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Structural characterization of hemicellulose A from wheat (*Triticum aestivum*) varieties differing in their *chapati*-making quality

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ABSTRACT

Wheat varieties such as DWR-162, GW-322 (good *chapati*-making quality) and MACS-2496, HD-2189 (poor *chapati*-making quality) were used in the present study. Structural features of purified hemicellulose A was elucidated by a combination of methods such as methylation, periodate oxidation and optical rotation measurements. Hemicellulose A was mainly xylan type in nature along with small amounts of arabinoxylan type of polysaccharides having xylan backbone in β-(1,4) linkages. Monosubstituted and di-substituted xylosyl residues were present in these polysaccharides. Differences in the physico-chemical properties of dough in terms of *chapati* making qualities could be due to variations in their structures and also possible linkages with ferulic acid, uronic acid and other associated polysaccharides.

1. Introduction

Hemicelluloses which include hemicellulose A, B and C are mainly pentosans including arabinoxylans along with xyloglucan and β-glucans and represent major group of cell wall polysaccharides in wheat and other cereals. These are known to play significant role in various cereals and perform physico-chemical properties (Dupont & Selvendran, 1987; Oraphin, Pawadee, & Krisda, 2004). As one of the major constituents of dietary fiber, they are known to reduce the incidence of diseases such as diabetes, colon cancer, atherosclerosis, etc. (Lu, Walker, Muir, Mascara, & Kerin, 2000). Hemicelluloses are usually associated with other cell-wall components such as cellulose, cell-wall proteins, lignin and other phenolic compounds by covalent and hydrogen bonds, by both ionic and hydrophobic interactions (Meuser & Suckow, 1986; Rybka, Sitarski, & Raczynska-Bojanowska, 1993).

The hemicelluloses found in cereals such as wheat, rice, maize, oat, rye and barley are generally structurally more diverse and complex (Ebringerova & Heinze, 2000; Subbarao & Muralikrishna, 2006). The reported industrial applications for plant hemicellulose include their use as viscosity modifiers, gelling agents, tablet binders or wet strength additives (Ebringerova, 2006; Whistler, 1993). Hemicelluloses are also used as nutraceuticals and chiral separations (Fang, Sun, Salisbury, Fowler, & Tomkinson, 1999). Hemicellulosic polysaccharide consists of xyloglucan, β-glucan and arabinoxylan in rice endosperm cell wall (Ebringerova, 2006; Shyamprasad Rao & Muralikrishna, 2007).

Depending on the sequence of extraction, hemicelluloses are categorized as hemicelluloses A, B and C (designated Hem A, Hem B and Hem C, respectively), which vary in their sugar composition, physical and functional properties (Dupont & Selvendran, 1987; Subbarao & Muralikrishna, 2006). They can be extracted by alkaline solutions that cleave predominantly ester and hydrogen linkages by which different polymers are interconnected in the cell wall network, leaving cellulose microfibrils in the alkali-insoluble residue (Maes & Delcour, 2001). Thus, the hemicelluloses released from water-unextractable material (WUM) by alkaline treatment usually become water soluble after purification. However, in some cases when specific structural features allow their self-association, the aggregates formed during neutralization and dialysis are readily precipitated from aqueous solution (Meuser & Suckow, 1986; Subbarao & Muralikrishna, 2006).

Hemicelluloses (arabinoxylans including xylans) in different varieties of wheat are known to vary and have relation to bread making quality (Cleemput, Roels, Vanoort, Grobet, & Delcour, 1993). Differences in molecular features of arabinoxylans, including degree of branching, spatial arrangement of arabinosyl residues along the xylan backbone, and the ferulic acid content, could alter the visco-elastic properties of the gels (Izydorczyk & Biliaderis, 1995). Previously, we have reported on the rheology of dough, water holding capacity of dough and carbohydrate composition of pentosans from wheat varieties, in relation to their *chapati*-making quality (Hemalatha, Manu, Bhagwat, Leelavathi, & Prasada Rao, 2007; Revanappa, Bhagwat, & Salimath, 2007). In the present

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study, hemicelluloses A from wheat varieties having differences in *chapati* making qualities are purified and their structural features are elucidated by a combination of methods.

2. Materials and methods

2.1. Materials

DWR-162 variety of wheat (*Triticum aestivum* L.) was obtained from University of Agricultural Sciences, Dharwad and MACS-2496, GW-322, HD-2189 varieties were procured from Agharkar Research Institute, Pune, India. Termamyl (EC 3.2.1.1. from *Bacillus licheniformis*) was procured from Novo, Nordisk, Denmark. Glucoamylase and dialysis bags (Cellulose membranes, 12,000 MW cut-off) were procured from Sigma, St. Louis, USA. All other chemicals and reagents used were of analytical grade.

2.2. Isolation of hemicellulose A from whole-wheat flours

Hemicelluloses were extracted by following the previously described method (Nandini & Salimath, 2001). Briefly, flour was treated with 70% alcohol to remove free sugars. The residue was cooked with water to gelatinize starch and then subjected to glucoamylase digestion. The starch free residue was then taken for the extraction of hemicelluloses using 10% NaOH under nitrogen atmosphere. The extract was acidified with acetic acid (50%) in ice-cold temperature to pH 4.5 to get precipitate (hemicellulose A) and it was washed till neutral pH and dialyzed and lyophilized. It was further purified by alcohol precipitation under acidic (pH 3.0) condition followed by glucoamylase digestion at 60 °C for 4 h. After the digestion, the polysaccharides were precipitated by adding three volumes of alcohol and the residue was collected by centrifugation and it was uniformly dispersed in water and lyophilized (Nandini & Salimath, 2003).

2.3. Analytical methods

Estimation of total sugars was done by phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and uronic acids by carbazole method (Dische, 1947). Sugar composition in the flour and water-insoluble fractions was analyzed after solubilization with 72% sulfuric acid (in ice-cold temperature) followed by hydrolysis in 10% sulfuric acid at boiling water bath temperature for 6–8 h. The water-soluble fractions were hydrolyzed with 2 N trifluroacetic acid in sealed tubes at 100 °C for 5–6 h. The sugars were analyzed by gas–liquid chromatography as alditol acetates (Sawardekar, Slonekar, & Jeanes, 1967) on an OV-225 column at column temperature of 200 °C using a Shimadzu GLC and ferulic acid was quantified by HPLC upon alkaline hydrolysis (Shyamprasad Rao & Muralikrishna, 2007). Optical rotation of polysaccharides was determined at 20 °C using a Perkin-Elmer (model 243) polarimeter.

2.4. Methylation analysis

Polysaccharides (10 mg) were methylated by following the method of Hakomori (1964). Permethylated polysaccharides were hydrolyzed with formic acid and sulfuric acid, successively, acetylated and the partially permethylated alditol acetates were analyzed by GLC–MS. GLC–MS analysis was performed on a Shimadzu (Model QP 5000) GLC–MS using a SP-2330 capillary column (ϕ 0.31 mm $\times$ 30 mm) operating at an ionization potential of 70 eV with a temperature programme (180–200 °C, 4 °C rise per min). Chloroform was used as solvent and the carrier gas was helium.

2.5. Periodate oxidation

Polysaccharide solution (10 mg in 5 ml) was mixed with sodium meta periodate (5 ml, 20 mM) and kept at 4 °C in dark for 48 h. Aliquots were withdrawn from the sample at regular intervals (4 h) and the amount of periodate remaining was determined by following the earlier described method (Avigad, 1969). Formic acid liberated from the polysaccharide sample on oxidation was estimated by titrimetric method using sodium hydroxide.

2.6. Smith degradation

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

3. Results and discussion

3.1. Carbohydrate composition of native and purified pentosans from hemicellulose A

The carbohydrate composition of native and purified pentosans from hemicellulose A is given in Table 1. The carbohydrate content was higher in all the fractions of purified hemicellulose A compared to native samples (Table 1). Uronic acid contents were higher in HD-2189 (4.7%) and MACS-2496 (4.3%). Sugar composition analysis indicated that these fractions are rich in xylose residues along with moderate levels of arabinose and glucose in all the wheat varieties. Native fractions had substantial amount of glucose, a part of which may be coming from undigested starch molecules and other associated polysaccharides such as xyloglucans and glucans (Dupont & Selvendran, 1987). Small amounts of galactose might be due to galactoarabinoxylans and arabinogalactans, the presence of which has been reported in members of graminae (Izydorczyk & Biliaderis, 1995). The lower arabinose to xylose ratio in all these fractions might be due to the presence of higher unsubstituted xylan chains or xylan type of polysaccharides might exist. Presence of glucuronic acid in small amount along with arabinose and xylose, indicate them to be glucurono-arabinoxylans and a xyloglucan type of polysaccharide (Maes & Delcour, 2001; Nandini & Salimath, 2003). Higher amounts of glucose were present in poor *chapati*-making quality varieties (MACS-2496 and HD-2189). Wheat varieties such as DWR-162 and HD-2189 are having higher arabinose to xylose ratios.

3.2. Methylation analysis of purified pentosans

The pentosans from hemicellulose A were methylated by the method of Hakomori (1964) to study the nature of glycosidic linkages. Methylation and subsequent hydrolysis revealed that the xylose residues were present in three forms; un-substituted, monosubstituted and di-substituted (Table 2). Methylation analysis revealed xylose residues in the main chain with 1,4 linkages. Most of the xylose residues were un-substituted as evidenced by the presence of large amounts of 2,3-Me_2-Xyl. This was more in MACS-2496 and HD-2189 varieties which are having poor *chapati* making characteristics. Small amounts of terminal xylose residues were present in the pyranose form, as indicated by the presence of 2,3,4-Me_3-Xyl. The xylan backbone was substituted by arabinofuranosyl residue at O-3 position, as indicated by the presence of

Table 1
Carbohydrate composition of native and purified pentosans from hemicellulose A of whole-wheat flour of different varieties.

Samples	Total sugar	Uronic acid	Ara[a]	Xyl	Gal	Glc	Ara/Xyl ratio
DWR-162							
Native	88.1	2.6	10.8	66.5	1.2	21.5	0.16
Purified	90.0	3.7	19.2	74.6	1.0	5.2	0.25
GW-322							
Native	88.0	6.7	4.3	79.3	–	16.4	0.05
Purified	92.5	3.1	19.5	68.2	1.3	7.0	0.28
MACS-2496							
Native	73.8	6.0	5.8	50.5	–	43.7	0.11
Purified	86.0	4.3	11.2	78.0	2.4	8.4	0.14
HD-2189							
Native	95.5	3.3	5.0	60.4	–	34.6	0.08
Purified	94.5	4.7	12.8	76.5	1.5	9.2	0.16

[a] Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose.

Table 2
Methylation analysis of purified pentosans (%) from hemicellulose A polysaccharides.

Mode of linkage	Alditol acetates	DWR-162	GW-322	MACS-2496	HD-2189
$Ara_f \rightarrow 1$[a]	2,3,5-Me_3-Ara	10.7	10.2	8.7	8.2
$5 \rightarrow Ara_f \rightarrow 1$	2,3-Me_2-Ara	4.5	4.3	1.5	2.2
$2,3,5 \rightarrow Ara_f \rightarrow 1$	Ara	5.8	5.2	4.6	3.6
$Xyl_p \rightarrow 1$	2,3,4-Me_3-Xyl	11.7	12.5	10.8	12.2
$4 \rightarrow Xyl_p \rightarrow 1$	2,3-Me_2-Xyl	52.6	51.3	60.2	58.5
$3,4 \rightarrow Xyl_p \rightarrow 1$	2-Me-Xyl	10.2	11.6	7.8	8.7
$2,3,4 \rightarrow Xyl_p \rightarrow 1$	Xyl	3.5	4.0	2.0	3.0
$4 \rightarrow Glcp \rightarrow 1$	2,3,6-Me_3	0.8	0.5	3.2	2.3
$3 \rightarrow Glcp \rightarrow 1$	2,4,6-Me_3	0.2	0.4	1.2	1.3
–	$\frac{2,3\text{-}Me_2\text{-}Xyl}{2\text{-}Me\text{-}Xyl+Xyl}$	3.8	3.28	6.14	5.0

[a] Abbreviations as in Table 1.

2-Me-Xyl. Doubly substituted xylose residues were also found in minor amounts in all the wheat varieties as indicated by the presence of xylitol among the methylated species. Di-substituted xylose residues have been reported in wheat (Nandini & Salimath, 2003), rice (Shyamprasad Rao & Muralikrishna, 2007) and sorghum (Nandini & Salimath, 2002). Small amounts of arabinosyl residues were present as terminal sugars, indicated by the presence of 2,3,5-Me_3-Ara. Main chain arabinosyl residues (2,3-Me_2-Ara) were also observed in minor amounts in all the wheat varieties. Short arabinosyl chains have been reported in branched arabinoxylans (Izydorczyk & Biliaderis, 1995). Variations were observed in the degree of branching as indicated by the ratio of unbranched to branched xyloses. The ratio was higher in MACS-2496 and HD-2189 indicating that pentosans in these varieties are less branched compared to DWR-162 and GW-322 wheat varieties. The hemicellulosic polymers from the cell walls of wheat bran sequentially extracted with alkali showed both high and low degree of branching (Nandini & Salimath, 2003; Schooneveld, Beldman, & Voragen, 1999). In addition to xylopyranose and arabinofuranose, glucopyranosyl residues linked at C-3 and C-4 were also detected, suggesting that glucose in pentosan fractions from hemicellulose A might originate from β-(1 → 3), (1 → 4), glucans and their concentration was higher in MACS-2496 and HD-2189 poor varieties (Izydorczyk & Biliaderis, 1995).

3.3. Periodate oxidation and Smith degradation

The arrangement of branching in pentosans was also investigated by the periodate oxidation of pentosans. Periodate consumed per mole of anhydrosugar was lowest in GW-322 (0.50) followed by DWR-162 (0.54, Table 3) indicating higher degree of branching in these polysaccharides, which is also evident from their higher arabinose content (Table 1). Highly branched arabinoxylans obtained from sorghum were shown to consume about 0.64 mol of periodate over 24 h of oxidation (Nandini & Salimath, 2003). Trace amount of formic acid was released. Glycerol, arabinose and xylose were the major Smith degradation products identified. The products obtained by the Smith degradation further substantiated periodate oxidation results. Smith degradation analysis of the glucurono-arabinoxylans from sorghum showed high amount of glycerol and xylose (Woolard, Rathbone, & Novellie, 1976). Arabinoxylans from native and malted ragi showed high amount of glycerol and xylose (Subbarao & Muralikrishna, 2006). Blocks of two neighbouring di-substituted xylose residues have also been reported in wheat arabinoxylans (Hoffmann, Kamerling, & Vliegenhart, 1992).

Table 3
Moles of periodate consumed, ferulic acid contents (μg/g) and optical rotation of purified pentosans from hemicellulose A.

Wheat variety	Moles of periodate consumed	Ferulic acid (μg/g)	$[\alpha]_D$
DWR-162	0.54	240.0	−100.21
GW-322	0.50	250.5	−84.54
MACS-2496	0.65	205.6	−66.33
HD-2189	0.68	210.4	−80.42

3.4. Optical rotation measurements

Optical rotation values were −100.2° and −84.5° for DWR-162 and GW-322, respectively, while MACS-2496 and HD-2189 had −66.4° and −80.4°, respectively (Table 3). High negative optical

rotation value indicates preponderance of β-linkages in the xylan backbone. These results are in agreement with the optical rotation values reported in the literature for arabinoxylans (Nandini & Salimath, 2002; Subbarao & Muralikrishna, 2006).

3.5. Ferulic acid content

The ferulic acid content (μg/g) of purified pentosans is given in Table 3. DWR-162 had highest ferulic acid content (240 μg/g) followed by GW-322 (220.5 μg/g). The values reported here are low compared to other pentosans reported in the literature (Izydorczyk & Biliaderis, 1995; Rybka et al., 1993; Shyamprasad Rao & Muralikrishna, 2007). In wheat, ferulic acid is esterified to arabinose residues of cell wall arabinoxylans. Ferulic acid may be involved in cross-linking of cell wall polysaccharides in wheat through ester and ether bonds (Hatfield, Ralph, & Grabber, 1999; Smith & Hartley, 1983).

Chapati, the flat unleavened baked product prepared from whole-wheat flour, is the main traditional staple food in the Indian subcontinent. Wheat varieties, GW-322 and DWR-162 revealed good *chapati* making characteristics, while, MACS-2496 and HD-2189 had poor *chapati*-making quality are reported earlier (Revanappa et al., 2007). Pentosans from hemicellulose A of wheat varieties consists of mainly xylan backbone in β-(1,4) linkages to which small amounts of arabinose residues were attached at O-3 and/or O-2 and O-3 positions by α-linkages. Pentosans from wheat varieties such as DWR-162 and GW-322 had higher degree of xylan substitution by arabinose residues compared to MACS-2496 and HD-2189. It is generally accepted that differences in AX structure, even relatively small, can result in changes of chains of conformation and intermolecular association, which may have an impact on functionality of these polysaccharides (Ordaz-Ortiz, Devaux, & Saulnier, 2005).

Poor *chapati* making varieties had high amounts of glucose compared to good varieties. High amounts of glucose could be arising from β-glucans which are strongly associated with the arabinoxylans. The strong association between alkali-extractable arabinoxylans and β-glucans is believed to be caused by their non-covalent interactions between them and also with other cell wall material such as cellulose and lignin (Maes & Delcour, 2001). Their insolubility nature may be due to diferulic acid cross-links occurring between arabinoxylan chains (Fincher & Stone, 1986; Rybka et al., 1993). Pentosans from good roti making qualities of sorghum and bajra varieties are highly branched in nature (Nandini & Salimath, 2001, 2002). The contents of arabinose and xylose were reported to be higher in varieties of wheat that have good tandoori *roti* making quality (Saxena, Salimath, & Haridas Rao, 2000). Ferulic acid association with pentosan components has been known to be involved in oxidative gelation reaction (Izydorczyk & Biliaderis, 1995). Ferulic acid content was more in good *chapati* varieties compared to poor varieties.

4. Conclusions

Thus, the result presented in this communication, indicated variation in the structural feature of pentosans from hemicelluloses A between the wheat varieties differing in their *chapati* making potential. Pentosans mainly consist of xylan backbone to which arabinose are substituted to greater degree in good *chapati* making varieties (DWR-162 and GW-322). Pentosans from poor *chapati* making varieties (MACS-2496 and HD-2189) are mainly xylan in nature and have strong association with β-glucans and cellulosic polysaccharides. Higher ferulic acid substitution in good *chapati* making varieties (GW-322 and DWR-162) may also be one of the contributing factors to the changes in functional properties in terms of *chapati*-making quality.

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