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Non-starch polysaccharide composition of two cultivars of banana (*Musa acuminata* L.: cvs Mysore and Nanicão)

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Abstract

Fruits represent a rich source of soluble and insoluble fibre, and the pectin is the most common and known soluble fraction from the cell wall solubilization occurring during fruit ripening. Banana fruit, for example, is one of the most consumed fruits in the world, but its non-starch polysaccharide composition is almost unknown. Despite few works have been carried out about the enzymes concerning cell wall loosening focusing banana ripening, there is no knowledge about the composition of the banana cell wall. Moreover, there is no information about the influence of the cultivar in that composition. Nanicão and Mysore cultivars were chosen for this work because of their differential accumulation of both starch during development and amounts of total fibre in the ripe fruit. Nanicão and Mysore had their fibres subfractioned and their composition analysed. Results showed that the cultivars are distinct not only in terms of starch and soluble sugars accumulation, but also in non-starch polysaccharides amounts and composition. Non-starch polysaccharides are similar in total amounts in both banana cultivars (~3.5), but substantially different in the content of CDTA and NaOH-4M soluble fractions and also in the molecular mass distribution of WSP and CDTA. Nanicão has more calcium-linked pectin than Mysore, which in turn is richer in hemicellulose-like polysaccharides. Both cultivars likewise cereals polysaccharides seem to be composed of galacturonans and arabinoxylans.

Keywords: Banana; Cell wall; Dietary fibre; Non-starch polysaccharides

1. Introduction

Dietary fibre (DF) is a collective term for a variety of plant substances that are resistant to digestion by human gastrointestinal enzymes. Edible plant cell wall polysaccharides, lignin and associated substances make up the bulk of the fibre fraction. Based on this definition, resistant starch, wheat bran, oat bran and fructo-oligosaccharides are included in the category of fibre (Englyst & Englyst, 2005). DF terminology and classification can differ, considering the various countries and the knowledge about food components resistant to the human digestion. For instance, the term "non-starch polysaccharides" has been used instead DF, in the United Kingdom, in nutritional labelling. Recently, "total fibre" was classified as Dietary Fibre and Functional Fibre. The former was defined as "the non-digestible carbohydrate and lignin that are intrinsic and intact in plants" and the latter, "isolated, non-digestible carbohydrates that have beneficial effects in humans" (Jenkins, Marchie, Augustin, Ros, & Kendall, 2004).

In spite of its resistance to human digestion, DF is far from being inert. Depending on its components and consequent solubility in water, it is classified to soluble or insoluble forms which can provide many health benefits. Because of its multiple components with different physical and chemical properties, DF has the capacity to either sorb or bind many harmful substances, as cholesterol, glucose, hydrochloric acid and heavy metals, reducing their levels in the organism. For this, DF consumption has been linked

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to the prevention and treatment of atherosclerosis, coronary heart diseases, diabetes and large intestine cancer (Aldoori et al., 1998; Bosaeus, 2004; Jenkins et al., 2004).

Fruits are a rich source of soluble and insoluble fibre, being the pectin the most common and known soluble fraction from the cell wall solubilization occurring during fruit ripening. Banana fruit, for example, is one of the most consumed fruits in the world, but its non-starch polysaccharide composition is almost unknown. Despite few works have been done in the enzymes concerning cell wall loosening focusing banana ripening (Pathak, Mishra, & Sanwal, 2000; Lohani, Trivedi, & Nath, 2004; Asif & Nath, 2005), there is no knowledge about the composition of the banana cell wall. Kayisu, Hood, and Vansoest (1981) and Wade, Satyan, and Kavanagh (1993) worked in this subject employing harsh cell wall extraction procedures, which nowadays are not acceptable because soluble cell wall fraction can be seriously damaged, leading to considerable losses.

Moreover, there is no information about the influence of the cultivar in that composition. Based on the knowledge of other banana carbohydrates, the amount and composition of non-starch polysaccharides can be very different among cultivars. Nanicão and Mysore cultivars were chosen because they accumulate different amounts of starch (~20 and less than 12%, respectively) during development, which accounts for different amounts of soluble sugars accumulation (~18% and 10%) during ripening and their flours also differ in DF content. The latter showing three times more insoluble fraction in its composition (Mota, Lajolo, Ciacco, & Cordenunsi, 2000).

The aim of this work was to characterize the non-starch polymers composition of two banana cultivars that differs in terms of dietary fibre and a possible relationship with a positive effect of these compounds to the human health.

2. Material and methods

2.1. Material

Banana fruit (*Musa acuminata* L.) cultivars Mysore (AAB) and Nanicão (AAA), were harvested in a plantation located in Itapetininga (São Paulo State, Brazil). Bananas with approximately 110 days after anthesis (daa) related to the physiological maturity were stored at temperature (20 °C) and moisture (~90%) under control. Based on their respiration and ethylene production, ripe bananas were peeled, sliced and immediately frozen in liquid N_2, and stored at −80 °C. At the time of analysis, samples were thoroughly homogenised by powdering in liquid nitrogen. Each sample was composed of at least 10 banana fingers.

2.2. Cell wall isolation

The water soluble polymers (WSP), the pectins immobilised by calcium (CDTA soluble polymers) and 4M NaOH soluble polymers were isolated according to the chemical-enzymatic method as described in Shiga and Lajolo (2006).

2.3. Ion-exchange chromatography

Approximately 10 mg of the fraction water soluble polymers (WSP) were injected on a 5 mL HiTrap™ Q-Sepharose™ FF (Amersham Pharmacia Biotech, Uppsala, Sweden), equilibrated in phosphate buffer (0.02 M, pH 6.8) containing 5 mM NaN_2 and 20 mM NaCl. Polymers were eluted sequentially with 12 mL of equilibrating buffer and phosphate buffer (0.02 M, pH 6.8) containing 0.5 M and 1 M NaCl. The fractions were collected and assayed for total sugar (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and uronic acid content (Filisetti-Cozzi & Carpita, 1991). The fractions were dialysed, freeze dried and weighed.

2.4. Size exclusion chromatography

Size exclusion chromatography was performed in the fractions water soluble polymers and CDTA soluble polymers isolated from Mysore and Nanicão cultivars on a C16/70 column, 16 mm × 70 cm (Pharmacia, Sweden), and packed with Sephacryl S-400 (Pharmacia). The column was equilibrated and eluted with phosphate buffer (pH 7.0; 20 mM NaN_2; 500 mM NaCl). The void and included volumes of the columns were determined by blue dextran (Pharmacia) and glucose. The molecular weight standards used were dextran polymers (fractionation range 50–3500 kDa, Fluka, Switzerland). The flow was fixed in 0.2 mL/min and 2 mL fractions were collected.

2.5. Uronic acids (UA) determination

The UA content was determined according to Filisetti-Cozzi and Carpita (1991).

2.6. Neutral sugars (NS) determination

The neutral sugars rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal) and glucose (Glc), except for the glucose derived from the cellulose (CEL) released by acid hydrolysis of all polysaccharide fractions obtained from both cultivars, were quantitatively determined by gas–liquid chromatography after reduction and acetylation (Blakeney, Harris, Henry, & Stone, 1983; Carpita & Whittern, 1986; Fox, Morgan, & Gilbart, 1989). All sugar standards were purchased from Sigma–Aldrich Co. (Saint Louis, MO, USA). The derivatives were dissolved in ethyl acetate and injected into HP6890 gas chromatograph (GC) equipped with a flame ionisation detector (FID) (Hewlett–Packard, USA). The alditol acetates were separated using a SP-2330 fused-silica capillary column (0.25 mm × 30 m) with a 0.20 μm film thickness (Supelco, Inc., Bellefonte, PA, USA). The column temperature was programmed from 170 °C to 240 °C at 10 °C/min with a 20 min hold at the highest temperature. Injector and detector temperatures were set at

250 °C. Helium was used as carrier gas at a flow rate of 30 mL/min.

2.7. Soluble sugars

Soluble sugars were extracted three times with 80% ethanol at 80 °C. After centrifugation, the supernatants were combined and the ethanol was evaporated under vacuum. The residues were reconstituted with water, filtered through 0.22 μm membrane filters and analysed by a high pressure liquid chromatography with pulse amperometric detection (HPAE-PAD – Dionex, Sunnyvale, CA, USA), using PA_1 column (Dionex, Sunnyvale, CA, USA) in an isocratic run of 18 mM NaOH during 25 min.

3. Results and discussion

3.1. Carbohydrates composition of Mysore and Nanicão banana cultivars

According to Mota, Lajolo, and Cordenunsi (1997) 'Mysore' accumulates less starch than 'Nanicão' during development (12% and 20%), the amount of soluble sugars accumulated during 'Nanicão' ripening is bigger. The same result was observed in this work (Table 1). As usual, sucrose was the predominant sugar over fructose and glucose, in both cultivars. The amounts of the soluble fibre fraction were similar among cultivars, but the insoluble fraction was twice higher in 'Mysore' than 'Nanicão'. Residual starch was higher in Nanicão but, according to our experience, both cultivars achieve very low starch levels when full ripen. No other monosaccharide besides glucose and fructose could be detected by HPLC-PAD.

3.2. Cell wall polysaccharides recovery

Cell wall polymers account for about 3.5% of whole banana pulp in both cultivars (Table 2), while the dietary fibre values detected by DF-specific methodology, were quite different between both cultivars: 2.7% for Nanicão and 4.2% for Mysore (Table 1). The difference may be credited to differences in the methodologies used for each procedure. The water soluble polymers (WSP) and the residue fractions were similar in quantities among cultivars, but different in terms of CDTA and 4M-NaOH fractions content. The former was 75% higher in Nanicão than Mysore, while the latter was 60% lower. Table 3 helps to understand the difference between cultivars taking into account the relative percentage of carbohydrate polymeric fractions in total cell wall extracted: CDTA-fraction corresponds to 27% of total cell wall in Nanicão and only 7% in Mysore.

Table 2
Relative percentage of polymeric carbohydrate fractions (g/100g of fresh fruit) from two banana cultivars

Fractions	Nanicão (g/100 g fruit pulp)	Mysore (g/100 g fruit pulp)
WSP	0.6 ± 0.05	0.7 ± 0.16
CDTA	0.8 ± 0.05	0.2 ± 0.02
4M	1.0 ± 0.08	1.6 ± 0.03
Residue	0.4 ± 0.02	0.4 ± 0.02
Total	3.5 ± 0.14	3.6 ± 0.14

WSP means water soluble polymers (WSP), CDTA, polymers solubilised by CDTA solution; 4M, polymers solubilised by 4M NaOH.

Table 3
Relative percentage of carbohydrate polymeric fractions (g/100g of cell wall) of total cell wall extracted from two banana cultivars

Fractions	Nanicão (%)	Mysore (%)
WSP	22.2 ± 1.23	24.2 ± 0.16
CDTA	27.2 ± 1.11	6.7 ± 0.02
4M	36.9 ± 1.45	55.3 ± 3.06
Residue	13.8 ± 0.43	13.8 ± 1.11
Total	100.0	100.0

WSP means Water soluble polymers (WSP), CDTA, polymers solubilised by CDTA solution; 4M, polymers solubilised by 4M NaOH.

3.3. Water soluble polymers (WSP)

The WSP extracted from both cultivars were mainly composed of uronic acids (UA) (~140 μg/mg of material), and low quantities (less than 29 μg/mg of material) of arabinose, xylose, galactose and glucose (Fig. 1). Mysore contains two times more arabinose and one time more xylose than Nanicão. In spite of their low quantities, the cultivars showed substantial differences in the contents of these monosaccharides. On the other hand, the amount of UA, galactose and glucose were, respectively, 18%, 30% and 200% lower in the same cultivar. Most of these polymers are concentrated in a range of molecular mass between 5–85 and 20–150 kDa for 'Nanicão', and 'Mysore', respectively (Fig. 2). The Ion-exchange chromatography profile (Fig. 3) showed that most of the Water-soluble fraction was composed of Neutral-polymers (69% and 77% for 'Mysore' and 'Nanicão', respectively), being the Acidic-polymers the minor fraction (31% and 23%, for 'Mysore' and 'Nanicão', respectively). The latter was predominantly composed of uronic acids with no great differences in the

Table 1
Carbohydrates (g/100 g) (Mean ± SD of triplicate assays) of two banana cultivars

Cultivar	Moisture	Soluble sugars			DF		Starch
		Glucose	Fructose	Sucrose	Soluble	Insoluble	
Nanicão	70.93 ± 0.48	2.34 ± 0.05	1.98 ± 0.22	15.2 ± 1.10	1.07 ± 0.24	1.68 ± 0.11	3.47 ± 0.11
Mysore	73.62 ± 0.36	3.4 ± 0.4	2.6 ± 0.29	8.1 ± 0.96	1.33 ± 0.2	2.86 ± 0.14	1.69 ± 0.07

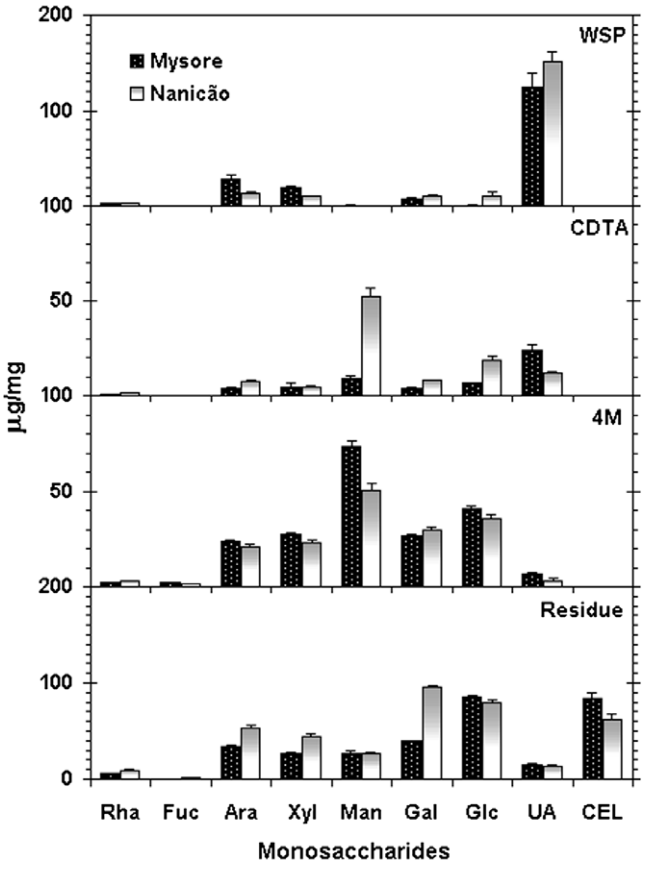

Fig. 1. Banana cell wall polymers profile. WSP, water-soluble polysaccharides; CDTA, CDTA-soluble polymers; 4M, 4M NaOH-soluble polymers; Residue, cellulose-rich residue.

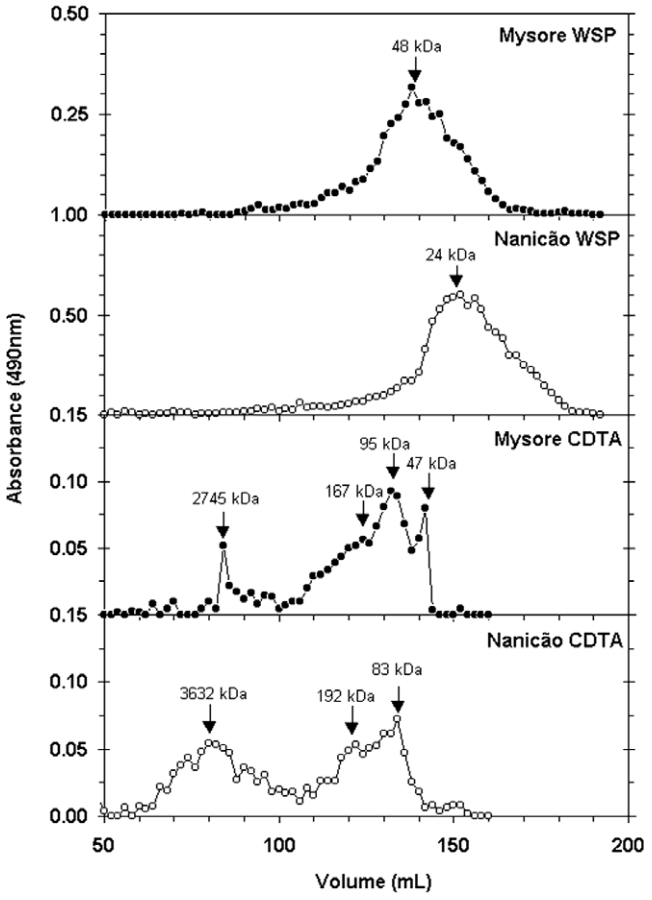

Fig. 2. Neutral and acidic sugar profile in Nanicão and Mysore cultivars cell wall polysaccharides. Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid.

cultivars. On the contrary, the neutral-polymers, mostly composed of uronic acid, glucose, mannose, arabinose, xylose and galactose, were very distinct among cultivars. The monosaccharide profile of the water soluble polymers (WSP) suggest that this fraction is mainly composed of polygalacturonans (Fig. 1). The ratio of arabinose to xylose was nearly 2:1 for Mysore and 1:1 for Nanicão, suggesting the presence of arabinoxylan in the composition in the water soluble polymers (Tables 4 and 5).

3.4. CDTA-soluble polymers

The main monosaccharide in CDTA-fraction was mannose (52 µg/mg) in Nanicão, and UA (24 µg/mg) in Mysore. The other sugars were arabinose (4 and 7 µg/mg for Mysore and Nanicão, respectively), xylose (5 and 4.5), galactose (4 and 8) and glucose (6 and 18). The cell wall polymers solubilized by CDTA solution is supposed to be calcium-linked pectins which match with the monosaccharide composition found in Mysore-CDTA fraction. Although, the large amounts of mannose found in Nanicão-CDTA are not usual in this fraction. The ratio of mannose to glucose (Tables 4 and 5), strongly suggest glucomannan composition in the CDTA-soluble polymers of Nanicão. The ratio of arabinose to xylose was nearly 2:1 for Nanicão and 1:1 for Mysore, which suggests arabinoxylan composition in the pectin-fraction. This composition is usual for commelinoid monocots, and is commonly found in the Poaceae (grasses and cereals) (Smith & Harris, 1999). Banana is a commelinoid monocot from the order of Zingiberales and subclass of Zingiberidae and, likewise Poales, has cell wall type II composed largely of glucuronoarabinoxylans. The similarity of their non-starch polysaccharides with those found in cereals leads to the presumption that the effects of these polymers in the intestinal function may be quite close.

The polymers of the Mysore-CDTA fraction seem to be concentrated in a range of low molecular mass (between 40 and 450 kDa), while the polymers of Nanicão were mostly distributed in two distinct ranges of molecular mass: a high one (290–7300 kDa), and low one (60–290) (Fig. 2).

3.5. 4M-NaOH soluble polymers

Similarly to CDTA-fraction, the alkali-soluble polymers also showed low sugar recovery, with about 17–20% of the total mass analysed. The bulk of carbohydrates in both cul-

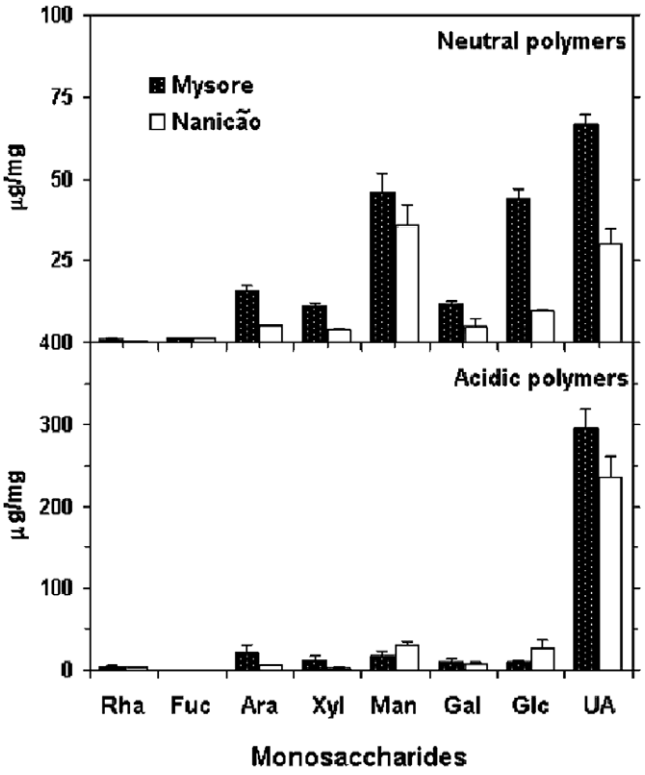

Fig. 3. Neutral and acidic sugar profile in Nanicão and Mysore cultivars cellulose-rich residues. Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acid, CEL, cellulose-rich residue.

Table 4
Sugar composition of the Mysore banana cell wall polysaccharides

Sugar	WSP (mM/g)	CDTA (mM/g)	4M (mM/g)	Residue (mM/g)
Rha	13.6	3.5	13.0	31.7
Fuc	–	–	13.8	–
Ara	191.1	26.4	155.5	222.3
Xyl	127.2	32.2	183.8	180.2
Man	0	55.2	446.4	163.5
Gal	39.7	20.5	146.2	218.5
Glc	0	35.3	225.5	472.7
UA	642.3	124.6	34.9	74.4
CEL	0	0	0	468.0
Ara/Xyl	1.5	0.8	0.8	1.2
Man/Glc	–	1.6	2.0	0.3

tivars, Mysore and Nanicão, was composed of mannose rich polymers (~73 and 51 µg/mg, respectively) (Fig. 1). The mannose to glucose ratio (Tables 4 and 5) suggest (similarly to CDTA-fraction), glucomannan composition in this fraction. This fraction was also composed by high amounts of arabinose (23 and 21), xylose (27 and 23), galactose (26 and 29) and glucose (40 and 35). The monosaccharide composition suggests that the alkali-soluble polymers are composed of hemicellulose described in literature as a heterogeneous group of substances made up of β-glucans, xylans and mannans (Carpita & McCann, 2000). In both cultivars, remarkable amounts of glucose appeared in NaOH-soluble polymers, which could be considered starch contamination. To avoid this artefact, special care was taken as follows: additional steps of enzymatic hydrolysis were performed when the presence of starch granules was detected by light microscopy in the pellet resultant of the cell wall fractionating steps. It was not possible to solubilize the 4M-fraction in the chromatography conditions in order to determine their polymers molecular mass.

Table 5
Sugar composition of the Nanicão banana cell wall polysaccharides

Sugar	WSP (mM/g)	CDTA (mM/g)	4M (mM/g)	Residue (mM/g)
Ram	19.6	8.4	15.4	52.0
Fuc	–	–	10.1	7.9
Ara	92.2	48.4	140.5	354.2
Xyl	64.2	30.2	155.0	294.7
Man	0	318.1	308.8	163.5
Gal	56.8	46.4	162.7	530.4
Glc	61.0	102.3	195.4	443.1
UA	779.7	61.4	15.8	68.4
CEL	0	0	0	340.5
Ara/Xyl	1.4	1.6	0.9	1.2
Man/Glc	–	3.1	1.6	0.4

3.6. Insoluble fraction

The residue after the cell wall solubilization in water, CDTA and NaOH was a cellulose-rich one composed of large amounts of glucose probably released from hemicellulose (85 and 80) and cellulose (84 and 61) (Fig. 1). Substantial amounts of galactose (39 and 96), xylose (27 and 44), mannose (both 27), arabinose (33 and 53) and UA (14 and 13) were also found. Rhamnose (6 and 9) and fucose were found in low amounts, suggesting the presence of galacturonans.

4. Conclusions

Non-starch polysaccharides are similar in total amounts in both banana cultivars (~3.5), but differ substantially the content of CDTA and NaOH-4M soluble fractions and also in the molecular weight distribution of WSP and CDTA. Nanicão has more calcium-linked pectin than Mysore, which, in turn, is richer in hemicellulose-like polysaccharides. Both cultivars, likewise cereals polysaccharides seem to be composed of galacturonans and arabinoxylans.

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