standard (Tollier & Robin, 1979) and for UV-absorbance at 280 nm.

The latter was used as a measure for the presence of lignin-like material.

Cross-linking of glucuronoarabinoxylans

Hydrogen peroxide-pyroxidase
Samples were cross-linked in 0.1 M sodium phosphate buffer of pH 6, by the addition of 0.05 mg horse-radish peroxidase (Type 1, Art. P-8125, Sigma, St Louis, CA) and 2 μmoles of hydrogen peroxide per ml of solution. The concentration of the samples ranged from 5 to 15 mg/ml. The samples were diluted to the appropriate concentration with phosphate buffer containing 0.05% sodium azide. The reaction was performed at 25°C.

Ammonium persulphate
Cross-linking of samples by the addition of ammonium persulphate at a concentration of 0.01 M was performed in distilled water containing 0.05% sodium azide. The concentration of the samples and reaction temperature was similar as described above.

Viscometry
Viscosities of the solvent and of the polysaccharide solutions before and after addition of reagents were measured with an Ubbelohde capillary viscometer (Schott, Mainz, Germany) with a capillary diameter of 0.63 mm, submerged in a thermostatically controlled waterbath at 25°C. Measurements were taken 15 min or 24 h after addition of peroxide and ammonium persulphate, respectively.

Analytical methods

Neutral sugar composition and uronic acid content of the wheat bran materials, extracts and residues were determined as described previously (Bergmans *et al.*, 1996).

Ferulic acid content
Samples were saponified with 5 ml of 0.5 M potassium hydroxide for 16 h at room temperature in nitrogen atmosphere and shielded from light. *p*-Hydroxybenzoic acid was added as internal standard. The samples were acidified with 0.75 ml of 6 M hydrochloric acid and extracted twice with 4 ml ethylacetate. The combined ethylacetate extracts were dried under nitrogen. The residues were dissolved in 1 ml methanol and analysed by HPLC. A SpectraSYSTEM P4000 gradient pump equipped with a membrane degasser (Thermo Separation Products, Fremont, CA) was used with a reversed phase Spherisorb 10 ODS column. The column was operated at room temperature at a flow rate of 1 ml/min. Elution was performed with a gradient of 0.01 M acetic acid of pH 5 and methanol. Starting conditions were 5% methanol in 0.01 M acetic acid for 5 min, then linear gradients were applied of 5%–50% methanol in 0.01 M acetic acid during 20 min and 50% –90% methanol in 0.01 M acetic acid during 10 min. This final condition was kept for the following 5 min. The column effluent was monitored by a SpectraSYSTEM UV1000 detector (Thermo Separation Products, Fremont, CA) at a wavelength of 280 nm. A standard of *trans*-ferulic acid was used to quantify the amounts present in the samples. As isomerization of the *trans* to the *cis*-isomer can occur during sample work-up, part of the standard solution was irradiated for 4 h at 360 nm in order to give partial conversion to the *cis*-isomer. By this means quantification of both isomers was performed.

High-performance size-exclusion chromatography (HPSEC)
Molecular weight distributions of the extracts were determined by HPSEC using three Bio-Gel TSK columns in series as described elsewhere (Bergmans *et al.*, 1996).

RESULTS AND DISCUSSION

Cold water extraction

Water-extractable material was isolated from wheat bran by cold water in an attempt to obtain feruloylated glucuronoarabinoxylans. In Table 1 the yield and composition of this extract is given. Only 1% of the original bran appeared to be extractable in cold water. The sugar and ferulic acid content of the extract were 54% and 0.1%, respectively. The extract contained 1.5% of the glucuronoarabinoxylans originally present in the bran. From Table 1 it can be calculated that 1 out of 100 arabinose residues in the extract was esterified with ferulic acid, for the whole bran it was 1 out of 20 arabinose residues. This showed that ferulic acid was predominantly bound to polymers, which were not water-extractable. These polymers are most probably held in the cell wall matrix by covalent linkages. Ferulic acid is known to be a bridging unit in lignified grass cell walls between polysaccharide and lignin by means of an ester and ether-linkage, respectively (Scalbert *et al.*, 1985; Lam *et al.*, 1990). However, esterified ferulic acid is also known to be predominantly present in the aleurone layer (Fulcher *et al.*, 1972; Pussayanawin & Wetzel, 1987), which contains hardly any lignin (Bacic & Stone, 1981; Akin, 1995). The insolubility of the feruloylated arabinoxylans of the aleurone layer is very likely caused by extensive hydrogen bonding, because these arabinoxylans have a low degree of substitution (Bacic & Stone, 1981; Schooneveld-Bergmans *et al.*, submitted).

The Ara/Xyl-ratio of 0.62 for WE was low compared to previous alkaline extracts obtained from wheat bran (Bergmans *et al.*, 1996). As this ratio corresponds quite well with the ones reported for water-extractable wheat flour arabinoxylans (Izydorczyk *et al.*, 1990; Cleemput et al., 1993), WE or part of it may as well have originated from residual endosperm material in the bran. The amount of starch in the bran of 12% (w/w) (Bergmans *et al.*, 1996) is indicative for this assumption, however it was not studied

Table 1. Yield and composition of extracts obtained from wheat bran (WB) by water extraction, from destarched wheat bran by steam treatment or from wheat bran WUS by dilute alkali extraction

	Yield[a]	Ferulic acid content[b]	Total sugar content[c]	Molar composition[d]						
				Ara	Xyl	Man	Gal	Glc	UA	Ara/Xyl
WB as is										
WE	1.2	0.1	53.8	32.2	52.2	1.3	5.1	5.8	3.3	0.62
WB destarched										
$180SE^{10}$	20.6	0.6	46.1	35.5	37.7	0.5	2.5	19.9	3.9	0.94
$190SE^{5}$	19.5	0.6	54.4	35.8	38.1	0.6	2.4	19.5	3.6	0.94
$190SE^{10}$	30.7	0.5	44.2	26.2	51.2	0.7	2.2	15.6	4.1	0.51
$190SE^{15}$	31.1	0.5	42.1	31.0	47.1	0.5	2.4	14.6	4.4	0.66
$200SE^{5}$	20.6	0.6	51.8	34.4	38.7	0.5	2.4	20.5	3.5	0.89
WB WUS										
$0.02CE_4$	2.0	0.3	75.8	42.9	46.2	0.3	2.0	5.7	3.0	0.94
$0.05BE_2$	2.9	0.2	72.8	41.6	51.9	0.2	1.5	2.6	2.2	0.80

[a]Expressed as weight percentage (dm) of original wheat bran.
[b]Expressed as weight percentage (dm) of each fraction.
[c]Neutral sugars + uronic acids expressed as weight percentage (dm) of each fraction.
[d]Expressed as percentage (mole per 100 mole).

any further. HPSEC-analysis of the extract showed the presence of populations of varying hydrodynamic volumes [Fig. 2(a)].

From these results it can be inferred that for the isolation of large amounts of feruloylated glucuronoarabinoxylans from wheat bran techniques need to be used that disintegrate the dense cell wall structure without hydrolysis of ester-linked ferulic acid.

Steam explosion

From literature it is known that disintegration of lignified cell walls can be achieved by steam explosion treatments, resulting in solubilization of partially depolymerized hemicelluloses without loss of esterified ferulic acid (Puls *et al.*, 1985; Puls & Poutanen, 1989). Preliminary experiments of mild steam treatments for 10 min at 180°C with wheat bran resulted in strong browning of the material. This was most probably caused by decomposition of starch, because conversion of sugars to furfural-like compounds is known to occur during severe steaming of wood chips (Puls *et al.*, 1985). Moreover, formation of Maillard and tannin-like condensation products during heat treatment of cereals has also been reported (Theander & Westerlund, 1984; Castro *et al.*, 1994). Browning appeared to be reduced when destarched bran was used for steam treatments. However, as a result of the destarching treatment a loss of approximately 12% of the glucuronoarabinoxylans of the original bran was observed (results not shown). This was most likely caused by the hot water incubation and autoclave treatment. Previously, a loss of approximately 10% of the glucuronoarabinoxylans originally present in wheat bran WUS was reported as a result of a 1 h autoclave treatment (Bergmans *et al.*, 1996).

By applying different steam treatments, which varied in temperature and time, it was attempted to solubilize polymeric feruloylated glucuronoarabinoxylans from destarched wheat bran. In Table 1 the yields and compositions of the

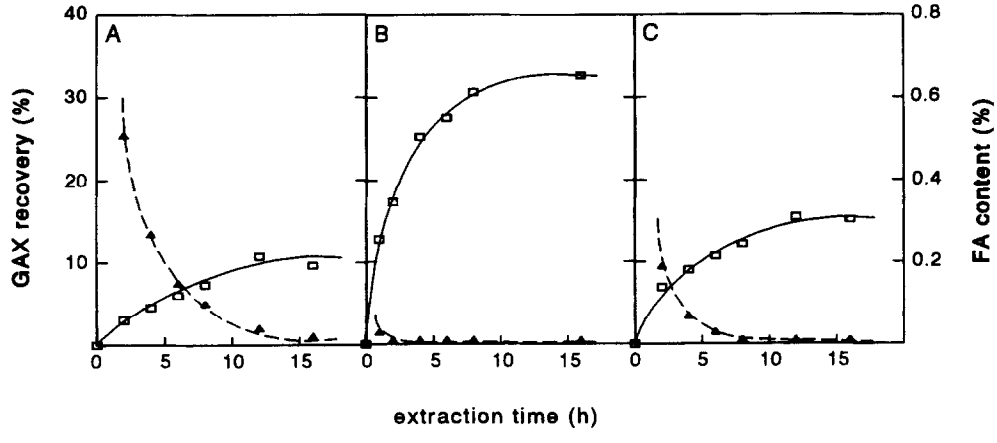

Fig. 1. Recovery of glucuronoarabinoxylan (————) and ferulic acid content of the extract (— — —) during dilute alkali extraction of wheat bran WUS. A: saturated calcium hydroxide at 20°C; B: saturated calcium hydroxide at 70°C; C: 0.05 M barium hydroxide at 20°C.

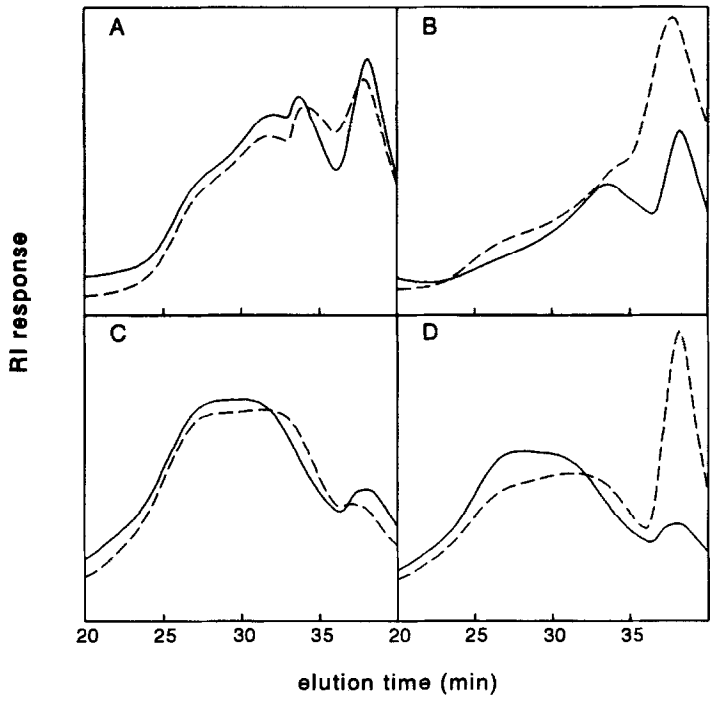

Fig. 2. HPSEC-elution patterns of extracts of feruloylated glucuronoarabinoxylans from wheat bran before (— — —) and after purification (————————) by acetone precipitation. A: WE; B: 200SE[5]; C: 0.02CE$_4$; D: 0.05BE$_2$.

steam extracts are presented. The yields of the extracts ranged from 20% to 31%, and the extracts contained 19% to 28% of the glucuronoarabinoxylans present in the parental bran. The purity of the extracts was not very high, because their total sugar contents were only approximately 50%, of which 15–20 mole% was accounted for by coextracted glucose. The glucose may originate from starch, as the destarched bran had a residual starch content of 4% (w/w), or it may originate from (1 → 3)(1 → 4)-β-glucans. Cellulose has been shown not to get solubilized by steam treatment (Puls *et al.*, 1985). The ferulic acid content of the steam extracts was considerably higher than that of the water-extractable fraction. It was calculated that 1 out of 10 to 20 arabinose residues in these extracts was esterified with ferulic acid, which corresponds well with the ratio in the whole bran. Approximately 20% of the ferulic acid originally present in the bran was recovered in the extracts. From Table 1 it can be observed that higher temperature and longer time of steaming resulted in a higher dry matter yield, but also in a lower total sugar content of the extract. The latter is likely caused by the aforementioned decomposition of sugars to furfural-like compounds and the formation of condensation products. HPSEC-analysis of the steam extracts showed that only 180SE[10], 190SE[5] and 200SE[5] contained a minor fraction eluting at relatively high hydrodynamic volume. For the other extracts the steaming conditions appeared to be too rigorous to yield polymeric material. In Fig. 2(b) the HPSEC-elution pattern of 200SE[5] is shown.

From these results it can be stated that steam explosion can be used for the extraction of feruloylated glucuronoarabinoxylans from destarched wheat bran. However, steaming conditions need to be mild in order to recover any polymeric material.

Dilute alkali extraction

As a third attempt to isolate feruloylated glucuronoarabinoxylans from wheat bran, dilute alkali extraction was used. Although it is generally known that esterified ferulic acid is hydrolysed in alkaline solutions, it is also reported that 0.1 M sodium hydroxide treatment for 1 h released only 10 to 20% of the alkali labile ferulic acid of grass cell wall materials (Hartley & Morrison, 1991). The extractability of feruloylated glucuronoarabinoxylans from wheat bran WUS was therefore followed in time for three different alkali treatments. Bivalent hydroxides, such as barium and calcium hydroxide, were preferred for the extraction, because of their previously observed selectivity (Gruppen *et al.*, 1991; Bergmans *et al.*, 1996). The use of sodium borohydride, which further improved the selectivity of the extractants and prevented alkaline peeling, was now omitted. Literature data on the possible reduction of the α,β-unsaturated carbonyl side chain of hydroxycinnamic acids by borohydride were contradictory. Carpita (1986) claimed that the unsaturated bond was reduced, whereas Ford (1989) recovered almost all phenolic acids without modification when 0.2 M borohydride solution was used as extractant for polysaccharides from pangola grass. Because the majority of the extractable glucuronoarabinoxylans from wheat bran WUS are highly substituted (Schooneveld-Bergmans *et al.*, submitted) alkaline peeling was not expected to play a role during extraction. It was therefore decided not to use sodium borohydride.

In Fig. 1 the yields of extracted glucuronoarabinoxylans and the ferulic acid content of the extracts are presented. Fig. 1(a) shows that saturated calcium hydroxide, of which the concentration is approximately 0.02 M, extracted up to 10% of the glucuronoarabinoxylans of WUS in 16 h. After 6 h of extraction the ferulic acid content of these polysaccharides was 0.1%. Extraction at 70°C with the same extractant resulted in a maximum yield of more than 30% of the glucuronoarabinoxylans originally present in WUS. However, already after 1 h of extraction the ferulic acid content of the solubilized material was less than 0.1%. The effect of temperature on ferulic acid recovery in the extracted material was further investigated by performing extractions with saturated calcium hydroxide at temperature intervals of 10°C in the range of 20°C to 70°C. Substantial losses of ferulic acid were observed starting at 40°C (results not shown). Extraction at a slightly higher hydroxide concentration was performed with 0.05 M barium hydroxide at 20°C. In 16 h 15% of the glucuronoarabinoxylans of WUS were extracted. After 4 h of extraction the ferulic acid content of the extract was less than 0.1%.

Greenshields & Rees (1993) described the use of 0.1–0.6 M sodium or potassium hydroxide typically for 0.5–5 h at 60°C–85°C for extraction of feruloylated polysaccharides from testaceous plant material. Based on the results described above it is suspected that their range of extraction conditions is not appropriate to prevent the loss of ferulic acid in all cases. However, minor differences in the extraction procedure, such as solid to liquid ratio and particle size of the plant material, may have an effect on the results, but this was not further investigated.

From the series of extractions shown in Fig. 1 two extraction conditions were selected for enlarged scale extraction of feruloylated glucuronoarabinoxylans from wheat bran WUS. The conditions were saturated calcium hydroxide for 4 h ($0.02CE_4$) and 0.05 M barium hydroxide for 2 h ($0.05BE_2$), both at 20°C. The yields and compositions of these extracts are presented in Table 1. Only 2% and 3% of the glucuronoarabinoxylans present in the bran were extracted by calcium and barium hydroxide, respectively. This corresponds with 4% and 7% of the glucuronoarabinoxylans of WUS as is shown in Fig. 1. The selectivity of the extractions appeared to be quite high compared to the steam treatments, because the total sugar contents amounted to more than 70% of the dry matter and coextraction of only 3–6 mole% of glucose was observed. The extracts contained approximately 0.3% of ferulic acid. As the ferulic acid content of the residues of extraction were also determined (results not shown), the total ferulic acid recovery after extraction was determined to be approximately 60%. HPSEC analysis showed the presence of a relatively high content of material of high hydrodynamic volume [Fig. 2(c,d)].

In conclusion. dilute alkali can be used for the extraction of polymeric feruloylated glucuronoarabinoxylans from wheat bran, as long as the concentration of alkali, time and temperature of extraction are properly balanced.

Purification of extracts

Preliminary studies on the viscosity behaviour of the extracts upon addition of hydrogen peroxide and peroxidase showed no increase of the viscosity even at high concentrations of glucuronoarabinoxylans. Use of 80% ethanol (v/v) is reported for purification of feruloylated arabinoxylans from wheat flour, resulting in a considerable improvement of the gelling capacity (Moore *et al.*, 1990). Studies on the homogeneity of the dilute alkaline extracts of wheat bran WUS ($0.02CE_4$ and $0.05BE_2$) by fractionation using graded ethanol precipitation, showed that the fractions soluble at high concentrations of ethanol had low sugar and ferulic acid contents (results not shown). Because of this, these highly ethanol soluble fractions were assumed not to participate in the cross-linking, on the contrary they may even limit cross-linking as a result of the presence of lignin and protein, which may scavenge radicals needed for coupling of ferulic acid residues. Purification of the various extracts was performed on a small scale by precipitation at 70% or 80% (v/v) ethanol, methanol or acetone. Generally, when acetone was used the sugar recovery and UV-absorbance were highest. The lowest recovery of UV-absorbance was observed for methanol, but it also resulted in great losses of carbohydrates. Although ethanol gave intermediate results, the use of acetone was preferred because it reduced the UV-absorbance relatively well without great losses of sugars (results not shown).

Table 2. Yield and composition of acetone purified extracts obtained from wheat bran by water extraction (WE·P), from destarched wheat bran by steam treatment ($200SE^5$·P), or from wheat bran WUS by dilute alkali extraction ($0.02CE_4$·P and $0.05BE_2$·P)

	Yield[a]	Ferulic acid content[b]	Total sugar content[c]	Molar composition[d]						
				Ara	Xyl	Man	Gal	Glc	UA	Ara/Xyl
WE·P	79.8	0.1	44.6	33.2	55.6	0.7	5.6	1.4	3.4	0.60
$200SE^5$·P	49.7	0.7	46.0	24.4	44.4	0.3	2.9	21.5	6.5	0.55
$0.02CE_4$·P	67.5	0.5	85.3	43.7	48.3	0.2	2.4	1.5	4.0	0.90
$0.05BE_2$·P	53.3	0.4	78.8	40.1	53.8	0.2	1.2	1.4	3.3	0.75

[a]Expressed as weight percentage (dm) of original extract.
[b]Expressed as weight percentage (dm) of each fraction.
[c]Neutral sugars + uronic acids expressed as weight percentage (dm) of each fraction.
[d]Expressed as percentage (mole per 100 mole).

For large scale purification of the extracts the use of acetone was chosen. In Table 2 the yields and compositions of the purified extracts are shown. Comparison of the Ara/Xyl-ratios of the extracts before (Table 1) and after purification (Table 2), showed a decrease for all extracts. This is mainly caused by the higher solubility of highly substituted arabinoxylans in organic solvents compared with lowly substituted populations (Gruppen *et al.*, 1992; Schooneveld-Bergmans *et al.*, submitted). The very sharp decrease in Ara/Xyl-ratio for the steam extract is most probably also caused by the extraction method. It has been reported that mild acidic conditions can occur during steam treatment (Puls *et al.*, 1985), as a result of which the highly acid-sensitive furanosidic linkages can be hydrolysed. In the subsequent purification the hydrolysed arabinose was lost in the supernatant as a result of its solubility in 80% acetone. For the water extract purification resulted in a loss of glucose, and 80% of the glucuronoarabinoxylans of the original extract were recovered. However, an increased sugar content was not obtained by purification. This latter observation was also noticed for the steam extract, which consisted of only 50% of the glucuronoarabinoxylans of the parental extract. This low recovery was predominantly a result of the solubility of the mono- and oligosaccharides produced during steaming in high concentrations of organic solvent. Both alkaline extracts showed an increased total sugar content as well as ferulic acid content. A recovery of 80% and 60% of the original glucuronoarabinoxylans was obtained for $0.02CE_4$ and $0.05BE_2$, respectively, whereas almost all ferulic acid was recovered.

It is, therefore, concluded that the best purification was obtained for the alkaline extracts, because an increase in total neutral sugar content and ferulic acid content coincided with a loss of coextracted glucose and non-sugar material.

Molecular weight distribution

The molecular weight distributions of the extracts before and after purification are shown in Fig. 2. It is clear that purification resulted in a loss of material eluting at retention times of 35 min and further. This is most clear for $0.05BE_2$ [Fig. 2(d)]. From the purification of the steam extract [Fig. 2(b)] it is also obvious that the relative amount of material of low hydrodynamic volume decreased. Both alkaline extracts contained relatively more material of high hydrodynamic volume than the water and steam extracts. After purification molecular weight distributions of the former two extracts narrowed, which was not observed for the water and steam extracts.

Cross-linking of extracts

Crude extracts

The relative viscosity of all crude extracts was not affected in the concentration range of 5–15 mg/ml by the addition of hydrogen peroxide and peroxidase. As was mentioned before this may be the result of the presence of phenolic compounds that scavenge radicals. However, inhibition of the enzyme by phenolic compounds may also have occurred. In case of ammonium persulphate as cross-linking agent, an increase in viscosity was observed for both alkaline extracts (results not shown). The production of sulphate ion radicals from persulphate therefore appears not to be limited by the aforementioned phenolics. When the viscosity increase of the alkaline extracts of corresponding concentration were compared, it appeared to be highest for the barium hydroxide extract for the whole range of concentrations tested. As the barium hydroxide extract had a slightly lower glucuronoarabinoxylan and ferulic acid content compared with the calcium hydroxide extract, the arabinoxylan and ferulic acid content appear not to be the only crucial factors in the cross-linking. For wheat flour arabinoxylans the intrinsic viscosity (Ciacco & d'Appolonia, 1982a,b; Izydorczyk & Biliaderis, 1992a,b) and the Ara/Xyl-ratio (Izydorczyk & Biliaderis, 1992a) were also reported to have an effect on the cross-linking. From Fig. 2(c,d) it can be observed that the molecular weight distribution of both extracts differed only in the low molecular weight region, which is not expected to have a major effect on their intrinsic viscosities. From Table 1 it is clear that the barium hydroxide extract had a lower Ara/Xyl-ratio than the calcium hydroxide extract. This may have caused the observed difference in viscosity increase upon cross-linking.

Purified extracts

After purification, all extracts showed increased viscosities upon addition of hydrogen peroxide and peroxidase. In Fig. 3 the relative viscosities of the extracts before and after addition of the reagents are presented. From this figure it is clear that for the water and steam extracts the low viscosities only increase slightly. This is most probably attributable primarily to the low purity of the extracts (Table 2) and the relatively low content of high molecular weight material (Fig. 2). Fig. 3 also shows that the increase in viscosity is larger at higher extract concentration, with the exception of the calcium hydroxide extracted material.

By determination of the ferulic acid recovery after cross-linking it was investigated whether the amount of monomeric esterified ferulic acid residues consumed by the reaction was correlated with the viscosity increase. No correlation was observed (results not shown), which may indicate that not all dimers formed contribute to the viscosity increase to a similar extent or that not all ferulic acid residues consumed result in the formation of a dimer. As viscosities were only measured at one point in time after addition of the reagents, it is not known whether oxidative degradation of arabinoxylan, which can take place competitively with the cross-linking reaction as a result of the hydroxyl radicals produced from the hydrogen peroxide (Ciacco & d'Appolonia, 1982a), influenced the viscosities of the extracts also.

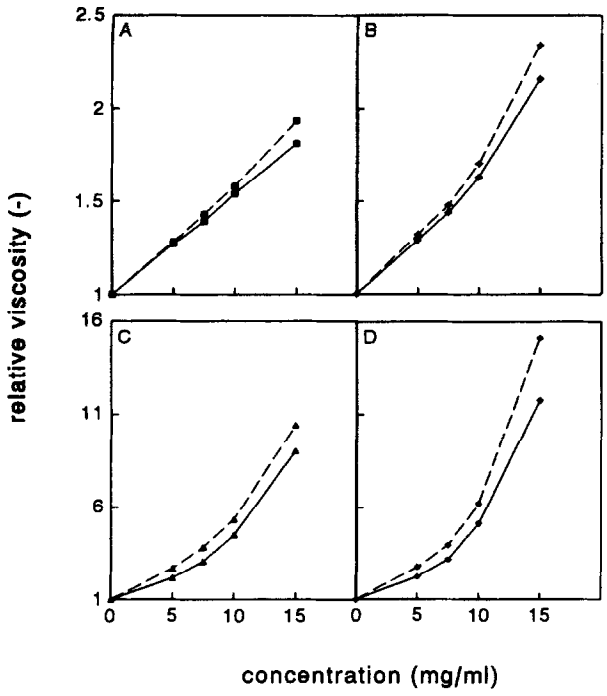

Fig. 3. Viscosities of purified extracts of feruloylated glucuronoarabinoxylans at different concentrations before (————) and after (— — —) the addition of hydrogen peroxide and peroxidase, expressed relative to the viscosity of the solvent. A: WE·P; B: 200SE^5·P; C: 0.02CE$_4$·P; D: 0.05BE$_2$·P.

Cross-linking of the purified extracts by addition of ammonium persulphate resulted in no increased viscosities for the water and steam extracts at all concentrations, whereas the dilute alkali extracts formed gels over the whole range of concentrations tested. The flow time of these gels could not be measured in a capillary viscometer and it was not attempted to characterize possible differences originating from the differences in concentration or composition of the extracts by any other means. However, it can be stated that the cross-linking ability of feruloylated wheat bran glucuronoarabinoxylans is dependent on their molecular weight distribution, Ara/Xyl-ratio, the purity and the type of cross-linking reagent used. Determination of the types and amounts of ferulic acid dimers formed, as was recently reported by Ralph *et al.* (1994), most likely leads to a better understanding of the mechanism of coupling and is currently under investigation.

CONCLUSIONS

Extraction of feruloylated glucuronoarabinoxylans from wheat bran appeared to be most successful by dilute alkali extraction, in relation to their viscosity behaviour upon oxidative cross-linking. Extracts obtained by water and steam extraction had a lower glucuronoarabinoxylan content, contained relatively less high molecular weight material and showed hardly any increase in viscosity after addition of cross-linking reagents, when compared to the dilute alkali extracts. During alkali extraction the concentration of hydroxyl ions, the temperature and time of extraction appeared to be of major importance in the recovery of ferulic acid esterified to the glucuronoarabinoxylans. Purification of the extracts by acetone precipitation resulted in a higher increase of the viscosity upon cross-linking compared with the crude extracts. Substantial differences were found in the increase of viscosity when hydrogen peroxide and peroxidase was compared with ammonium persulphate as cross-linking reagent. Apart from the purity of the extract and the type of cross-linking reagent, the Ara/Xyl-ratio was also shown to have an effect on the increase of viscosity. A high Ara/Xyl-ratio resulted in a smaller viscosity increase.

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