Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 1160–1170
Food Chemistry
www.elsevier.com/locate/foodchem

Structural characteristics of water-soluble feruloyl arabinoxylans from rice (*Oryza sativa*) and ragi (finger millet, *Eleusine coracana*): Variations upon malting

R. Shyama Prasad Rao, G. Muralikrishna *

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India

Received 28 October 2006; received in revised form 23 November 2006; accepted 12 January 2007

Abstract

Water-soluble feruloyl arabinoxylans (feraxans), isolated from native and malted (96 h) rice (*Oryza sativa*) and ragi (*Eleusine coracana*) grains, were fractionated on DEAE-cellulose, followed by purification on Sephacryl S-300 and the homogeneity was ascertained by high performance size exclusion chromatography, cellulose acetate and capillary electrophoresis. Structural characterization of the purified polysaccharides by methylation, followed by GLC–MS, and also by ^{1}H NMR and ^{13}C NMR spectroscopy, indicated very high branching and presence of high amounts of O-2 substituted xylans. The amount of O-2, 3 disubstituted xylopyranosyl residues and the arabinose:xylose ratio was higher in malt feraxans. All feraxan samples consumed almost equal amounts of periodate (4.02–4.30 μmol/mg). High amount of xylose (~40%), as identified by Smith degradation, further substantiated the high branching of feraxans. A model is presented depicting the structure of water-soluble feraxans from rice and ragi and their changes upon malting.

Keywords: Arabinoxylan; Cereals; Ferulic acid; Malting; Ragi; Structure

1. Introduction

In recent years, the complex carbohydrates/polysaccharides role has been increasingly recognized in therapeutics and nutrigenomics fields. Arabinoxylans, the chief non-starch polysaccharides in cereals (Izydorczyk & Biliaderis, 1995), are particularly interesting and important as they exhibit a wide range of functional properties and health benefits. Conventionally, they are bracketed under the broad definition of dietary fibre (soluble and insoluble) and are known to have many beneficial roles in human nutrition and health, such as lowering of cholesterol, reducing the disease symptoms of constipation and reducing the risk of diabetes, atherosclerosis and colorectal cancer (Morris, Marr, & Clayton, 1977; Plaami, 1997; Willett, 1994). They are also known to influence the quality of bakery products due to their physicochemical properties, e.g. viscosity and water-holding capacity (Izydorczyk & Biliaderis, 1995). Arabinoxylans are also considered for edible coatings and films.

The bioactive potential of arabinoxylans has been lately recognized. Rice bran arabinoxylan is shown to sensitize human T cell leukemia cells to necrosis, and is active against HIV (Ghoneum & Gollapudi, 2003). Being potent natural immunomodulators and prebiotic, they have recently been considered as functional food ingredients (Charalampopoulos, Wang, Pandiella, & Webb, 2002). Arabinoxylans are also proposed to have wound-dressing potential. Recently, feruloyl arabinoxylans were shown to be highly antioxidant, and this property is correlated with their molecular architecture (Rao & Muralikrishna, 2006).

As the function is intimately related to the structure, characterization of highly complex and diverse polysaccharides such as arabinoxylans is highly desirable. For

* Corresponding author. Tel.: +91 821 2514876; fax: +91 821 2517233.
E-mail address: krishnagm2002@yahoo.com (G. Muralikrishna).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.015

example, the fine structure of arabinoxylans, like the distribution of arabinose residues on the xylan backbone, is thought to bring about variations in properties and functions (Dervilly-Pinel, Tran, & Saulnier, 2004).

Despite the large amount of knowledge on the structure of arabinoxylans (Ishii, 1997; Izydorczyk & Biliaderis, 1995), elucidation of the fine structures of arabinoxylans from varied sources and diverse conditions still remains as a matter of immense interest and importance. Rice and ragi, the major cereal and millet, respectively, are important sources of arabinoxylans and are consumed by millions of people across the world. Earlier, we have shown the potent antioxidant activity of water-soluble feraxans from these two grains (Rao & Muralikrishna, 2006). Although structures of water-insoluble alkali-soluble arabinoxylans from rice (Shibuya & Iwasaki, 1985) and ragi (Subba Rao & Muralikrishna, 2004) are elucidated, detailed structural characterization of water-soluble feraxans from these two cereals is lacking. Further, comparative analysis of feraxans in these two grasses, and the changes brought about by malting/germination, have not been studied under similar conditions. Thus, water-soluble feraxans were obtained from native and malted rice and ragi and characterized using diverse chromatographic and spectrometric analyses to achieve a fine structural insight of these polysaccharides, in order to correlate the structure and function relationship, with specific reference to the bound ferulic acid.

2. Materials and methods

2.1. Materials

Rice (*Oryza sativa* var. Jaya) and finger millet – ragi (*Eleusine coracana* var. Indaf-15) were procured from V.C. Farm of the University of Agricultural Sciences, located at Mandya, Karnataka. Fine chemicals were from Sigma Chemical Company, USA and Pharmacia Co., Sweden. HPLC and GLC–MS columns were from Shimadzu Corporation, Tokyo, Japan and Pierce Chemical Company, Rockford, USA. All the solvents and other chemicals used were of analytical grade.

2.2. Malting

Rice and ragi seeds were cleaned, steeped in double-distilled water for 16 h at 25 °C, and germinated under controlled conditions at 25 °C for 96 h in a B.O.D. incubator, as reported earlier (Nirmala, Subba Rao, & Muralikrishna, 2000). After germination, seeds were kilned at 50 °C for 24 h and powdered to obtain malted flour. Ungerminated seeds were powdered to obtain native flour.

2.3. Extraction and purification of feraxans

Water-soluble feraxans were obtained from native and malted rice and ragi flours, as described earlier (Rao and Muralikrishna, 2006). In brief (Fig. 1), flour was extracted with water (200 ml × 4 at 25 °C) and the supernatant obtained after centrifugation (3000*g* for 20 min) was precipitated with 3 volumes of ethanol. Precipitate was separated, dialyzed (~8 kDa cutoff) and lyophilized. This water-extractable non-starch polysaccharide (NSP) was further dissolved (10%, w/v) in water and the insoluble portion was separated by centrifugation. The soluble portion was heated (95 °C for 10 min) to denature enzymes and precipitate proteins. It was further centrifuged and the supernatant thus obtained was dialyzed and lyophilized to obtain water-soluble NSP.

Water-soluble NSP was fractionated on a DEAE-cellulose anion-exchange column by eluting successively with water, 0.1 and 0.2 molar ammonium carbonate (AC) and 0.1 and 0.2 M NaOH. The major (0.1 M AC-eluted) fraction (water-soluble feraxans) was purified on a Sephacryl S-300 column. The molecular mass of purified feraxans was determined on a Sephacryl S-300 column. T-Dextran (T-10, T-40, T-70, T-150, T-500 and T-2000 kDa) standards were used for obtaining molecular weight calibration

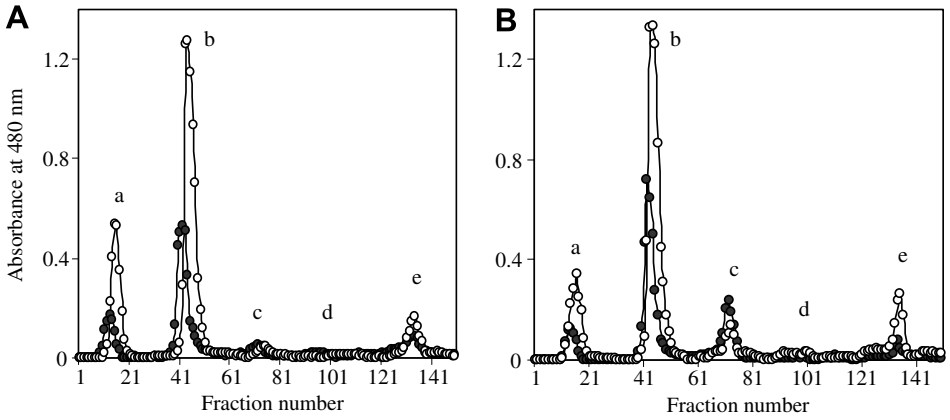

Fig. 1. Fractionation profile on DEAE-cellulose of water-soluble NSP from native (●) and malted (○) rice (A) and ragi (B): water-eluted fraction (a), 0.1 M ammonium carbonate-eluted fraction (b), 0.2 M ammonium carbonate eluted fraction (c), 0.1 M NaOH-eluted fraction (d) and 0.2 M NaOH-eluted fraction (e) (fraction size, 5 ml).

curve. Upon elution, fractions were dialyzed and lyophilized. The homogeneity of the feraxans was further ascertained by cellulose-acetate paper electrophoresis (Subba Rao & Muralikrishna, 2004), capillary electrophoresis (silica column, ϕ 75 μm × 100 cm; borate buffer, 0.5 molar, pH 8.3; column pressure 100 mbar; voltage 20 kV; detection at 253 nm) (Soga & Serwe, 2000) and high performance size exclusion chromatography (E-linear, ϕ 7.8 × 300 mm and E-1000, ϕ 3.9 × 300 mm column; water elution; RI detector) (Gruppen, Kormelink, & Voragen, 1993).

2.4. Analytical methods

Polysaccharides were acid-hydrolyzed and derivatized to determine the neutral sugar composition by GLC and ferulic acid was quantified by HPLC upon alkaline hydrolysis (Rao and Muralikrishna, 2004). Uronic acid content was quantified by the carbazole method (Dische, 1947). UV-absorption spectra of water-soluble feraxans were recorded from 200 to 400 nm, using a UV–Vis spectrophotometer (Shimadzu, Tokyo, Japan). Optical rotation of feraxans was determined at 20 °C using a Perkin–Elmer (model 243) polarimeter (Subba Rao and Muralikrishna, 2004). IR-spectra of feraxans in KBr pellets were recorded from 4000 to 400 cm^{-1} (4 cm^{-1} resolution), using a Perkin–Elmer 2000 GC-IR spectrometer (Norwalk, USA).

All the experimental values are averages of three independent experiments.

2.5. Methylation analysis

Purified polysaccharides (5 mg) were methylated by following the method of Hakomori (1964). Prior to methylation, carboxyl reduction of the polysaccharides (100 mg) was carried out (thrice) using carbodiimide and sodium borohydride for quantitative conversion of carboxyl group into primary alcohol (Subba Rao & Muralikrishna, 2004; Taylor & Conrad, 1972). Permethylated polysaccharides were hydrolyzed with formic acid and sulfuric acid successively, acetylated (Rao & Muralikrishna, 2004) and the resultant permethylated alditol acetates were analyzed by GLC–MS.

GLC–MS analysis was performed on a Shimadzu GC 17A QP-5000 system, using a SP 2330 capillary column (ϕ 0.31 mm × 30 m) operating at an ionization potential of 70 eV with a temperature programme (180–200 °C, 4 °C rise per min, 200 °C for 50 min). Mass range from 40 to 400 amu (m/e) was taken for analysis. Helium was used as the carrier gas.

2.6. Periodate oxidation – Smith degradation

Polysaccharide solution (10 mg in 5 ml water) was mixed with sodium meta periodate (5 ml, 20 mmolar) and kept at 4 °C in the dark for 48 h. Aliquots (0.5 ml) were withdrawn from the sample at regular intervals (4 h) and the amount of periodate remaining was determined by the 2,4,6,-tri-2-pyridyl-*S*-triazine (TPTZ) method (Avigad, 1969).

Formic acid liberated from the polysaccharide sample on oxidation was estimated by a titrimetric method using sodium hydroxide (Brown, Halsall, Hirst, & Jones, 1948).

Polysaccharide sample (10 mg in 5 ml water) was oxidized with sodium meta periodate (5 ml, 20 mM) at 4 °C in the dark for 48 h, treated with ethylene glycol (0.1 ml) and then reduced with sodium borohydride (100 mg) at room temperature for 16 h. The sample was dialyzed, hydrolyzed with sulfuric acid (0.5 N) at room temperature for 48 h, acetylated and the resultant Smith degradation products were analyzed by GLC–MS (Abdel-Akher, Hamilton, Montgomery, & Smith, 1952).

2.7. ^{13}C and ^{1}H NMR studies

Polysaccharide solution (100 mg/ml in D_2O) was placed in a sample probe (ϕ 5 mm × 15 cm) and the resonance spectrum was recorded in a Bruker AMX 400 MHz spectrometer operating at 60 °C for 4 h with a spectral width of 22,272 Hz and about 3000 scans. Deuterium resonance was used as a field frequency lock and the shifts were recorded with reference to external TMS (Hoffmann, Kamerling, & Vliegenthart, 1992).

3. Results and discussion

3.1. Characterization of feraxans

Non-starch polysaccharides were isolated from native and malted (96 h) rice and ragi as reported earlier (Rao & Muralikrishna, 2004). They were designated as water-extractable (WEP) and water-unextractable feraxans (WUP), based on their solubility in water. WUP contained 90% of bound phenolic acids and the rest (~10%) were associated with WEP. Bound ferulic acid was 16-(1426 μg/g of WUP) and 8-(1519 μg/g of WEP) fold higher in WUP of rice and ragi, respectively, than in their respective WEP's (209 μg/g of ragi WEP, 104 μg/g of rice WEP) as published earlier (Rao & Muralikrishna, 2004).

Water-soluble feraxans were fractionated by DEAE-cellulose ion-exchange column chromatography (Fig. 1), followed by gel filtration on Sephacryl S-300, resulting in eight feraxan fractions (Fig. 2). Homogeneity of these purified water-soluble feraxans was tested by different methods: (a) reloading each fraction obtained on Sephacryl S-300 on the same column (b) HPSEC (c) cellulose acetate paper electrophoresis (Figs not shown). Capillary electrophoresis of individual fractions indicated their homogeneity (Fig. 3) and accordingly this was used for structural characterization.

In the case of rice native feraxans (Table 1, Fig. 2A), average molecular weights are 231.5 kDa (peak 1, yield: ~65%), and 24.4 kDa (peak 2, yield: ~35%). Upon malting, average molecular weight of peak 1 decreased to 75.4 kDa (yield: ~50%) and that of peak 2 was slightly

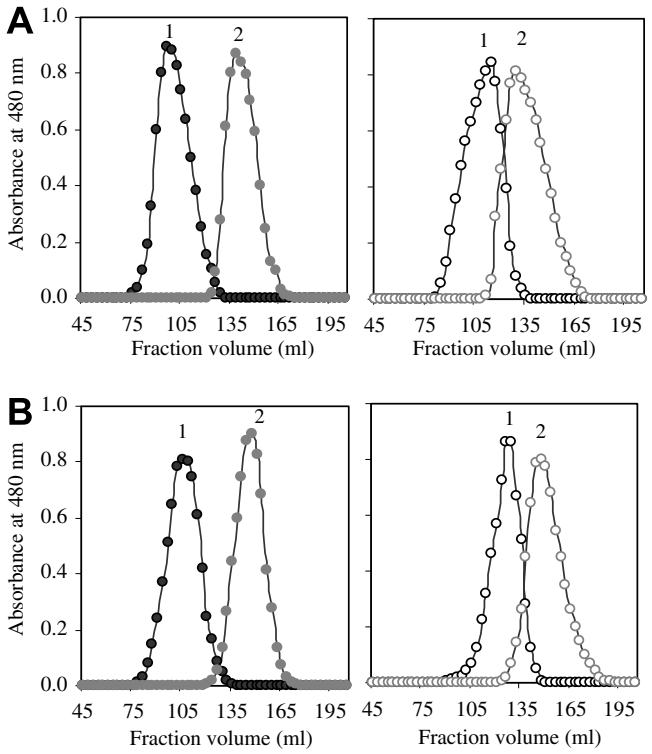

Fig. 2. Gel filtration profile on Sephacryl S-300 of individual feraxans from native (●) and malted (○) rice (A) and ragi (B). Peaks – 1 and 2.

increased to 39.6 kDa (yield: ~50%). Similarly, in ragi (Table 1), native feraxans have an average molecular weight of 139.9 kDa (peak 1, yield: ~65%) and 15.4 kDa (peak 2, yield: ~35%). Upon malting, average molecular weight of peak 1 decreased to 38.9 kDa (yield: ~35%) and that of peak 2 remained unchanged. However, its yield increased (~65%). These results show that malting causes many molecular changes in feraxans due to induced xylanase (Rao & Muralikrishna, 2006), which would act on large molecular weight feraxans, thus reducing their chain length (Table 1, Fig. 2). Water-soluble feraxans from both native and malted rice and ragi, are relatively small molecules compared with other arabinoxylans reported, especially from wheat (Dervilly-Pinel et al., 2004). The presence of a high amount of uronic acid (Table 1) seems to be a characteristic of water-soluble feraxans, especially ragi (Rao & Muralikrishna, 2006). Feraxans contained high amounts of bound ferulic acid (Table 1). The UV-absorption spectrum of feraxans showed a characteristic pattern with maximum absorption at around 320 nm, very similar to the spectrum obtained for trans ferulic acid. Interestingly, malt feraxans showed a high UV absorption compared to native (Rao & Muralikrishna, 2006), indicating a higher amount of bound ferulic acid. These spectra are also very similar to the spectra obtained for feruloyl oligosaccharides from wheat (Ralet, Faulds, Williamson, & Thibault, 1994).

The neutral sugar composition analysis showed chiefly, arabinose and xylose (Table 2). However, a considerable amount of galactose was also observed, which might be a part of the arabinoxylans as arabinoxylans are known to contain galactose (Izydorczyk & Biliaderis, 1995). Although a small amount of glucose is an inherent feature of arabinoxylans, feraxans, especially from rice contained very little glucose (Shyamprasad Rao & Muralikrishna, 2006).

As these purified water-soluble feraxans are found to contain high amounts of uronic acid (around 8–13%), carboxyl reduction was carried out prior to methylation using carbodiimide. Reduced feraxans contained about 2–3% uronic acid (rice 1.8, 1.5, 2.2 and 2.3; ragi 2.0%, 2.9%, 2.5% and 2.8% for peak 1 and 2 from native and malt

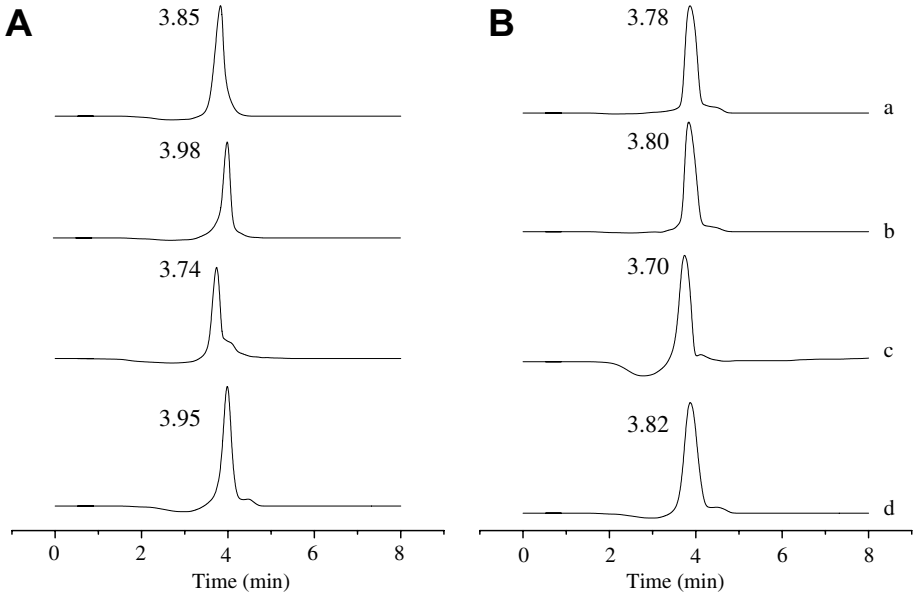

Fig. 3. Capillary electrophoresis profile of rice (A) and ragi (B) feraxans: NP1 (a), NP2 (b), MP1 (c) and MP2 (d).

Table 1
Yield, molecular weight, ferulic acid, uronic acid and specific rotation of purified water-soluble feraxans obtained from native and malted rice and ragi

		Yield (%)[a]	Molecular weight (kDa)	Ferulic acid (μg/g)	Uronic acid (%)	Specific rotation $[a]_D$
Rice						
N	P1	65.0	231.5	130.9	8.0	−5.1
	P2	35.0	24.4	78.5	8.3	−7.4
M	P1	50.0	75.4	1388.2	9.5	−5.9
	P2	50.0	39.6	1471.6	8.7	−5.9
	P1	60.5	139.9	161.4	9.0	−1.6
	P2	34.7	15.4	54.0	13.4	−0.3
M	P1	33.5	38.9	843.8	8.2	−2.3
	P2	64.6	15.4	949.9	12.9	−0.8

[a] Percentage of sample loaded on Sephacryl S-300, N, native; M, Malt; P, Peak.

Table 2
Neutral sugar composition (%) of purified water-soluble feraxans obtained from native and malted rice and ragi

		Rha	Ara	Xyl	Man	Gal	Glc	Ara/Xyl	P/H
Rice									
N	P1	0.4	42.3	48.5	0.0	8.8	0.0	0.87	10.3
	P2	0.0	40.5	50.3	1.3	7.9	0.0	0.81	9.9
M	P1	0.5	45.4	47.6	0.0	6.5	0.0	0.95	14.3
	P2	0.5	43.4	48.1	0.0	8.0	0.0	0.90	11.4
Ragi									
N	P1	0.0	39.7	49.3	1.8	9.2	0.0	0.81	8.1
	P2	1.3	40.1	45.6	1.5	7.0	4.5	0.88	6.6
M	P1	0.7	46.6	44.2	0.0	8.5	0.0	1.05	10.7
	P2	1.0	43.5	43.7	0.0	8.2	3.6	1.00	7.4

Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; P/H, pentose/hexose.

feraxans, respectively). High amounts of uronic acid present in arabinoxylans of rice bran (Shibuya & Iwasaki, 1985), sorghum endosperm cell walls (Verbruggen, Beldman, & Voragen, 1995) and ragi (Subba Rao & Muralikrishna, 2004) were reduced prior to methylation study for the quantitative conversion of uronyl group to primary alcohol.

3.2. Methylation analysis

The interglycosidic linkages between monosaccharide residues of eight purified feraxans were investigated by methylation analysis. At least nine species of permethylated alditol acetates were identified, based on retention time and mass spectral patterns, and their molar ratios are given in Table 3. Arabinose is chiefly terminally linked, however, mono and di-substituted arabinose residues were also detected (~30%), similar to those reported in maize (Saulnier, Vigouroux, & Thibault, 1995), sorghum (Woolard, Rathbone, & Novellie, 1976) rye (Vinkx, Stevens, Gruppen, Grobet, & Delcour, 1995) and ragi (Subba Rao & Muralikrishna, 2004) arabinoxylans. These residues might be present as short side-chains on the xylan backbone and they also provided a site for the covalent attachment of ferulic acid, the major bound phenolic acid in feraxans (Rao & Muralikrishna, 2006).

On the other hand, mono (2/3), di and un-substituted xylose were detected in almost equal amounts (Table 3). A small amount of trimethylated xylose might have originated as a result of terminally linked xylose or end-residue. Arabinose residues are linked to xylose at O-3 or O-2 or both at O-2 and O-3, as indicated by similar amounts of mono and di-substituted xylose residues. Galactose and glucuronic acid (as glucose upon carboxyl reduction) (Bergmans, Beldman, Gruppen, & Voragen, 1996; Shibuya & Iwasaki, 1985) are chiefly terminally linked to the xylan backbone.

Although xylose residues are predominantly mono (3) substituted in maize (Saulnier et al., 1995), sorghum (Woolard et al., 1976) rye (Vinkx et al., 1995) and ragi (Subba Rao & Muralikrishna, 2004), water-insoluble arabinoxylans and water-soluble feraxans from rice and ragi, especially from malts, showed high amounts of disubstituted xylose residues – almost equalling the mono and unsubstituted xylose residues. Similar to this, a heteroxylan isolated from wheat kernel pericarp was shown to be a highly substituted glucuronoarabinoxylan in which 80% of the xylose residues are mono or di-substituted (Brillouet & Joseleau, 1987). These results are contrary to the reports on wheat (Cleemput et al., 1995; Izydorczyk & Biliaderis,

Table 3
Methylation analysis (peak area, mol%) of purified water-soluble feraxans obtained from native and malted rice and ragi

O-Methyl ether	Linkage	Rice				Ragi			
		NP1	NP2	MP1	MP2	NP1	NP2	MP1	MP2
2,3,5-Ara	Terminal	25.9	27.5	29.0	28.9	25.4	29.1	31.5	29.2
2,3-Ara	1,5	4.9	4.2	6.1	4.8	5.6	4.3	6.7	7.1
2-Ara	1,3,5	8.5	5.7	6.4	6.4	7.2	5.5	5.2	2.1
2,3,4-Xyl	Terminal	1.1	1.3	1.4	1.3	1.8	1.9	1.4	1.3
2,3-Xyl	1,4	13.5	18.7	18.4	18.0	18.9	15.5	13.5	13.2
2/3-Xyl	1,3/2,4	22.8	17.6	9.2	12.5	15.6	16.3	11.7	12.2
Xyl	1,2,3,4	7.5	9.3	14.6	12.8	9.8	9.5	14.1	12.6
2,3,4,6-Gal	Terminal	7.0	6.3	5.5	6.5	7.1	5.4	6.9	6.0
2,3,4,6-Glc	Terminal	5.0	5.4	5.9	5.1	5.3	8.2	4.6	9.8
2,3-Xyl/(2(3)-Xyl + Xyl)		0.45	0.70	0.77	0.71	0.74	0.60	0.52	0.53
Xyl/2(3)-Xyl		0.33	0.53	1.59	1.02	0.63	0.58	1.21	1.03

1995; Shiiba, Yamada, Hara, Okada, & Nagao, 1993) and barley (Han, 2000) arabinoxylans, which are very much less branched, as indicated by high amounts of unsubstituted xylose.

GLC–MS analysis of the carboxyl-reduced feraxans, upon acid hydrolysis and acetylation, showed the presence of 4-O-Me-glucose (diagnostic MS fragments – 129, 189 and 217), indicating uronic acid to be in the 4-O-Me-glucuronate form, similar to the earlier report on ragi (Subba Rao & Muralikrishna, 2004). Cereal arabinoxylans are known to contain very high amounts of uronic acid in the 4-O-Me-glucuronate form (Saulnier et al., 1995; Shibuya & Iwasaki, 1985).

3.3. Periodate oxidation – Smith degradation

Eight purified feraxans consumed from 4.02 to 4.30 μmol of periodate per mg of polysaccharide, indicating that about 60–65% of sugars have adjacent free hydroxyl groups. Periodate consumption was high initially (5 h) but reached a plateau after 24 h (figure not shown). Periodate consumption by malt feraxans was slightly lower, indicating their higher branched nature, which is also evident from their higher arabinose content (Table 2). Similar to this, highly branched glucuronoarabinoxylans obtained from sorghum husk were found to consume about 0.64 mol of periodate over 27 h of oxidation (Woolard et al., 1976). In a recent study, Dervilly-Pinel et al. (2004) showed almost equal consumption (4.27 and 4.11 μmol/mg AX) of periodate by two arabinoxylan populations with different levels of substitution (Ara/Xyl = 0.38 and 0.82), wherein di-substitution increased with a higher Ara/Xyl ratio.

There was no detectable level of formic acid in the reaction mixture. This indicated absence/low amount of three consecutive hydroxyl groups in the sugars. This also suggested that the high amounts of uronic acid (~10%) present in the feraxans are chiefly in the 4-O-methyl form, which is substantiated by GLC–MS analysis. Galactose/glucose residues present in short side-chains (as evident by the presence of trace amounts of 2,3,6-Me_3-galactose/glucose) further reduced three contiguous –OH groups and thus oxidation.

Glycerol and xylose were the major Smith degradation products identified (Table 4). Similar to this study, Smith degradation analysis of the glucuronoarabinoxylans from sorghum husk showed a high amount of glycerol and mild acid hydrolysis yielded many oligosaccharides with different xylose values (Woolard et al., 1976). Based on periodate oxidation and Smith degradation study of wheat water-unextractable arabinoxylans, Gruppen et al. (1993) reported that most of the branched residues were present as isolated units of blocks of two contiguous substituted xylose residues. Here, Smith degradation analysis of malt feraxans showed a high amount of xylose, indicating its high substitution.

Table 4
Analysis of Smith degradation products (%) obtained for purified water-soluble feraxans

		Glycerol	Ara	Xyl	Ara/Xyl
Rice					
N	P1	63.1	10.5	26.4	0.40
	P2	59.1	3.7	37.2	0.10
M	P1	58.9	2.5	38.6	0.07
	P2	61.5	2.1	36.4	0.06
Ragi					
N	P1	55.6	7.6	36.8	0.21
	P2	54.5	7.4	38.1	0.19
M	P1	48.1	2.1	49.8	0.04
	P2	50.0	3.7	46.3	0.08

3.4. ^{13}C and ^{1}H NMR studies

The ^{13}C NMR spectra obtained for eight purified feraxans are very much alike (Fig. 4) and similar to the spectra of other cereal arabinoxylans (Izydorczyk & Biliaderis, 1995; Subba Rao & Muralikrishna, 2004). They showed distinguishable clusters of signals (Hoffmann et al., 1992). The chemical shift values of the signals for anomeric carbon of Ara_f ($\delta = 108.8$–110.7 ppm) and Xyl_p ($\delta = 102.6$–104.7 ppm) indicated that Ara_f had α and Xyl_p had β configuration (Bock & Pedersen, 1983; Hoffmann et al., 1992). C-1 signals for mono and di-substituted xylose residues might be observed at around 104.7 and 102.6 ppm, respectively. Signals were seen for Ara_f C-2, C-4 and C-5 (ring carbon atoms) at around 82.6, 85.2 and 62.3 ppm, respectively (Table 5). However, signal intensities could not clearly be assigned to the relative abundance of the different residues. Otherwise, signal intensities of these 'structural-reporter-group' regions could give information regarding the relative abundance of mono and di-substituted xylose (Hoffmann et al., 1992).

Signals observed at around 98.8 and 72.1 ppm could be assigned to the C-1 and C-3 of α-D-glucuronic acid (4-O-Me), respectively. Low intensity signals at around 59.5 and 18.0 ppm might arise from –O–CH_3 of 4-O-Me-α-D-glucuronic acid (Brillouet & Joseleau, 1987). A low intensity signal for the >C=O (C-6 carbonyl group) of 4-O-Me-α-D-glucuronic acid was detected at around 178.0 ppm. Similar observations have been made with the ^{13}C NMR spectra obtained for ragi arabinoxylans (Subba Rao & Muralikrishna, 2004). ^{13}C NMR spectra of feraxans did not show prominent signals that could be assigned to bound ferulic acid. However, signals seen at around 178.0 and 59.5 ppm may be assigned to ferulic acid.

In ^{1}H NMR spectra (Fig. 5a and b), a peak at around δ 5.47 ppm represented the anomeric proton of arabinose linked to the O-3 position of xylose residues, while the two peaks at around δ 5.34 and δ 5.18 ppm are from anomeric protons of arabinose residues linked to O-2 and O-3 of the same xylose residue. The unresolved signals or shoulders downstream of the peaks at δ 5.34 and δ 5.18 ppm

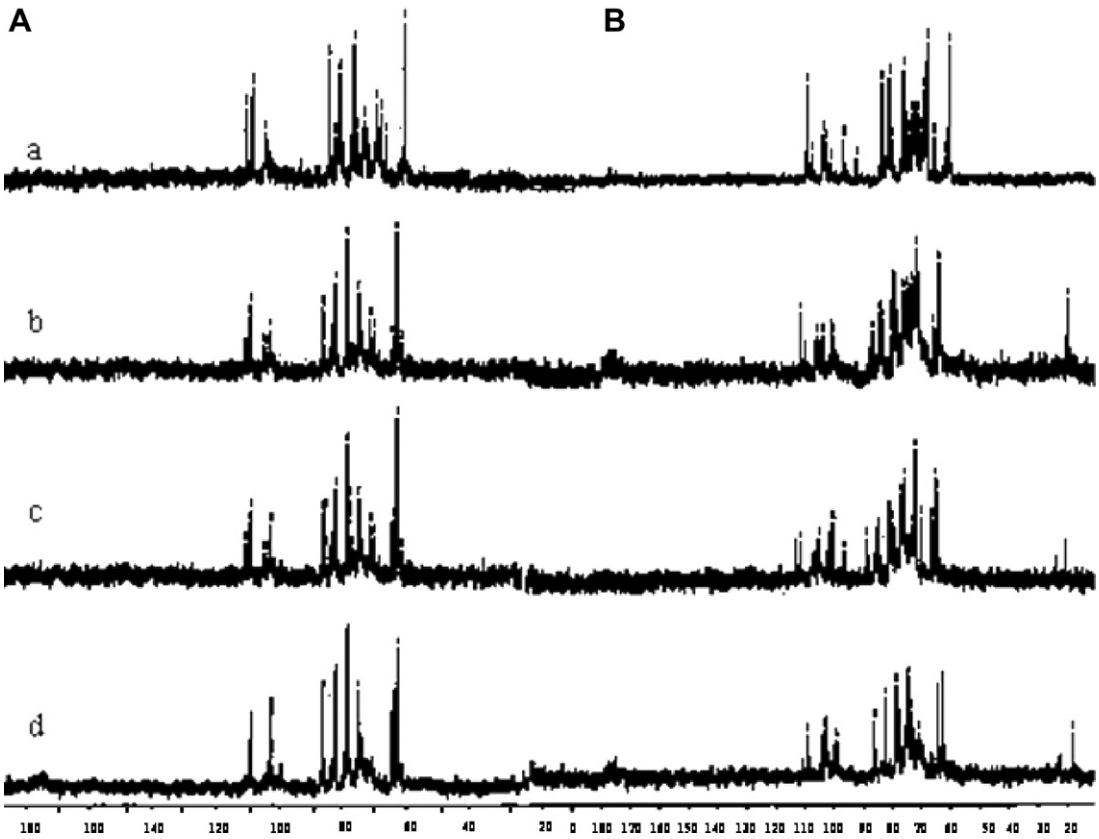

Fig. 4. ^{13}C NMR spectra of water-soluble feraxans obtained from native and malted rice (A) and ragi (B) feraxans: NP1 (a), NP2 (b), MP1 (c) and MP2 (d).

resulted from two neighbouring di-substituted xylose residues in the arabinoxylan chain (Hoffmann et al., 1992; Vinkx et al., 1995), which indicated that the feraxans contained both isolated and paired di-substituted xylose residues, similar to other arabinoxylans, especially from wheat (Cleemput et al., 1995) and barley (Trogh, Courtin, and Delcour, 2004). The presence of an unresolved signal or shoulder downstream of the peak at around δ 5.47 ppm represented the presence of O-3 mono-substituted xylose next to di-substituted xylose (Hoffmann et al., 1992). The content of O-2 mono-substituted xylose is estimated as the difference between the integrals of the two peaks of arabinose residues linked to di-substituted xylose (Oscarsson, Andersson, Salomonsson, and Aman, 1996; Vinkx et al., 1995).

The proportions of un-mono-(O-2 and O-3) and di-substituted xylose in the purified feraxans are given in Table 6. The amount of di-substituted xylose is quite high and ranged from 17.6% to 38.2%. This value is comparably higher than the di-substitution level observed for other arabinoxylans, especially from barley (~24%) (Oscarsson et al., 1996; Trogh et al., 2004). While the un-substituted xylose residues remained, overall, the same levels of di-substituted xylose residues were comparably higher for malt feraxans as was evident from their higher ratio of di/mono-substitution (Table 6).

Table 5
Assignments[a] of ^{13}C NMR signals (chemical shifts, ppm) obtained for feraxans from rice and ragi

Residue	Chemical shifts (ppm)				
	C-1	C-2	C-3	C-4	C-5
β-D-Xyl_p	104.7	73.2	74.8	77.0	64.1
β-D-Xyl_p-(adj)	104.0				64.1
Element A					
β-D-Xyl_p	102.6	73.1			63.8
α-L-Ara_f-(1 → 2)	110.5	82.7		85.2	62.3
α-L-Ara_f-(1 → 3)	108.8	81.6		85.2	62.3
Element B					
β-D-Xyl_p	104.7	73.8	78.6	74.3	63.8
α-L-Ara_f-(1 → 3)	108.8	81.6	78.6	85.2	62.7

Element A = →4)[α-L-Ara_f-(1 → 2)][α-L-Ara_f-(1 → 3)]-β-D-Xyl_p(1 →
Element B = →4)[α-L-Ara_f-(1 → 3)]-β-D-Xyl_p(1→
β-D-Xyl_p = → 4)-β-D-Xyl_p(1 →
β-D-Xyl_p-(adj)= → 4)-β-D-Xyl_p(1→ adjoining element A and element B at the non-reducing end).

[a] Assignments are based on Hoffmann et al. (1992) and references therein.

The four structural elements in the xylan backbone, namely, un-mono-(O-2), mono-(O-3) and di-substituted xylose are correlated with the Ara/Xyl ratio and results are shown in Fig. 7A. It is observed that overall levels of un- and mono-(O-2) substituted xylose residues remained

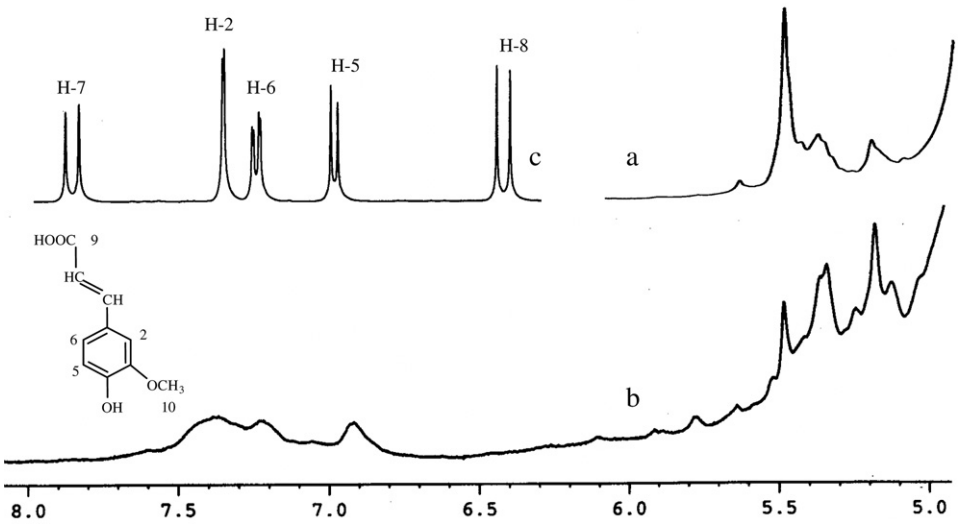

Fig. 5. ^{1}H NMR spectra (anomeric signals of arabinose) of water-soluble feraxans obtained from native (P2) (a) and malted (P2) (b) rice. MP2 spectrum shows signals arising from bound ferulic acid. Spectrum for ferulic acid is shown (c).

Table 6
Substitution pattern of xylose in feruloyl arabinoxylans

		u-xyl	2-xyl	3-xyl	2,3-xyl	Di/mono	Un/substituted
Rice							
N	P1	30.6	13.9	37.9	17.6	0.34	0.44
	P2	40.4	3.7	34.5	21.4	0.56	0.68
M	P1	41.8	7.0	14.5	36.7	1.71	0.72
	P2	41.1	11.6	16.2	31.1	1.12	0.70
Ragi							
N	P1	42.2	11.5	23.1	23.2	0.67	0.73
	P2	36.7	5.3	33.3	24.7	0.64	0.67
M	P1	33.1	10.3	18.4	38.2	1.33	0.50
	P2	34.2	5.3	26.3	34.2	1.08	0.52

u-xyl, unsubstituted xylose; 2-xyl, 2-O-substituted xylose; 3-xyl, 3-O-substituted xylose; 2,3-xyl, 2,3-O-substituted xylose.

constant with increasing Ara/Xyl ratio. However, the level of mono-(O-3) substituted xylose residues decreased and di-substitution increased with increase in the Ara/Xyl ratio. Similar relationships were reported previously for wheat and rye water-extractable arabinoxylans (Cyran, Courtin, & Delcour, 2003; Dervilly, Saulnier, Roger, & Thibault, 2000; Dervilly-Pinel et al., 2004; Vinkx et al., 1995). Since malt feraxans have higher Ara/Xyl ratios, their di-substitution level is higher, to accommodate the extra arabinose without much change in the level of un-substituted xylose.

The relationships: Ara/Xyl ratio, un/substituted xylose and di/mono-substituted xylose with those of molecular weight of feraxans are plotted and the results are shown in Fig. 7B. It is clear that the Ara/Xyl ratio and di-substitution decreased with increasing molecular weight of the feraxans, whereas the un-substitution level remained steady or slightly increased. A similar trend was observed with the literature data for barley (Cyran et al., 2003) and wheat (Dervilly-Pinel et al., 2004) arabinoxylans (figures not shown). Arabinoxylan fractions obtained with increased concentrations of ethanol/ammonium sulphate were observed to have higher Ara/Xyl ratios and lower molecular weights (Izydorczyk & Biliaderis, 1995). It may be noted that graded precipitation of arabinoxylans takes place by virtue of hydrophobic interactions, which in turn are governed by the molecular weight and Ara/Xyl ratio. Arabinoxylan precipitates when either the molecular weight is higher or the Ara/Xyl ratio is lower than the general pool.

The ^{1}H NMR spectrum of rice feraxan (MP2) (Fig. 5b) showed signals at around δ 6–8 ppm, which may be assigned to bound ferulic acid (Fig. 5c) (Cyran et al., 2003).

In IR spectra of feraxans (Fig. 6), signals observed at around 1417.0 and 2930.0 cm^{-1} are due to $-CH_2$ and –CH stretching vibrations, respectively, and the signal observed at around 3365.0 cm^{-1} is due to –OH stretching vibrations of polysaccharide, and water involved in hydrogen bonding (Kacurakova, Belton, Wilson, Hirsch, & Ebringerova, 1998). The signal at around 1415.0 cm^{-1} is due to C–C, C–O and C–O–H bending vibrations. Signals at this region are known to show variations, depending on the amount of substitution at the O-2 and O-3 positions. The intensity of signals in this region decreases (coupled with the loss of peak multiplicity) with increased substitution (Kacurakova et al., 1998). A peak at around 1728.6 cm^{-1} corresponds to the signal from the $\gtrdot$C=O group of the uronic acid residue.

Optical rotation values of purified feraxans obtained from native and malted rice and ragi ranged from −0.3 to −7.4 (Table 1). The negative value indicates that the polymer is primarily β-linked. However, this value is low compared to the high negative values of other arabinoxylans (Saavendra, Karacsonyi, & Alfoldi, 1988; Subba Rao & Muralikrishna, 2004), which may be partly because feraxans contain more α linkages, due to their high arabinose, galactose and uronic

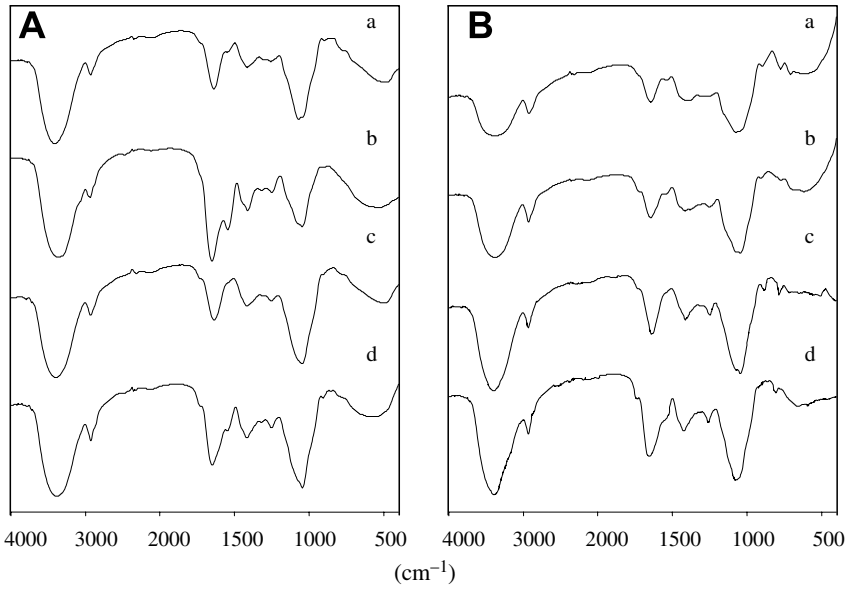

Fig. 6. Infrared spectra of water-soluble feraxans from native and malted rice (A) and ragi (B). NP1 (a), NP2 (b), MP1 (c) and MP2 (d).

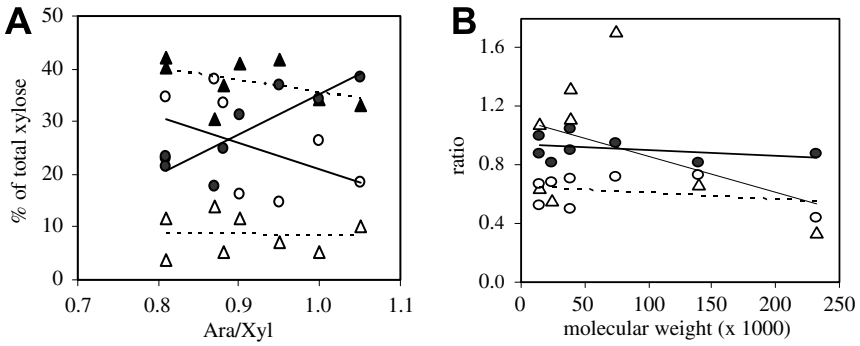

Fig. 7. Relationship between the relative proportion of differently linked xylose residues (unsubstituted, ▲; O-2 substituted, △; O-3 substituted, ○ and O-2, 3 substituted, ●) and the ratio of A/X for water-soluble feraxans obtained from native and malted rice and ragi (combined) (A). Relationship of molecular weight with ratios of A/X (●), un-substituted/substituted xylose (○) and di/mono-substituted xylose (Δ) in water-soluble feraxans obtained from native and malted rice and ragi (combined) (B).

acid contents. On the other hand, primarily α-linked polymers are known to have high positive optical rotation values (Saavendra et al., 1988).

3.5. Structural model for water-soluble feraxans

A model is presented depicting the structural characteristics of water-soluble arabinoxylans from native and malted rice and ragi (Fig. 8A). These feraxans are of low molecular weight compared to many other cereal arabinoxylans (Izydorczyk and Biliaderis, 1995) and have higher arabinose contents (nearly equal to xylose). These two factors made them particularly water-soluble. Feraxans also contained high amounts of galactose and uronic acid, whose content is slightly higher in malts. They also contained high amounts of bound ferulic acid, which is several-fold higher in malts.

Due to their low branching/substitution, native feraxans may contain 2 or more contiguous un-substituted xylose residues, which perhaps serve as the easy access point for the cleavage of native feraxans by xylanase (Rao and Muralikrishna, 2006). On the other hand, although malt feraxans are of low molecular weight, since they are highly branched, their further degradation might require synergistic action of xylanolytic enzymes. It is interesting to note that malt feraxans contained very high amounts of ferulic acid. This is probably made possible through the partial degradation of very high molecular weight feruloyl arabinoxylans, which might be otherwise insoluble in water. Xylanase may preferentially cleave the high molecular weight feraxans in the un-substituted or mono/low substituted regions. This leads to the formation of water-soluble, highly feruloylated small molecular weight arabinoxylans (feraxans) during malting (Fig. 8B). It also leads to overall increase in the level of arabinose substitution and/or di-substitution. With their high substitution levels, rice and ragi water-soluble feraxans were structurally more similar to rye (Bengtsson, Andersson, Westerlund, and Aman,

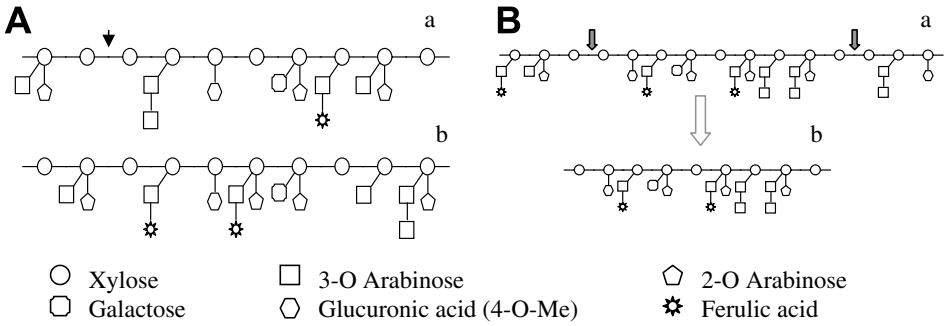

Fig. 8. Possible structural models for feraxans obtained from native (a) and malted (b) rice and ragi. Native feraxan is less branched and has easy access point for xylanase (arrowhead) (A). Partial biodegradation of high molecular feruloyl arabinoxylan [having easy access points for xylanase (small arrows)] (a) leading to highly feruloylated low molecular weight arabinoxylan (b) with higher arabinose:xylose ratio (B).

1992) and maize (Saulnier et al., 1995) arabinoxylans than to wheat arabinoxylans (Izydorczyk and Biliaderis, 1995).

4. Conclusions

In summary, water-soluble feraxans from rice and ragi are shown to be low molecular weight polysaccharides with high amounts of arabinose, galactose, uronic acid and ferulic acid. Malting led to decreased molecular weight (rice: 232 kDa to 75.4 kDa; ragi: 140 kDa to 38.9 kDa) but increased ferulic acid content (rice: 131 μg/g to 1472 μg/g; ragi: 161 μg/g to 950 μg/g) of feraxans, due to the action of xylanase. Their high branching is also characterized by high O-2 and di-substitution and resembled the highly branched rye and maize arabinoxylans. Malting is shown to bring about dynamic changes in the structural features of these water-soluble feraxans.

Acknowledgements

We thank Dr. V. Prakash, Director, CFTRI, Mysore, for his keen interest in the work, and encouragement. We thank the Chairperson, Sophisticated Instruments Facilities, IISc, and Bangalore for providing NMR facilities. R.S.P.R. thank the Council of Scientific and Industrial Research (CSIR), New Delhi, for the grant of a Junior/Senior Research Fellowship.

References

Abdel-Akher, M., Hamilton, J. K., Montgomery, R., & Smith, F. (1952). A new procedure for the determination of the fine structure of polysaccharides. *Journal of American Chemical Society, 74*, 4970–4971.

Avigad, G. (1969). Rapid, sensitive determination of periodate. *Carbohydrate Research, 11*, 119–123.

Bengtsson, S., Andersson, R., Westerlund, E., & Aman, P. (1992). Content, structure and viscosity of soluble arabinoxylans in rye grain from several countries. *Journal of the Science of Food and Agriculture, 58*, 331–337.

Bergmans, M. E. F., Beldman, G., Gruppen, H., & Voragen, A. G. J. (1996). Optimization of the selective extraction of (glucurono) arabinoxylans from wheat bran: use of barium and calcium hydroxide solution at elevated temperatures. *Journal of Cereal Science, 23*, 235–245.

Bock, K., & Pedersen, C. (1983). Carbon-13 nuclear magnetic resonance spectroscopy of monosaccharides. *Advances in Carbohydrate Chemistry and Biochemistry, 41*, 27–66.

Brillouet, J.-M., & Joseleau, J.-P. (1987). Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of wheat kernel. *Carbohydrate Research, 159*, 109–126.

Brown, T., Halsall, T. G., Hirst, E. L., & Jones, J. K. N. (1948). The structure of starch. The ratio of non-terminal to terminal groups. *Journal of Chemical Society, Part I*, 27–32.

Charalampopoulos, D., Wang, R., Pandiella, S. S., & Webb, C. (2002). Application of cereals and cereal components in functional foods: a review. *International Journal Food Microbiology, 79*, 131–141.

Cleemput, G., van Oort, M., Hessing, M., Bergmans, M. E. F., Gruppen, H., Grobet, P. J., et al. (1995). Variation in the degree of D-xylose substitution in arabinoxylans extracted from a European wheat flour. *Journal of Cereal Science, 22*, 73–84.

Cyran, M., Courtin, C. M., & Delcour, J. A. (2003). Structural features of arabinoxylans extracted with water at different temperatures from two rye flours of diverse bread making quality. *Journal of Agricultural and Food Chemistry, 51*, 4404–4416.

Dervilly, G., Saulnier, L., Roger, P., & Thibault, J. F. (2000). Isolation of Homogeneous fractions from wheat water-soluble arabinoxylans. Influence of the structure in their macromolecular characteristics. *Journal of Agricultural and Food Chemistry, 48*, 270–278.

Dervilly-Pinel, G., Tran, V., & Saulnier, L. (2004). Investigation of the distribution of arabinose residues on the xylan backbone of water-soluble arabinoxylans from wheat flour. *Carbohydrate Polymers, 55*, 171–177.

Dische, Z. (1947). A new specific color reaction of hexuronic acids. *Journal of Biological Chemistry, 167*, 189.

Ghoneum, M., & Gollapudi, S. (2003). Modified arabinoxylan rice bran (MGN-3/Biobran) sensitizes human T cell leukemia cells to death receptor (CD95)-induced apoptosis. *Cancer Letters, 201*, 41–49.

Gruppen, H., Kormelink, F. J. M., & Voragen, A. G. J. (1993). Water-unextractable cell wall material from wheat flour. 3. A structural model for arabinoxylans. *Journal of Cereal Science, 18*, 111–128.

Hakomori, S. (1964). A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry (Tokyo), 55*, 205–208.

Han, J.-Y. (2000). Structural characteristics of arabinoxylan in barley, malt and beer. *Food Chemistry, 70*, 131–138.

Hoffmann, R. A., Kamerling, J. P., & Vliegenthart, J. F. G. (1992). Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydrate Research, 226*, 303–311.

Ishii, T. (1997). Structure and functions of feruloylated polysaccharides. *Plant Science, 127*, 111–127.

Izydorczyk, M. S., & Biliaderis, C. G. (1995). Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydrate Polymers, 28*, 33–48.

Kacurakova, M., Belton, P. S., Wilson, R. H., Hirsch, J., & Ebringerova, A. (1998). Hydration properties of xylans-type structures: an FTIR study of xylooligosaccharides. *Journal of the Science of Food and Agriculture, 77*, 38–44.

Morris, J. N., Marr, J. W., & Clayton, D. G. (1977). Diet and heart: a post-script. *British Medical Journal, 2*, 1307–1314.

Nirmala, M., Subba Rao, M. V. S. S. T., & Muralikrishna, G. (2000). Carbohydrates and their degrading enzymes from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf-15). *Food Chemistry, 69*, 175–180.

Oscarsson, M., Andersson, R., Salomonsson, A.-C., & Aman, P. (1996). Chemical composition of barley samples focusing on dietary fibre components. *Journal of Cereal Science, 24*, 161–170.

Plaami, S. P. (1997). Content of dietary fibre in foods and its physiological effects. *Food Reviews International, 13*, 29–76.

Ralet, M.-C., Faulds, C. B., Williamson, G., & Thibault, J.-F. (1994). Degradation of feruloylated oligosaccharides from sugar beet pulp and wheat bran by ferulic acid esterases from *Aspergillus niger*. *Carbohydrate Research, 263*, 257–269.

Rao, R. S. P., & Muralikrishna, G. (2004). Non-starch polysaccharide-phenolic acid complexes from native and germinated cereals and millet. *Food Chemistry, 84*, 527–531.

Rao, R. S. P., & Muralikrishna, G. (2006). Water soluble feruloyl arabinoxylans from rice and ragi: changes upon malting and their consequence on antioxidant activity. *Phytochemistry, 67*, 91–99.

Saavendra, F., Karacsonyi, S., & Alfoldi, J. (1988). Studies on the polysaccharides of sugar cane (*Saccharum officinarum*): structural features of the water-insoluble D-xylans. *Carbohydrate Research, 180*, 61–71.

Saulnier, L., Vigouroux, J., & Thibault, J.-F. (1995). Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydrate Research, 272*, 241–253.

Shibuya, N., & Iwasaki, T. (1985). Structural features of rice bran hemicellulose. *Phytochemistry, 24*, 285–289.

Shiiba, K., Yamada, H., Hara, H., Okada, K., & Nagao, S. (1993). Purification and characterization of two arabinoxylans from whet bran. *Cereal Chemistry, 70*, 209–214.

Soga, T., & Serwe, M. (2000). Determination of carbohydrates in food samples by capillary electrophoresis with indirect UV detection. *Food Chemistry, 69*, 339–344.

Subba Rao, M. V. S. S. T., & Muralikrishna, G. (2004). Structural analysis of arabinoxylans isolated from native and malted finger millet (*Eleusine coracana*, ragi). *Carbohydrate Research, 339*, 2457–2463.

Taylor, R. L., & Conrad, H. E. (1972). Stoichiometric depolymerization of poly-uronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry, USA, 11*, 1383–1388.

Trogh, I., Courtin, C. M., & Delcour, J. A. (2004). Isolation and characterization of water-extractable arabinoxylan from hull-less barley flours. *Cereal Chemistry, 81*, 576–581.

Verbruggen, M. A., Beldman, G., & Voragen, A. G. J. (1995). The selective extraction of glucuronoarabinoxylans from sorghum endosperm cell walls using barium and potassium hydroxide solution. *Journal of Cereal Science, 21*, 271–282.

Vinkx, C. J. A., Stevens, I., Gruppen, H., Grobet, P. J., & Delcour, J. A. (1995). Physicochemical and functional properties of rye non-starch polysaccharides. VI. Variability in the structure of water-unextractable arabinoxylans. *Cereal Chemistry, 72*, 411–418.

Willett, W. C. (1994). Diet and health: what should we eat? *Science, 264*, 532–537.

Woolard, G. R., Rathbone, E. B., & Novellie, L. (1976). Studies on a glucuronoarabinoxylan from the husk of sorghum grain. *Carbohydrate Research, 51*, 239–247.