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Water-extracted polysaccharides of selected cereals and influence of temperature on the extractability of polysaccharides in sorghum

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Abstract

Non-cellulosic and non-starchy (I_2-KI negative) glucose-rich polysaccharides from wheat, ragi, rice and sorghum were extracted with water at ambient temperature (25°C). Ethanolic fractional precipitation of the polysaccharides yielded a number of fractions containing varying proportions of hexoses (0.7–98%) and pentoses (1–56%). Polysaccharides from sorghum were also extracted at 4, 55°C and at boiling water temperature. Prior amylolysis of the sorghum flour followed by extraction at boiling water temperature was found to give a better yield of non-starch glucose-rich polysaccharides.

Keywords: Cereals; Polysaccharides; Starch; Sorghum

1. Introduction

Knowledge on the nature of minor polysaccharide constituents of cereals, apart from starch and non-starch carbohydrates, is limited to their content and a preliminary sugar composition. Endosperm of cereals usually contains, in addition to various other polysaccharides, both α- and β-D-glucans (α- and β-(1→4)-linked, starch and cellulose, respectively). Aleurone, subaleurone and endospermic cell walls in cereals contain mainly mixed-linkage β-D-glucans (β-(1→3, 1→4)-D-glucans, MLG). It is reported that these glucans, having a structural function and at times also acting as an energy reserve (Mares and Stone, 1973) are both water soluble and alkali-soluble.

Beneficial implications of MLG in nutrition and health of the consumer have been well documented. These include cholesterol-lowering properties (Anderson, Cook, and Stone, 1978; Wood, 1984), modulatory effects on blood glucose, and hormone levels, suppression of colonic cancer and implications in micronutrient availability (Bhatty, 1993). On the other hand, in the malting of barley, the presence of MLG is highly disadvantageous, as it considerably decreases the wort filtration rate and the quality of resulting beer (Aman and Hesselman, 1985). Food enrichment with barley MLG is deleterious to the health of poultry, as it reduces the feed intake ratio (because of its high viscosity) and also it results in sticky dumplings (Walsh, Power, and Haedon, 1994). The methods of analysis of MLG are various and laborious, involving different enzymes and numbers of steps. The non-enzymatic method, using alkaline copper sulfate, has also been reported (Madacsi, Parrish, and Roberts, 1983). Different methods of aqueous extraction of MLG from cereals have shown the influence of temperature, time and pH on the solubility and overall yield (Fleming and Kawakami, 1977; Kato et al., 1983; Knuckles, Chiu, and Betschart, 1992; Woodward, Phillips, and Fincher, 1988). In barley and oats MLG has been almost entirely extracted with water (McCleary, 1988). In the present investigation, conventional non-enzymatic methods based on solubility differences (Wood, Fulcher, and Stone, 1983) have been adopted to extract glucose-rice polysaccharides from selected cereals. The influence of elevated temperature on the extractability of non-starchy glucan-rich polysaccharides from sorghum has also been examined.

2. Materials and methods

2.1. Materials

Authentic varieties (10 kg each) of cereal grains, viz., ragi (finger millet, *Eleusine coracana*, variety HR 911);

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wheat (*Tritium aestivum*, variety kalyansona); rice (*Oryza sativa*, variety vijaya with DM[1] 7.5 and 2.1, Desikachar, Raghavendra Rao, and Ananthachar, 1965) were procured from the local market of Mysore, whereas sorghum (*Sorghum bicolor*, variety SB-905) was procured from the Agricultural station, Dharwad. After cleaning they were sun-dried and ground in a standard plate mill to pass through a 60–100 mesh sieve. Flours were kept in the cold (4°C) till their usage. All chemicals used were of analytical reagent grade.

2.2. Methods

2.2.1. Extraction of polysaccharides

Cereal flours (500 g each) were thoroughly extracted for 3 h (×4) with water (0.8 L×3) at either ambient temperature (~25°C) or 4 or 55°C and centrifuged (1788 ***g***, 10 min). From the respective supernatants, the polysaccharides were precipitated using ethanol (3 vol). They were dissolved in water (25 ml), dialyzed overnight against distilled water and lyophilized.

2.2.2. Extraction at boiling water temperature

An aqueous suspension of sorghum flour (10%) was gelatinized (30 min in boiling water bath) and subjected to amylolysis using glucoamylase (E.C. No. 3.2.1.3, *A. niger*, Sigma, USA; sodium acetate buffer, 0.05 M, pH 4.8, at 60°C for 15 min). After centrifugation, the supernatant was discarded and the residue was subjected to aqueous extraction (0.40 L×3) at boiling water temperature (2 h×3). From the supernatant, boiling water-soluble polysaccharide (BWSP) was precipitated by ethanol, partially purified by dialysis of the aqueous solution and lyophilized.

2.2.3. Fractionation of polysaccharides

2.2.3.1. Precipitation with ethyl alcohol. To an aqueous solution (100 ml) of the polysaccharide (2%), ethanol in small aliquots of known volume was added slowly with stirring to incipient turbidity (see Tables 1 and 2 for individual concentration of ethanol). The solution was kept at 4°C to facilitate aggregation. It was centrifuged, the sediment washed with the same concentration of ethanol, solubilized in water and dialyzed and lyophilized (Fraction No. 1). To the supernatant, further additions of ethanol (up to ~75–82%) were continued and precipitate obtained at each step (Fraction Nos. 2–5) was separated, purified and dried. The final supernatant was concentrated to dryness (Fraction Nos. 4, 5 or 6, see Tables 1 and 2).

2.2.3.2. Precipitation with ammonium sulphate. To an aqueous solution (100 ml) of the polysaccharide (2%), solid ammonium sulphate, in small quantities was added (40–60% saturation) slowly with constant stirring till some turbidity appeared. It was kept at 4°C for 1 h to facilitate complete aggregation of the precipitate. After centrifugation, the precipitate, dissolved in water (10 ml) was dialyzed and lyophilized (Fraction No. 1). Ethanol precipitation (3 vol) of the supernatant gave Fraction No. 2.

2.2.3.3. Fractionation by DEAE—cellulose chromatography. An aqueous solution of the sample (1%, 10 ml) was applied on top of the DEAE-cellulose column (66 × 3.8 cm) and successively eluted with water, ammonium carbonate (0.5 M) and sodium hydroxide (0.2 M) solutions. Fractions (12–14 ml) collected were assayed for total sugars.

2.2.3.4. Fractionation by gel permeation chromatography. The polysaccharide solutions (10 mg/2 ml H_2O) were loaded on a Sephacryl S-400 column (100×1.5 cm) and eluted with water at a flow rate of 18 ml/h (Madhusudhan and Tharanathan, 1996). Fractions (3 ml) collected were analysed for total sugars.

2.2.4. Helix coil transition analysis (HCT)

Congo Red (88 μM in 0.001 M NaOH) was added to the polysaccharide solution (10 mg/ml in 0.001 M NaOH) and the absorbance (λ_{max}) of the resulting complex was determined by scanning from 400 to 650 nm (Williams et al., 1991).

2.2.5. General methods

Total sugar and D-glucose were determined by the modified phenol-H_2SO_4, (Rao & Pattabiraman, 1989) and D-glucose oxidase (Dahlqvist, 1964) methods, respectively. The polysaccharides were acid hydrolysed by the complete hydrolysis procedure (Ramadas Bhat & Tharanathan, 1986). The acid hydrolysates were neutralized (solid $BaCO_3$) reduced ($NaBH_4$), acetylated (pyridine-acetic anhydride, 1:1, v/v, 100°C, 1 h) and analysed by gas liquid chromatography on an OV-225 column (ss, 8 ft×118 in), 3% on Chromosorb W (100–200 ml) at an isothermal temperature of 190°C. Inositol was the internal standard used and the molar response factor was calculated for each sugar for accurate determination.

3. Results and discussion

3.1. Polysaccharides extracted at ambient (25°C) temperature

A number of polysaccharide fractions from ragi (Rg 1–6), sorghum (S 1–6), wheat (W 1–5), rice (R 1–5), rice bran (Rb 1–4) and wheat bran (Wb 1–6) were extracted with water and fractionated. From Table 1, it is clear

[1] DM = Degree of milling.

Table 1
Carbohydrate composition (%) of ethanol-precipitated fractions of cereal polysaccharides aqueous-extracted at 25°C

Cereal	Fraction No.		Ethanol (%)	Yield[a]	Total sugars[b]	Relative distribution of sugars (%)				
						Rha	Ara	Xyl	Gal	Glc
Ragi	Rg	1	50.0	0.01	39.3	–	16.3	4.5	7.2	11.2
		2	56.6	0.01	60.6	–	14.9	11.2	–	34.4
		3	61.5	0.02	98.8	1.8	22.0	7.5	–	67.4
		4	78.2	0.08	95.8	–	56.0	9.1	–	30.5
		5	81.8	0.11	90.2	–	43.6	12.8	11.2	22.4
		6	Supernatant	0.14	71.2	5.5	8.4	–	–	57.2
Sorghum	S	1	50.0	0.01	60.0	–	14.0	9.7	4.5	31.5
		2	58.3	0.01	71.2	–	15.2	8.9	–	46.9
		3	61.5	0.03	56.2	–	6.8	3.4	3.6	42.4
		4	66.6	0.07	84.3	–	24.6	4.5	–	55.0
		5	73.6	0.06	93.7	–	13.6	2.5	5.9	69.1
		6	Supernatant	0.12	90.0	–	4.9	–	–	83.5
Wheat	W	1	50.5	0.60	61.8	0.8	21.5	28.3	–	11.0
		2	56.5	0.21	97.8	2.2	22.9	21.9	–	50.4
		3	64.2	0.26	98.6	–	28.7	13.0	10.7	46.0
		4	76.7	0.20	93.7	–	24.7	1.9	37.5	26.9
		5	Supernatant	0.11	60.3	–	3.0	–	3.4	50.0
Rice (DM 2.1)	R	1	50.0	0.05	22.5	–	6.6	–	3.6	12.3
		2	61.5	0.12	86.2	2.2	2.8	–	–	80.9
		3	74.5	0.09	82.5	1.8	4.3	–	–	80.1
		4	81.9	0.01	77.7	1.1	1.0	–	–	75.4
		5	Supernatant	0.25	65.6	–	–	–	–	65.6
Rice (DM 7.5)	R	1	43.8	0.56	98.0	–	–	–	–	98.0
		2	52.2	0.10	97.5	–	–	–	–	97.5
		3	67.0	0.11	90.0	–	2.3	–	–	87.5
		4	74.8	0.07	58.1	–	5.5	–	–	52.5
		5	Supernatant	0.18	95.6	–	–	–	–	95.6

[a] The yield is expressed as weight percentage (as is basis).
[b] Total sugars expressed as weight percentage of each fraction.

that the recovery values of the polysaccharide fractions varied from 0.01 to a maximum of 0.6%. Their compositional analyses revealed considerable variations in that the first four or five fractions were predominantly of pentosan type, whereas the final supernatant fractions were exclusively rich in glucose. The latter, though negative to the I_2-KI blue colour test, were found to be hydrolysed (to ~30%) by glucoamylase, indicating a possible α-glucan contamination. Their negative reaction with I_2-KI could be attributed to lower iodine binding capacity (for example, starch hydrolysis products) as has been observed before (Cagampang, Battle Creek Kirleis, 1985). The presence of cellulosic material in these fractions could be ruled out because these glucose-rich fractions were fairly soluble in water and could be completely acid hydrolysed with 1–2 N H_2SO_4.

Rhamnose was detected only in some fractions, viz. Rg 3 and 6: W 1 and 2; R(2.1) 2–4; R(7.5) 3 and 4; Rb(2.1) 2; and Rb(7.5) 1 and 3; whereas galactose, found in a few other flour fractions, viz. Rg 1&5; S I, 3&5; W 3–5; and R(2.1)1 was a common hexose constituent of bran polysaccharides (Tables 1 and 2). The bran fractions, particularly Rb(2.1) 2–4; Rb(7.5) 2 and Wb 1–3 were very low in glucose, instead they were rich in arabinose, xylose and galactose.

3.2. Sorghum polysaccharides

Fractional precipitation of the cold water-extracted polysaccharide of sorghum with ethanol yielded 5 fractions (see Fig. 1) and the yield ranged from 0.01 to 0.05% (Table 3). It is evident that the first two fractions had a relatively low concentration of total sugars. Though fraction 5 contained 70.4% glucose, its susceptibility to amylolysis was significant (66.6%). In the HCT analysis an insignificant Congo Red shift (λ_{max}) of only 1.5 nm was observed. Polysaccharides existing in an ordered conformation, preferably 1,3-linked glucose moieties, form a complex with Congo Red, which gives a significant shift in the λ_{max} (Ogawa and Hatano, 1978; Wood, 1991). The fractions (1 and 2) obtained by ammonium sulphate precipitation (Table 3) were also

Table 2
Carbohydrate composition (%) of ethanol-precipitated fractions of cereal bran polysaccharides aqueous-extracted at 25°C

Cereal	Fraction No.		Ethanol (%)	Yield[a]	Total sugars[b]	Relative distribution of sugars (%)				
						Rha	Ara	Xyl	Gal	Glc
Rice bran	Rb	1	50.0	0.29	7.5	–	7.5	–	–	–
(DM 2.1)		2	75.0	0.15	26.2	0.7	10.3	1.2	9.7	4.0
		3	83.3	0.18	41.2	–	19.9	6.2	10.3	4.7
		4	Supernatant	1.18	45.0	–	27.2	5.4	7.1	5.1
Rice bran	Rb	1	33.3	0.30	13.1	4.3	8.7	–	–	–
(DM 7.5)		2	80.0	0.15	45.0	–	2.0	3.3	15.1	2.2
		3	83.3	0.21	43.1	1.1	19.7	7.5	18.1	2.2
		4	Supernatant	0.71	39.3	–	–	1.7	23.5	15.7
Wheat bran	Wb	1	33.3	0.13	33.7	–	11.7	11.3	5.2	5.4
		2	55.5	0.75	60.0	–	20.8	30.6	4.5	4.0
		3	63.6	0.36	37.5	–	10.2	8.5	8.7	8.2
		4	71.0	0.37	82.5	–	21.3	7.2	20.3	33.4
		5	75.5	0.16	56.2	–	13.3	5.5	5.5	32.0
		6	Supernatant	0.36	55.0	–	2.6	9.8	3.0	39.6

[a] The yield is expressed as weight percentage (as is basis).
[b] Total sugars expressed as weight percentage of each fraction.

Table 3
Carbohydrate composition (%) of sorghum carbohydrates from aqueous extracts obtained at different temperatures

Extraction temperature	Fractionation type	Fraction No.	Yield (wt. % as is basis)	Total sugars[b]	Glucose[a] (%)	Amylolysis	Congo Red shift $\Delta\lambda$ (nm)
4°C	Ethanol	1	0.03	10.1	2.6	–	–
		2	0.02	19.7	7.8	–	–
		3	0.05	48.7	4.4	5.9	2.5
		4	0.01	44.7	1.8	6.4	2.0
		5	0.03	76.5	70.4	66.6	1.5
	Ammonium sulphate	1	0.02	32.4	68.0	70.3	(−)1.5
		2	0.04	84.1	65.0	68.5	(−)6.5
55°C	Ethanol	1	0.45	90.0	89.8	74.5	1.0
		2	0.31	82.5	73.4	100.0	1.5
		3	0.08	80.9	64.0	75.7	2.5
		4	0.09	90.0	86.8	100.0	2.5
	Ammonium sulphate	1	0.03	70.3	80.2*	81.5	6.5
		2	0.60	100.0	55.7*	56.0	7.7
	DEAE-cellulose	1	0.16	88.0	50.6*	60.0	6.0
Boiling water bath	DEAE-cellulose	1	0.20	90.0	65.8	–	5.5

[a] Glucose by g.l.c as alditol acetate except* by the TGO method (Madhusudhan & Tharanathan, 1996).
[b] Total sugars expressed as weight percentage of each fraction.

found susceptible to the amylolysis to a greater extent (~70.3%). Surprisingly, the shifts in the λ_{max} of the Congo Red complex with these fractions were negative, for reasons unknown.

To facilitate better recovery, the sorghum flour was subjected to aqueous extraction at 55°C and then fractionated (Table 3). Though these fractions (1–4) contained considerable amounts of total sugars and particularly glucose (64 to 89.8%), their susceptibility to amylolysis (74.5%) indicated significant contamination with α-glucan. The latter may be partially due to the solubilized amylose fraction (at 55°C) leaching into the supernatant. It is known that fractional precipitation with ethanol, though simplest and easiest, leads to co-precipitation of the associated polymeric fractions (Whistler & Sannella, 1965), thus contributing to their heterogeneity. However, the bathochromic shift of 7.7 nm ($\Delta\lambda$) observed for the major ammonium sulphate-precipitated fraction (No. 2) revealed the presence of β-D-glucan. Nevertheless, ammonium sulphate has been shown to selectively precipitate the β-glucan polymers (~3–10.6%) in barley (Stephen, 1983).

On DEAE-cellulose (CO_3^{2-}) chromatography the hot water-soluble polysaccharide gave a neutral fraction eluted with water, which was rich in protein (24%) and susceptible to amylolysis to an extent of ~60%. Its high

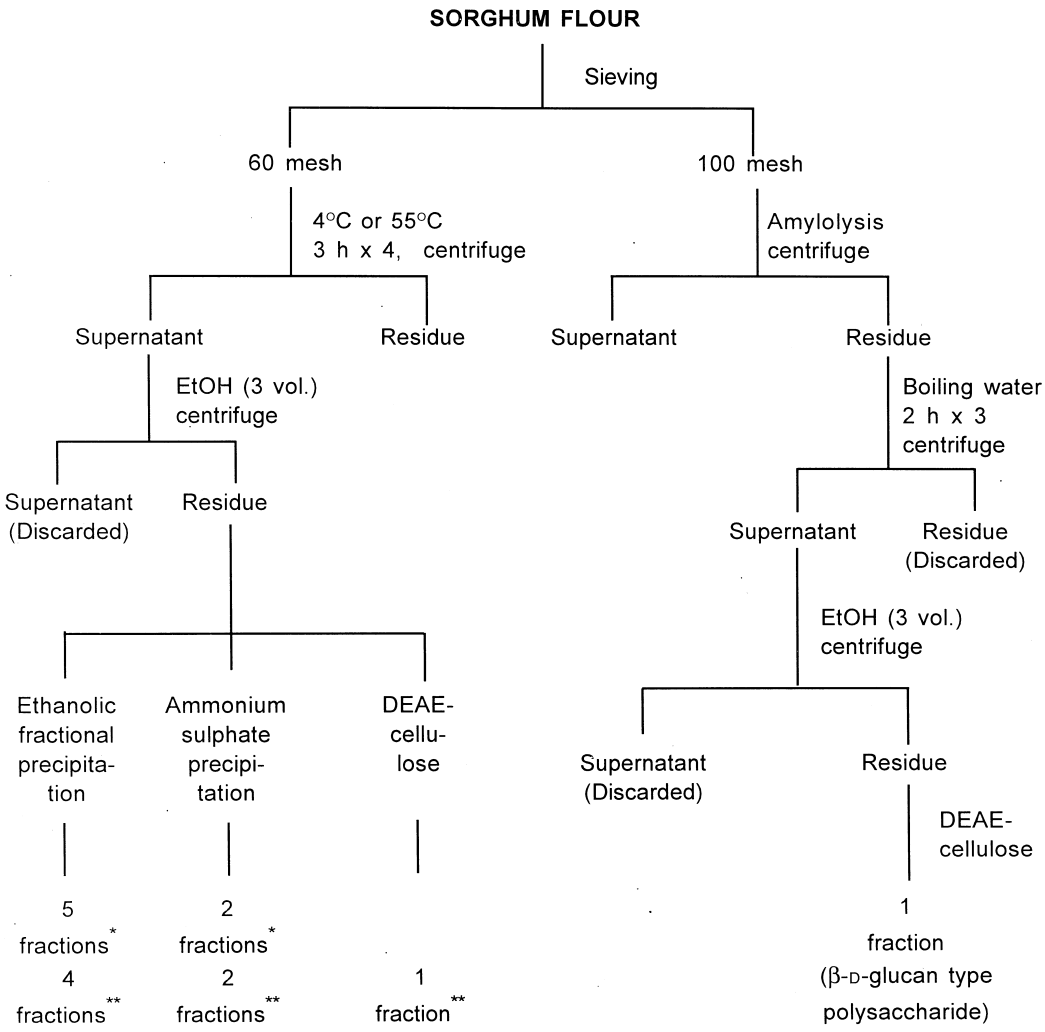

Fig. 1. Scheme of extraction and fractionation of polysaccharides from sorghum flour; * from 4°C, ** from 55°C extracted polysaccharides (see Table 3 for details).

protein content could be attributed to the firmly linked peptide sequences in the native cell wall (Forrest and Wainwright, 1977), as shown in the case of barley MLG (Fincher and Stone, 1986). Thus obtaining a homogeneous MLG-type polysaccharide from sorghum by normal aqueous extraction and fractionation procedures was found to be a difficult objective, because of the presence of large amounts of starch and other non-starch carbohydrates.

Extraction of sorghum polysaccharide at boiling water temperature and fractionation on DEAE-cellulose gave a neutral fraction (0.2% yield) containing ~65.8% glucose (Table 3). Its negative response on further amylolysis, indicated the possible occurrence of MLG in it. However, its protein content was quite considerable (~10%). The Congo Red shift in λ_{max} observed was 5.5 nm. Though, in barley, an exponential relationship between MLG content and temperature of extraction has been reported (Fleming & Kawakami, 1977), the overall content of this glucose polymer in the grain per se appears to differ considerably. The relatively lower content of MLG in sorghum is additionally supported by our histochemical localization studies (to be published elsewhere). From these data it may be inferred that prior amylolysis of the cereal flour followed by aqueous extraction at higher temperature favours a better recovery of non-starch glucose-rich polysaccharides.

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