JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Hemicelluloses of Ragi (Finger Millet, *Eleusine coracana*, Indaf-15): Isolation and Purification of an Alkali-Extractable Arabinoxylan from Native and Malted Hemicellulose B

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Hemicelluloses (A and B) were isolated from an Indo-African hybrid variety of finger millet (ragi, *Eleusine coracana*) by extracting the starch-free residue with 10% sodium hydroxide under a continuous stream of nitrogen, and changes in their sugar composition during malting for 96 h were studied. Hemicellulose B, obtained in higher yield from both native (N) and malted (M) flours, was found to be completely soluble in water, richer in uronic acid, and more viscogenic than hemicelullose A. Fractional precipitation of hemicellulose B by ammonium sulfate resulted in four precipitable fractions (F-60, F-70, F-80, and F-100) and a nonprecipitable (NP) fraction varying in their yield and arabinose, xylose, galactose, and glucose contents. A progressive increase in the pentose-to-hexose ratio (P:H) from 0.42:1.0 in F-60 to 1.94:1.0 in NP was observed in native hemicellulose B fractions; however, in malted hemicellulose B the P:H ratio increased from 0.43:1.0 in F-60 to 1.56:1.0 in F-80 and then decreased to 1.13:1.0 in NP. The major fraction, F-70 (N, 44.5%; M, 38.5%), was separated into eight subfractions on DEAE-cellulose by successive elution with water, ammonium carbonate (AC) (0.1, 0.2, and 0.3 M AC), and sodium hydroxide (0.1 and 0.2 M) differing in their yield and neutral sugar composition. The purity of the major glucuronoarabinoxylan fraction (0.1 M AC eluted) was ascertained by Sepharose CL-4B, HPSEC, cellulose acetate, and capillary electrophoresis methods. A significant decrease in the molecular mass of arabinoxylan from 1200 to 1120 kDa upon malting for 96 h is an indication of cell wall degradation by the inducible cell wall degrading enzymes.

KEYWORDS: Arabinoxylans; malting; finger millet; hemicellulose

INTRODUCTION

Hemicelluloses are alkali-extractable polysaccharides present in close association with cellulose microfibrils and other matrix polysaccharides in cereal and millet cell walls. They consist of mixed-linkage (1→3/1→4)-β-D-glucans, arabinoxylans, arabinogalactans, arabinogalactan-proteins, and arabinogalactan-peptides (*1*). Depending on the sequence of extraction, hemicelluloses are categorized as hemicelluloses A, B, and C (designated hemi-A, hemi-B, and hemi-C, respectively), which vary in their sugar composition and physical and functional properties (*2*). The isolation of hemicelluloses is a two-stage process, involving alkaline hydrolysis of ester groups, especially the ester groups present between the ferulic acid and arabinose residues of feruloylarabinoxylans, followed by their extraction into aqueous medium (*3*). It is known that high concentrations of hydroxyl ions liberated from alkali solutions cause swelling of cellulose, hydrolysis of ester linkages, and disruption of intermolecular hydrogen bonds between cellulose and hemicelluloses, thereby facilitating the extraction of hemicelluloses from the other cell wall components (*4*). Some of the methods developed for the effective extraction of hemicelluloses from cereals and millets are extraction with (a) barium or calcium hydroxide at elevated temperatures (*4*), (b) sodium or potassium hydroxide (*5*), and (c) alkaline hydrogen peroxide (*3*). The quantity, composition, structure, and physicochemical and functional properties of these hemicelluloses vary not only from species to species but also from variety and place of origin within the species (*6*).

Arabinoxylans, also known as pentosans, are one of the major constituents of hemicelluloses of cereals and millets. They are of growing importance not only because of their health-beneficial effects but also because of their role as a major influencing factor in various biotechnological and industrial processes (*7*). Arabinoxylans are composed of a main chain of (1→4)-linked β-D-xylopyranosyl residues with various degrees of substitution on O-2 or O-3 or at both O-2 and O-3 by arabinose and uronic and ferulic acids (*1*). It is well-known that differences in the degree of substitution and molecular size influence arabinoxylan solubility, extractability, functionality, and, thereby, their utility in different technological and industrial

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10.1021/jf058144q CCC: $33.50
Published on Web 02/28/2006

processes including breadmaking and brewing (*8*). Arabinoxylans, because of their high solution viscosity, gelling capacity, and water absorption and water retention abilities are known to influence various rheological properties of dough such as dough stability, extensibility, retrogradation of starch, and, thereby, the quality of the end product (*1*). As one of the major constituents of dietary fiber, arabinoxylans may reduce the incidence of various diseases such as colon cancer, atherosclerosis, and diabetes (*9*). Like many other biomolecules, the functionality of arabinoxylans depends on their structure, the understanding of which requires a high-purity arabinoxylan preparation without any contamination from other associated cell-wall components. The major hurdles involved in obtaining high-purity arabinoxylans are removal of (a) contaminant starch, (b) mixed-linkage (1→3/1→4)-β-D-glucans and cellulose, (c) lignins, and (d) associated proteins. Several methods have been developed for the isolation and characterization of water-extractable and water-unextractable arabinoxylans from major food crops such as barley (*10*), wheat (*11*), rice (*12*), and sorghum (*13*, *14*). However, except for a few recent papers, not many studies have been carried out on the structural features of arabinoxylans from millets such as finger millet (*15*) and pearl millet (*16*) and their malts, the major source of food in developing countries such as India and Africa. The reported structural aspects of finger millet and pearl millet arabinoxylans demonstrated the presence of a β-(1,4)-linked xylopyranoside backbone substituted primarily with arabinofuranoses at C-3 via α-(1,3) linkage. Substitutions at C-2 and C-2/C-3 of xylose residues with arabinosfuranoses were also noted in these heavily substituted arabinoxylans (*15*, *16*). 4-(O-Me)-glucuronic acid is the other major substituent connected to C-2 of xylose residues in finger millet arabinoxylans (*15*).

Ragi (finger millet, *Eleusine coracana*), one of the most widely used millets in southern parts of India, is a richer source of dietary fiber (18%), phenolic compounds (1%), and calcium (0.34%) compared to cereals such as barley, rice, maize, and wheat (*17*). Its native and malted forms have been in routine use for the preparation of various geriatric and infant foods. Ragi is also consumed in the form of muddae (a thick porridge), dosa (a pan cake), biscuits, and malt by many South Indians, especially in Karnataka state (*18*). Several beverages and pharmaceutical preparations have been formulated by using germinated ragi flours (*19*). Extensive studies have been carried out on various changes that take place during malting of ragi pertaining to (a) carbohydrate-degrading enzymes and characterization of α-amylases (*20*, *21*), (b) nonstarch polysaccharides and their functional properties (*22*), (c) phenolic compounds and their antioxidant properties (*23*), and (d) free sugars, amino acids, phytic acid, minerals, and dietary fiber content (*20*, *24*).

In the present study, we have isolated, fractionated, and partially characterized hemicelluloses from a fast-germinating Indo-African hybrid variety of ragi, that is, Indaf-15, developed a method for obtaining high-purity alkali-extractable arabinoxylans from ragi, and studied the changes that occurred in their contents and composition during malting. Because ragi is a richer source of dietary fiber and, the major part of it, after cellulose, is contributed by hemicelluloses, the present study of isolation and characterization of hemicelluloses from native and malted conditions is believed to be important in the preparation of functional foods and also in providing a method for the purification of arabinoxylans from dietary fiber-rich millets.

MATERIALS AND METHODS

Finger millet (Indaf-15) seeds were procured from V.C. farm of the University of Agricultural Sciences, located at Mandya, Karnataka, India. DEAE-cellulose (0.99 mequiv/g, medium flow), Sepharose CL-4B (4% cross-linked), Amberlite IR-120-P (8% cross-linked, 16−50 mesh), anhydrous sodium borohydride, carbazole (purified by repeated crystallization from benzene), glucoamylase (EC 3.2.1.3) from *Aspergillus niger*, glucose oxidase (EC 1.1.3.4) from *A. niger*, and peroxidase (EC 1.1.11.7) from horseradish were purchased from Sigma Chemical Co., St. Louis, MO. Sugar standards, rhamnose, fucose, arabinose, xylose,mannose, galactose, glucose, galacturonic acid, and inositol, were obtained from ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, OH. T-series dextran standards (T-10, T-20, T-40, T-70, T-150, T-500, and T-2000) were from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals used in this study were of analytical grade. Gas chromatography column, OV-225 (3%, 3.2 mm × 15.2 cm), was purchased from Pierce Chemical Co., Rockford, IL. E-linear (7.8 mm × 30 cm) and E-1000 (3.9 mm × 30 cm) gel permeation chromatographic columns were obtained from Waters Associates, Milford, MA. A Beckman microzone cellulose acetate electrophoresis unit and cellulose acetate membranes were obtained from Beckman Instruments International, S.A., Geneva, Switzerland. A plain uncoated silica capillary column was procured from Prince Co., Emmen, The Netherlands.

Malting. Malting was carried out as published earlier (*20*). In brief, ragi seeds (100 g each) were cleaned, and allowed to germinate, after 24 h of steeping, in a BOD incubator set at 25 °C. Germinated seeds were withdrawn at 24 h intervals up to 96 h and air-dried in a hot air oven maintained at 50 °C for 12 h. Dry seeds were powdered after removal of the growth portions by gentle manual brushing.

Analytical Determinations. Total carbohydrate and uronic acid contents were estimated by phenol−sulfuric acid (*25*) and carbazole methods (*26*), respectively.

Viscosity (η_r) Determination. Relative viscosity (with respect to water) of water-soluble fractions of hemi-A and -B at 1% concentration (w/v) was determined by Ostwald viscometer (*27*).

Isolation of Hemicelluloses. *Removal of Free Sugars, Free Phenolic Acids, Starch, Water and 0.5% EDTA-Soluble Polysaccharides.* Before extraction of hemicelluloses from native and malted ragi flours, contaminants such as free sugars, starch, water, and 0.5% EDTA-extractable polysaccharides (pectins) were removed as described earlier (*18*). In brief, ground flours (50 g) were extracted with 70% aqueous ethanol (500 mL × 3) followed by treating the residue successively with (a) water at room temperature, (b) glucoamylase (500 mg) at 55 °C in 0.05 M acetate buffer, pH 4.5 for 48 h (enzyme is supplemented at every 12 h), and (c) 0.5% EDTA at 85 °C for 2 h.

Extraction of Hemicelluloses. The starch free residue was extracted with 10% sodium hydroxide containing 1% (w/v) sodium borohydride (material/alkali 1:10 w/v) with continuous stirring under a stream of nitrogen for 4 h. Alkali-extractable polysaccharides (hemicelluloses) were separated from alkali-insoluble residue (celluloses) by centrifugation at 400*g* in a Sigma 202 C centrifuge for 10 min. Hemicelluloses were quantitatively recovered by repeating the sodium hydroxide extraction one more time. The extracts were combined, and the pH was adjusted to 4.5 by the addition of 50% glacial acetic acid with slow stirring. The polysaccharides precipitated (hemi-A) were recovered by centrifugation at 8000*g* for 30 min, dialyzed (using a 12000 Da molecular cutoff dialysis bag), and freeze-dried. The supernatant was adjusted to 75% ethanol concentration, and the precipitated polysaccharides (hemi-B) were dialyzed and freeze-dried. No polysaccharides were identified in the supernatant, and hence it was discarded.

Ammonium Sulfate Precipitation. Fractionation of hemicellulose-B using ammonium sulfate was carried out according to the method of Izydorczyk and Biliaderis (*28*). A 0.2% solution of hemi-B in 0.1 M phosphate buffer, pH 7.0, was adjusted to 60% saturation by adding solid ammonium sulfate with continuous stirring, and the polysaccharides were allowed to precipitate by leaving the solution for 4 h at room temperature. The precipitated polysaccharides (F-60) were recovered by centrifugation, and the supernatant was further adjusted successively to 70, 80, and 100% ammonium sulfate saturation to obtain

F-70, F-80, and F-100 fractions. The polysaccharides remaining in the supernatant were recovered by dialysis and freeze-drying and designated the nonprecipitable (NP) fraction.

DEAE-Cellulose Chromatography. F-70 of hemi-B and water-soluble hemi-A were fractionated on a DEAE-cellulose column (carbonate form, 3.5 × 25 cm) according to the method of Neukom and Kuendig (*29*). Polysaccharides (100 mg) were eluted stepwise with water, ammonium carbonate (AC) (0.1−0.3 M), and sodium hydroxide (0.1 and 0.2 M) at 60 mL/h. Fractions (10 mL) were collected, and suitable aliquots were analyzed according to the phenol−sulfuric acid. Completion of the elution with each eluant was tested by the phenol−sulfuric acid. Fractions containing carbohydrates were pooled, dialyzed, and freeze-dried.

Gel Permeation Chromatography on Sepharose CL-4B. Gel permeation chromatography was performed as described by Izydorczyk and Biliaderis (*30*). Polysaccharides (10 mg) were dissolved in 1 mL of 0.1 M NaCl containing 0.05% sodium azide and were analyzed on a precalibrated (with T-series dextran standards) Sepharose CL-4B column (1.6 × 92 cm) using 0.1 M NaCl containing 0.05% sodium azide as eluant. Fractions (3 mL) were collected using an LKB Bromma 2211 fraction collector at a 16 mL/h flow rate, and suitable aliquots were analyzed by the phenol−sulfuric acid. Fractions containing carbohydrate were pooled, dialyzed, and freeze-dried.

Hydrolysis of Polysaccharides. Hydrolysis of polysaccharides was carried out as follows. In brief, polysaccharides were suspended in water (0.5 mL) and solubilized with concentrated sulfuric acid (0.6 mL) for 2 h at ice-cold temperature. The sulfuric acid concentration in the reaction mixture was brought down to 8% with the addition of water, and the hydrolysis was carried out by refluxing in a boiling water bath for 12 h. After hydrolysis, the reaction volume was adjusted to 20 mL with distilled water and neutralized with solid barium carbonate, and the resultant white barium sulfate precipitated was removed by filtration through Whatman no. 1 filter papers. The clear filtrate thus obtained was concentrated, deionized with Amberlite (IR 120-P), and used for the preparation of alditol acetates.

Preparation and Analysis of Alditol Acetates. Alditol acetates were prepared as described earlier (*31*). The pH of the concentrated filtrate containing monosaccharides, obtained from the above step, was adjusted to 7.0 with 1 M sodium carbonate, and the reduction was carried out by adding solid anhydrous sodium borohydride (10 mg) followed by incubation at room temperature for 4 h. Excess sodium borohydride was neutralized with 2 N acetic acid, and the liberated borate ions were removed by repeated codistillation with methanol. Acetylation was carried out in a boiling water bath for 2 h by adding equal volumes (1 mL each) of anhydrous acetic anhydride and pyridine. Excess acetylating reagents were removed by flash evaporation; the alditol acetates were purified and dried by repeated washing with water and toluene. Purified alditol acetates thus obtained were extracted with chloroform (1 mL), dried, and analyzed on a 3% OV-225 column using a Shimadzu 14-B gas−liquid chromatograph equipped with a flame ionization detector and a CR-4 A recorder. The analysis was performed at 250 °C column temperature and 200 °C injector and detector temperatures with N_2 (40 mL/min) as carrier gas. The column was calibrated, and the response factors were calculated by injecting a sugar standard mixture containing rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, and inositol (used as internal standard). Sugars present in the samples were identified by comparing their relative retention times with standards and were quantified by using response factors. The sugar contents were expressed in mole percent.

High-Performance Size Exclusion Chromatography (HPSEC). HPSEC was carried out using a Shimadzu HPLC system (LC 6A) equipped with a refractive index detector and a CR-4A recorder. Polysaccharides (10 mg/mL) were analyzed on precalibrated (with T-series dextran standards) E-linear and E-1000 columns connected in series at a 0.6 mL/min flow rate using deionized, filtered (through 0.2 μm membranes) water as the eluant. Elution of the polysaccharides was monitored with a refractive index detector set at 8×10^{-6}AUFS.

Cellulose Acetate Electrophoresis. The electrophoretic mobility of polysaccharides was analyzed by using a Beckman Microzone electrophoretic cell (model R 101) and cellulose acetate membranes according to the method of Anderson et al. (*32*). In brief, the polysaccharides (10 mg/mL, 10 μL) were loaded onto a cellulose acetate membrane, prewetted with running buffer (50 mM ammonium carbonate−NaCl buffer, pH 9.3), using an applicator, and electrophoresed at constant voltage (180 V) for 30 min. The rate of migration was monitored by using porcion red dye, and the polysaccharides were visualized by staining with ruthenium red.

Capillary Electrophoresis (CE). Polysaccharides (0.1%, 10 μL) were dissolved in 0.2 M sodium borate buffer, pH 8.5, and were analyzed on a pure silica column (75 μm × 100 cm) maintained at 100 mbar pressure and 20 kV voltage using a Prince CE 56 model CE unit. Prior to analysis, the column was thoroughly washed with 0.1 M sodium hydroxide and equilibrated with 0.2 M sodium borate buffer, pH 8.5. Elution of the polysaccharides was monitored at 253 nm using a UV detector (model lambda 1010).

RESULTS AND DISCUSSION

Yield and Physicochemical Properties of Hemicelluloses from Native (N) and Malted (M) Finger Millet. Hemicelluloses A and B were isolated in different yields from native and malted finger millet. A 3-fold decrease (1.4% in native to 0.5% in malted finger millet) in the yield of hemi-A polysaccharides upon 96 h of malting indicated a substantial degradation of matrix cell-wall polysaccharides. This is in consonance with our earlier observation that showed a 3-fold decrease in the pentose-to-hexose (P:H) ratio in hemi-A upon malting for 96 h (*18*). This could be due to the elevated activities of various cell-wall-degrading enzymes such as arabinase and endo-xylanase during malting as reported previously in finger millet (*20*). Recently, increased endo-xylanase activity with simultaneous decrease in total arabinoxylan content was also reported in barley (*33*). However, no significant changes were observed in the total carbohydrate content and intrinsic viscosity of soluble hemi-A of native and malted conditions except a 2.5-fold increase in the uronic acid content of malted hemi-A (*18*). The yield of hemi-B is high compared to that of hemi-A in native and malted finger millet, and the increase is more pronounced (6-fold increase) in malted hemi-B, indicating the differential effects of malting on hemi-A and -B polysaccharides. In contrast to hemi-A, malting has brought a 1.5-fold increase (from 1.9 to 3.0%) in hemi-B content and a similar fold decrease in its intrinsic viscosity (from 3.0 to 2.0), further confirming the solubilization of cell-wall polymers by hydrolytic enzymes that are induced during malting, thereby the better extractability, increase in yield, and decrease in viscosity. Solubility and total carbohydrate and uronic acid contents were significantly higher in hemi-B than in hemi-A, indicating that they vary in their composition and structure. No significant changes were noted in the solubility and total carbohydrate and uronic acid contents of hemi-B fractions isolated from native and malted finger millet. Earlier, variations in the yield and composition of hemi-A and hemi-B extracted from wheat (*34*) and bajra flours (*14*) were reported.

Fractionation of Hemi-A. Hemi-A from N and M finger millet was separated into water-soluble (10 and 30% in N and M, respectively) and water-insoluble (90 and 70% in N and M, respectively) fractions, and their neutral sugar composition was determined. Both fractions are richer in glucose (~70%), along with minor contributions from arabinose, xylose, mannose, and galactose. High glucose content in hemi-A might have originated from (a) resistant starch, a kind of starch formed by amylose component that is resistant to amylases; (b) alkali-soluble celluloses; or (c) mixed-linkage glucans. Arabinose and xylose contents were 2-fold higher in soluble portions compared to their insoluble counterparts, indicating the possible presence of arabinoxylans. Slight decreases in the glucose content from 71

Table 1. Yield and Neutral Sugar Composition of Water-Soluble and Water-Insoluble Fractions of Native (N) and Malted (M) Hemicellulose A[a]

	soluble		insoluble	
yield[b]/sugar[c]	N	M	N	M
yield	10	30	90	70
Rha	1.0	2.0	1.0	4.0
Ara	10.0	11.0	5.0	5.5
Xyl	10.5	14.0	7.0	9.0
Man	4.0	4.0	8.0	8.0
Gal	3.0	7.0	3.0	3.0
Glc	71.5	62.0	76.0	70.5
Ara:Xyl	1.0:1.03	1.0:1.27	1.0:1.4	1.0:1.63
P:H	0.26:1	0.34:1	0.14:1	0.18:1

[a] Abbreviations: Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose, Glc, glucose; Ara:Xyl, arabinose-to-xylose ratio; P:H, pentose-to-hexose ratio. [b] Expressed as weight percentages. [c] Expressed as mole percentages (mol/100 mol). Results were obtained from triplicate experiments, and the coefficient of variation is <2%.

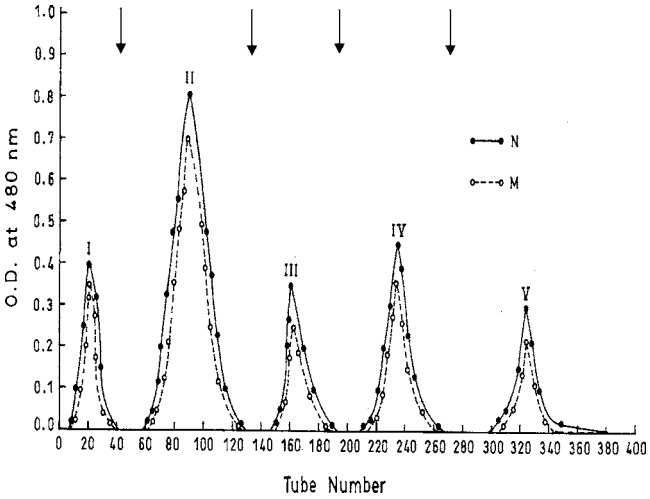

Figure 1. Elution profile of water-soluble hemi-A obtained from native (N) and malted (M) flours on DEAE-cellulose: I, water eluted; II, 0.1 M ammonium carbonate eluted; III, 0.2 M ammonium carbonate eluted; IV, 0.1 N sodium hydroxide eluted; V, 0.2 N sodium hydroxide eluted.

to 62% and from 76 to 70% were noted in soluble and insoluble fractions, respectively, upon 96 h of malting, which could be due to the degradation of either mixed-linkage glucans by glucanases or cellulose by cellulases that are induced during malting (*20*). However, no significant changes were noted in other sugar contents (**Table 1**).

Because the viscosity of soluble hemi-A is low and is relatively richer in arabinose and xylose content (20.5% in N and 25.0% in M) compared to its insoluble counterpart (12% in N and 14.5% in M), it was further fractionated on DEAE-cellulose into five fractions (water, 0.1 M AC, 0.2 M AC, 0.1 M NaOH, 0.2 M NaOH eluted) differing in their neutral sugar composition (**Figure 1** and **Table 2**). Among all of the fractions the 0.1 M AC eluted fraction was obtained in high yield (64% in N and 65% in M), was found to contain more pentoses (67.5% in N and 53% in M) than hexoses (31.5% in N and 45.5% in M), and was richer in uronic acid (~10%), indicating that the polysaccharides could be a glucuronoarabinoxylan type. A 1.8-fold decrease in the P:H ratio of this fraction, upon malting, indicated selective degradation of pentosans rather than hexosans as reported earlier (*20*). All other fractions were richer in hexoses than pentoses, the majority of which were contributed by glucose and did not exhibit any significant changes in either pentose-to-hexose or arabinose-to-xylose (Ara:Xyl) ratios. Further studies on the arabinoxylan richer 0.1 M AC eluted fraction by HPSEC, gel permeation chromatography, and cellulose electrophoresis revealed significant heterogeneity with respect to molecular weight and overall charge (data not shown), in both native and malted preparations; hence, no attempts were made to further purify and characterize arabinoxylans from this fraction. Because the yield and solubility of the hemi-A fraction from native and malted ragi are significantly low and the major fraction (0.1 M AC eluted) obtained upon DEAE-cellulose chromatography is heterogeneous, we have selected hemi-B, the other fraction richer in arabinose and xylose contents, to purify and characterize arabinoxylans.

Fractionation of Hemi-B. Unlike hemi-A, hemi-B obtained from native and malted flours is highly soluble (>90%), richer in uronic acid (10%), and more viscogenic and, hence, was fractionated with ammonium sulfate before separation on the DEAE-cellulose column. A 0.5% solution of hemi-B in phosphate buffer, pH 7.0, was fractionated by graded ammonium sulfate precipitation into four precipitable fractions (F-60, F-70, F-80, and F-100) and a nonprecipitable (NP) fraction in both native and malted conditions, and their yield and neutral sugar composition are shown in **Table 3**. Water-soluble and water-insoluble arabinoxylans of wheat (*28*) and rye (*7*, *35*) were fractionated using ammonium sulfate into various precipitable fractions differing in their neutral sugar composition.

Among the four precipitable fractions, F-70 is obtained in higher yield (N, 44.5%; M, 38.5%) compared to F-60, F-80, and F-100. The NP fraction presented as the second major fraction in both native (19%) and malted (24.6%) conditions. Except F-60, all other fractions obtained from native and malted hemi-B are soluble (~95%) in water and richer in total carbohydrate (>96%) and uronic acid contents (10%). F-60 obtained from native and malted hemi-B has lower solubility (42% in N and 46% in M) and uronic acid contents (4% in N and 5% in M). Neutral sugar composition analysis of fractions obtained from native hemi-B showed a progressive increase in arabinose content from 12% in F-60 to 43% in NP and a steady decrease in glucose content from 62.5% in F-60 to 34.5% in F-100 followed by a sharp decrease to 5.5% in the NP fraction. Similarly, malted fractions also showed an increase in arabinose content from 14% in F-60 to 32% in NP and a decrease in glucose content from 64% in F-60 to 32% in F-100 and to 12% in the NP fraction. Similarly, the xylose content also showed an increase from 17.5% in F-60 to 34% in F-80 in native fractions and from 16% in F-60 to 34.5% in F-80 of malted fractions. However, in contrast to arabinose and glucose contents, the xylose content decreased to 23 and 21% in the NP of N and M preparations, respectively. A progressive increase in P:H ratio from 0.42:1 in F-60 to 1.44:1 in F-100 of native hemi-B indicated the possible presence of high arabinoxylan contents in F-80 and F-100 (**Table 3**). However, the P:H ratios increased from 0.43:1 in F-60 to 1.56:1 in F-80 and then decreased slightly to 1.13 in NP in malted condition, indicating the differential behavior of native and malted hemi-B preparations to ammonium sulfate fractionation. An increase in pentose content upon ammonium sulfate fractionation was also noted in wheat and rye arabinoxylans, and the results obtained in the present investigation were in good agreement with the previous reports (*29*, *35*). No major changes were noted in the Ara:Xyl ratios of precipitable fractions from native and malted hemi-B, except in NP, wherein 2- and 1.5-fold higher arabinose contents are noted in both native and malted conditions, respectively. The presence of high galactose (28.5% in

Table 2. Yield and Neutral Sugar Composition of Fractions Obtained from DEAE-Cellulose Chromatography of Water-Soluble Native (N) and Malted (M) Hemicellulose A[a]

	water		0.1 M AC[b,c]		0.2 M AC[b]		0.1 M NaOH		0.2 M NaOH	
yield[d]/sugar[e]	N	M	N	M	N	M	N	M	N	M
yield	7.4	8.0	64.0	65.0	6.2	7.0	12.0	10.0	6.4	6.2
Rha	4.0	1.0	1.0	2.0	4.0	5.0	1.5	2.0	1.5	3.0
Ara	13.0	14.0	35.5	26.0	13.0	14.0	8.0	10.5	9.0	10.5
Xyl	14.0	11.5	32.0	27.0	20.5	18.0	15.0	16.0	20.5	22.0
Man	4.5	2.0	6.0	10.0	12.0	10.0	6.0	3.5	4.5	5.0
Gal	2.5	4.0	13.0	16.0	9.0	17.0	5.5	4.5	7.0	7.5
Glc	62.0	67.5	12.5	19.0	41.5	36.0	64.0	63.5	57.5	52.0
Ara:Xyl	1:1.07	1:0.82	1:0.9	1:1.04	1:1.58	1:1.28	1:1.87	1:1.52	1:2.27	1:2.09
P:H	0.39:1	0.35:1	2.14:1	1.17:1	0.54:1	0.51:1	0.30:1	0.37:1	0.43:1	0.50:1

[a] For abbreviations refer to **Table 1**. [b] AC, ammonium carbonate. Results were obtained from triplicate experiments, and the coefficient of variation is <2%. [c] Uronic acid content of 0.1 M AC eluted fraction from native and malted samples was found to be significantly high (9.0%) compared to all other fractions, wherein <1.0% of uronic acid was recorded (data not shown). [d] Expressed as percentage (weight) material recovered. [e] Expressed as mole percentages (mol/100 mol).

Table 3. Yield and Neutral Sugar Composition of Fractions Obtained upon Graded Ammonium Sulfate Precipitation of Native (N) and Malted (M) Hemicellulose B[a]

	fraction									
	F-60[b,c]		F-70		F-80		F-100		NP	
yield[d]/sugar[e]	N	M	N	M	N	M	N	M	N	M
yield	19	11.5	44.5	38.5	10	16	7.5	9.4	19.0	24.6
Ara	12.0	14.0	13.0	23.0	25.0	26.5	26.0	27.0	43.0	32.0
Xyl	17.5	16.0	20.0	26.0	34.0	34.5	33.0	30.0	23.0	21.0
Gal	8.0	6.0	9.0	6.0	5.0	8.0	6.5	11.0	28.5	35.0
Glc	62.5	64.0	58.0	45.0	36.0	31.0	34.5	32.0	5.5	12.0
Ara:Xyl	1.0:1.46	1.0:1.14	1.0:1.54	1.0:1.13	1.0:1.4	1.0:1.30	1.0:1.27	1.0:1.11	1.0:0.53	1.0:0.65
P:H	0.42:1.0	0.43:10	0.49:1.0	0.96:1.0	1.44:1.0	1.56:1.0	1.44:1.0	1.32:1.0	1.94:1.0	1.13:1.0

[a] For abbreviations refer to **Table 1**. [b] Solubility of F-60 in water, obtained from N and M samples, is 42 and 46%, respectively. All other fractions have >95% solubility in water. [c] Uronic acid content in the water-soluble fraction of F-60 obtained from N and M samples is 5 and 4%, respectively. All other fractions, obtained from N and M samples, have 9–10% uronic acid. Results were obtained from triplicate experiments, and the coefficient of variation is <2%. [d] Expressed as percentage (weight) material recovered. [e] Expressed as mole percentages (mol/100 mol).

N and 35% in M) and arabinose (43% in N and 32% in M) in NP fractions suggests the possible presence of arabinogalacto-proteins or arabinogalacto-peptides (**Table 3**). In a recent paper, Bulck et al. (*36*) isolated arabinogalacto-peptides from rye and barley water extracts by alcohol fractionation followed by ammonium sulfate precipitation. The results showed that the supernatant, obtained at 100% saturation with ammonium sulfate, was richer in arabinogalacto-peptides (*36*). High arabinose and xylose contents in F-80, F-100, and NP also suggest the possibility for the presence of high molecular weight di- and monosubstituted branched arabinoxylans as reported in wheat and rye (*7*, *28*). Earlier observations also demonstrated the role of both molecular weight and molecular structure in the non-covalent interactions between polysaccharides and their impact on the fractional precipitation by ammonium sulfate (*9*, *23*). Results similar to those of the present study were reported in rye arabinoxylans extracted with water at different temperatures, wherein the fractions obtained at 80 and 100% ammonium sulfate saturation levels were found to have high molecular weights and higher Ara:Xyl ratios compared to those obtained at 40 and 60% saturation levels (*7*, *35*).

Fractionation of F-70 on DEAE-Cellulose. The major fraction F-70 obtained with 44.5 and 38% yields from native and malted hemi-B, respectively, is water soluble (95%) and richer in total carbohydrate (96%) and uronic acid contents (10%) (**Table 3**). It was further fractionated on DEAE-cellulose by using water, ammonium carbonate (0.1, 0.2, 0.3 M), and sodium hydroxide (0.1 and 0.2 M) into five major fractions and two subfractions (P-2 of 0.1 and 0.2 M sodium hydroxide elution) (**Figure 2**). Unlike hemi-A, the recovery of hemi-B

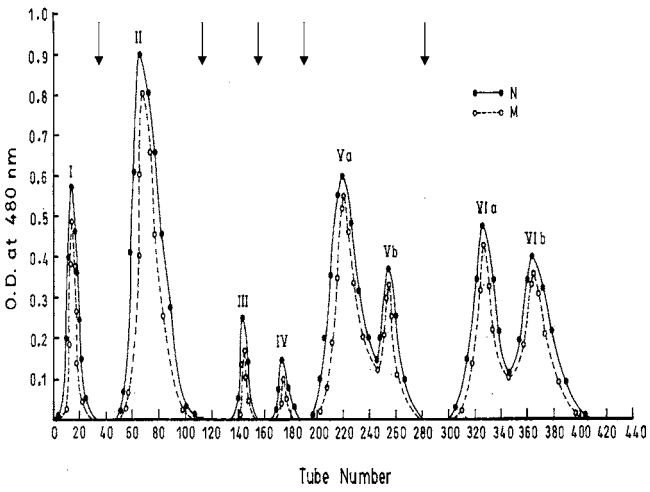

Figure 2. Elution profile of water-soluble F-70, obtained from native (N) and malted (M) hemi-B, on DEAE-cellulose: I, water eluted; II, 0.1 M ammonium carbonate eluted; III, 0.2 M ammonium carbonate eluted; IV, 0.3 M ammonium carbonate eluted; V, 0.1 N sodium hydroxide eluted (P-1); VI, 0.1 N sodium hydroxide eluted (P-2); VII, 0.2 N sodium hydroxide eluted (P-1); VIII, 0.2 N sodium hydroxide eluted (P-2).

was 65 and 71% in native and malted preparations, respectively, which could be due to its high binding affinity to DEAE-cellulose. Forty-six percent of the total recovered material is contributed by the 0.1 M AC fraction, followed by peak 1 of the 0.1 M sodium hydroxide fraction (18% in N and 15% in M), peak I of the native 0.2 M sodium hydroxide fraction

Table 4. Yield and Neutral Sugar Composition of Fractions Obtained from DEAE-Cellulose Chromatography of Native (N) and Malted (M) Hemicellulose B[a]

					0.1 N NaOH				0.2 N NaOH			
	water		0.1 M AC [b,c]		P-I		P-II		P-I		P-II	
yield[d]/sugar[e]	N	M	N	M	N	M	N	M	N	M	N	M
yield	5.0	6.0	30.0	33.0	11.5	10.6	5.0	8.5	8.0	7.0	5.6	6.0
Ara	10.0	10.5	45.0	50.0	15.5	13.0	30.0	26.0	23.0	20.0	30.0	30.0
Xyl	11.0	11.0	46.0	42.5	17.0	10.0	38.0	30.0	23.0	16.0	34.0	32.0
Gal	2.0	8.5	4.0	4.5	1.0	4.0	2.0	3.0	1.0	6.0	2.0	10.0
Glc	77.0	70.0	5.0	3.0	66.5	73.0	30.0	41.0	53.0	58.0	34.0	28.0
Ara:Xyl	1.0:1.1	1.0:1.05	1.0:1.0	1.0:0.85	1.0:1.1	1.0:0.77	1.0:1.27	1.0:1.15	1.0:1.0	1.0:0.8	1.0:1.1	1.0:1.06
P:H	0.26:1.0	0.27:1.0	10.1:1.0	12.3:1.0	0.48:1.0	0.3:1.0	2.1:1.0	1.3:1.0	0.85:1.0	0.67:1.0	1.8:1.0	1.63:1.0

[a] For abbreviations refer to **Table 1**. [b] AC, ammonium carbonate. Uronic acid content of 0.1 M AC fraction from native and malted samples was found to be 10.0 and 9.0%, respectively. However, no uronic acid was found in the water-eluted fraction. Fractions eluted with 0.1 and 0.2 M alkali have significantly less uronic acid content (data not shown). [c] Neutral sugar composition and uronic acid contents of 0.2 and 0.3 M AC eluted fractions were not determined. Results were obtained from triplicate experiments, and the coefficient of variation is <2%. [d] Expressed as percentage (weight) material recovered. [e] Expressed as mole percentages (mol/100 mol).

(12.3%), and peak II of the malted 0.1 M sodium hydroxide fraction (12%). Sugar composition data revealed that the water-eluted fraction was richer in glucose (77% in N and 70% in M) and was devoid of glucuronic acid, indicating the presence of a high amount of glucan type polymers. High glucose content was also noted in peak 1 of 0.1 M (66.5% in N and 73% in M) and 0.2 M (53.0% in N and 58.0% in M) sodium hydroxide eluted fractions. However, the glucose content was relatively low in peak 2 of 0.1 M (30% in N and 41% in M) and 0.2 M (34% in N and 28% in M) sodium hydroxide eluted fractions and was negligible in the 0.1 M AC eluted fraction. The 0.1 M AC fraction is exclusively richer in arabinose and xylose (91% in N and 92.5% in M), indicating the presence of arabinoxylans in this fraction (**Table 4**). A significant decrease in the P:H ratio of 0.1 and 0.2 M eluted fractions (both peaks 1 and 2) along with a slight decrease in the Ara:Xyl ratio was observed upon malting, indicating the possible degradation of arabinoxylans. Hemicelluloses from sorghum were fractionated on DEAE-cellulose into 13 fractions differing in their neutral sugar composition and uronic acid contents (*37*, *38*). In a similar way, water-soluble arabinoxylans from wheat were separated into six individual peaks differing in their neutral sugar composition and uronic acid contents by elution with water and varied concentrations of borate and sodium hydroxide (*39*).

Purity and Molecular Weight Determination of the 0.1 M AC Eluted Fraction. The completely water-soluble arabinose and xylose richer 0.1 M AC fraction, obtained in higher yield (46%) from native and malted samples, was found to be richer in total carbohydrate (98%) and uronic acid (10% in N and 9% in M) contents among all other DEAE fractions and was chosen for determining its purity and molecular weight (**Table 4**). Elution of this arabinoxylan richer fraction on precalibrated (with T-series dextran standards) Sepharose CL-4B gave a broad single peak with a molecular mass corresponding to 1200 kDa in N and 1120 kDa in M at peak maximum, indicating that alkali-extractable ragi hemi-B arabinoxylans are of high molecular weight and polydisperse (**Figure 3**). High molecular masses were reported for purified arabinoxylans obtained from wheat bran (*11*), barley (*40*), rice, and sorghum (*13*). The decrease in the molecular weight upon malting for 96 h could be due to the degradation of arabinoxylans by endoxylanases. Degradation of arabinoxylans during germination of cereals and millets is one of the prerequisites for the degradation of starchy endosperm as reported earlier in barley and other cereals and in millets (*18*). In an earlier observation, the decrease in the molecular weight of high molecular weight

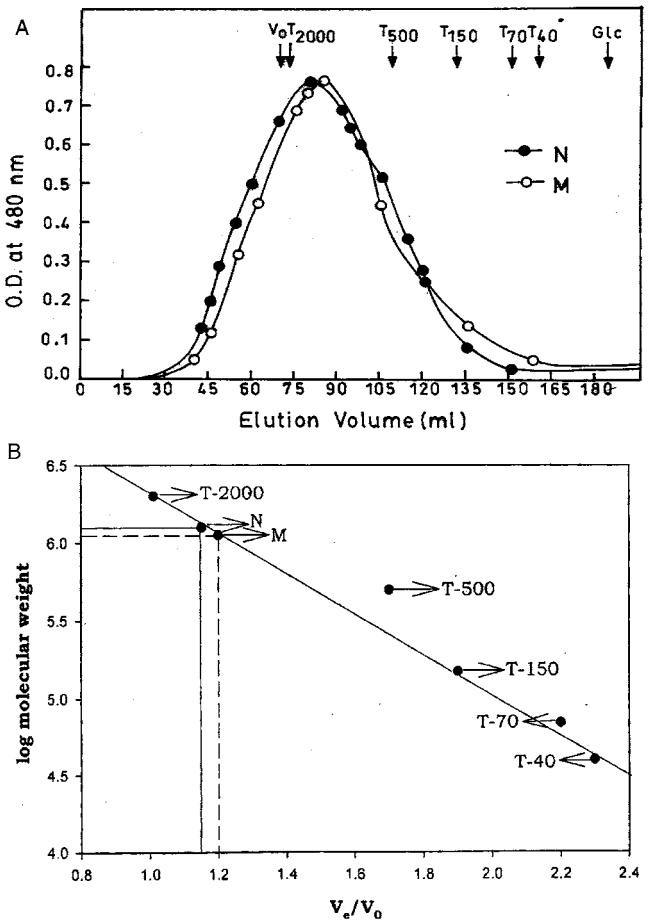

Figure 3. Purity and molecular weight determination of native (N) and malted (M) finger millet arabinoxylans: (**A**) elution profile of N and M finger millet arabinoxylans on Sepharose CL-4B; (**B**) calibration graph for the molecular weight determination of N and M finger millet arabinoxylans.

water-soluble arabinoxylans of barley was ascribed to the increased endoxylanase activity upon malting (*41*).

HPSEC. The elution of arabinoxylans on E-Linear and E-1000 columns, connected in series, was carried out using deionized water and was monitored with a refractive index detector. The arabinoxylans obtained from native and malted F-70 were eluted as single symmetrical peaks with retention times of 13.8 and 14.3 min, respectively, indicating that these arabinoxylans are highly homogeneous and were of high molecular weight (**Figure 4**). These results are in good

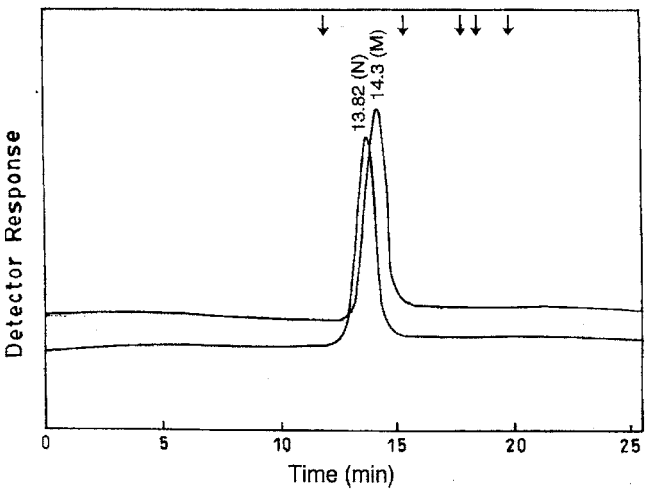

Figure 4. HPSEC analysis of native (N) and malted (M) finger millet arabinoxylans. Arrows from left to right indicate the elution position of T-series dextran standards T-2000, T-500, T-70, T-40, and T-10.

consonance with Sepharose CL-4B analysis, thereby confirming the high molecular weight of ragi alkali-extractable arabinoxylans. High molecular weights were reported to both water-soluble and water-insoluble arabinoxylans obtained from wheat (*11*), barley (*40*), sorghum (*14*), bajra (*16*), and rye (*7*, *35*) by HPSEC using both pullulan and T-series dextran standards, further validating our results obtained in the present investigation. A slight decrease in the retention time of malted arabinoxylan could be due to its degradation by endoxylanase during malting.

Cellulose Acetate Electrophoresis. Because the arabinoxylans isolated in the present study contain a significant amount of uronic acid (10% in N and 9% in M), the purity is also ascertained by their behavior under electric field. Electrophoresis was carried out on cellulose acetate membranes by using ammonium carbonate containing sodium chloride at 180 V. The samples from native and malted hemi-B were moved as single bands, indicating their homogeneity (**Figure 5A**). Not many papers are available regarding the confirmation of purity of arabinoxylans by cellulose acetate electrophoresis. Cellulose acetate electrophoresis is widely used to determine the purity of pectic polysaccharides.

CE. Of late, CE has emerged as one of the methods to establish the purity of proteins. However, not many papers are available about the use of this technique for determining the purity of polysaccharides. Hence, we made an attempt to use CE as one of the purity criteria to establish the homogeneity of arabinoxylans obtained from native and malted ragi hemi-B fractions, and the results are shown in **Figure 5B**. The arabinoxylans obtained from native and malted samples were eluted as single symmetrical peaks at 4.28 (N) and 4.03 (M) min. The early elution of malted arabinoxylan by 0.25 min could be due to either its decreased molecular weight or its low uronic acid content (9% in M compared to 10% in N). To the best of our knowledge this is the first report of the efficacy of CE in determining the purity of arabinoxylans.

On the basis of above results, the following conclusions were made: (a) malting has a significant impact on the content and physicochemical properties of hemicelluloses; (b) hemi-A is a heterogeneous mixture of glucans and arabinoxylans; (c) hemi-B from native and malted ragi, but not hemi-A, is the best source for the isolation of alkali-extractable arabinoxylans; and (d) alkali-extractable arabinoxylans from ragi were of high molecular weight and are prone to degradation by the induced endoxylanases during malting.

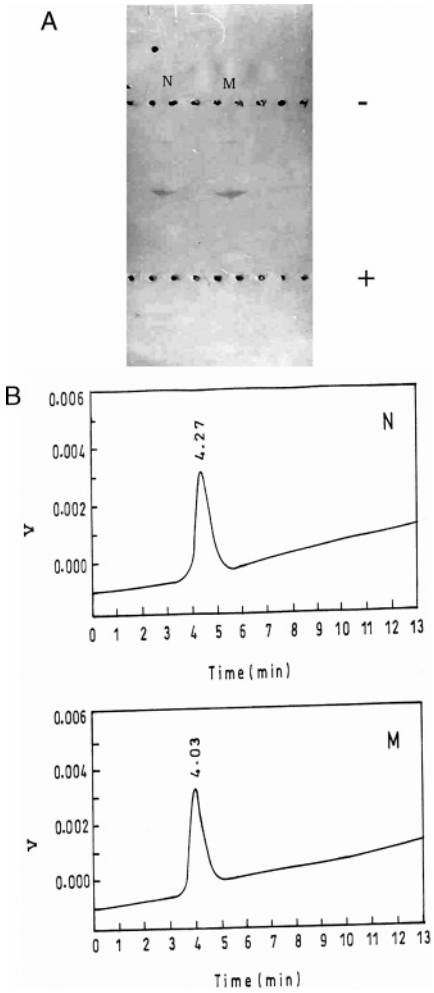

Figure 5. Migration of uronic acid rich arabinoxylans obtained from native (N) and malted (M) finger millet: (**A**) analysis by cellulose acetate electrophoresis; (**B**) analysis by CE.

We are of opinion that the results presented in this investigation about (a) the isolation and characterization of hemicelluloses and (b) the method developed to purify alkali-extractable glucuronoarabinoxylans from ragi might be helpful in the purification and characterization arabinoxylans from different cereals and millets and also contribute to the vast amount of arabinoxylan literature. The detailed structural analysis of purified ragi alkali-extractable arabinoxylans was reported recently (*15*).

ACKNOWLEDGMENT

We thank Dr. V. Prakash, Director, F.R.Sc, C.F.T.R.I. Mysore, for his keen interest and constant encouragement during this investigation.

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Received for review September 6, 2005. Revised manuscript received December 28, 2005. Accepted January 12, 2006. M.V.S.S.T.S.R. thanks the Council of Scientific and Industrial Research, New Delhi, India, for awarding Junior and Senior Research Fellowships.

JF058144Q