Content and Composition of Nonstarchy Polysaccharides in Endosperms of Sorghums Varying in Hardness

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ABSTRACT

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Nonstarchy polysaccharide content and composition in the endosperm of sorghum grains varying in hardness was estimated. Soft grains appeared to contain more water-soluble and water-insoluble nonstarchy polysaccharide, whereas the hard grains contained more cellulose. There was more pentose than hexose and more arabinose than xylose in the water-insoluble nonstarchy polysaccharide in soft grains than there was in hard grains. Similar but more pronounced differences were seen between hard and soft portions of the endosperm within kernels. Varietal differences in nonstarchy polysaccharide content and composition reflected compositional differences between the two endosperm types. The presence of linear hemicelluloses and cellulose in cell walls of the hard grains and hard portions of the endosperm may have contributed to rigidity and strength.

Sorghum (*Sorghum bicolor* (L.) Moench) is an important crop in the semiarid tropics that is mainly used at the home level as food after processing in different ways (Chandrashekar and Desikachar 1982). Sorghum has two distinct regions of endosperm—the outer corneous or hard endosperm and the inner floury or soft endosperm. The proportion of hard and soft endosperm depends on the variety and influences grain hardness. Grain hardness influences utility of grain. One of the fundamental differences between hard and soft endosperms is in the greater amount of protein in hard endosperms as well as the protein's interaction with the cell wall and starch (Chandrashekar and Kirleis 1988).

Hardness or texture in wheat plays an important role in flour milling (MacRitchie 1979). Bran separation from the endosperm is easier, the flour particle size is larger, and the starch damage is greater (Hoseney and Seib 1973) in hard wheat than in soft wheat during milling. Using light microscopy, Larkin et al (1952) reported that the cell wall thickness may be related to soft wheat millability.

The nonstarchy polysaccharides (cell wall polysaccharide) of cereal grains can be classified on the basis of their solubility in water and alkali and on their resistance to acid hydrolysis. Elder et al (1953), Weswig et al (1963), and Medcalf et al (1968) related acid-soluble pentosans to grain hardness. The water penetration rate was negatively related to grain hardness and arabinose to xylose ratio (Hale et al 1953, Butcher and Stenvert 1973, Lee and Stenvert 1973, Stenvert and Kingswood 1977).

The pentosan content of grain termed the "normal endosperm group" was positively correlated with kernel hardness in sorghum (Karim and Rooney 1972a). Karim and Rooney (1972b) reported that whole kernel and endosperm of sorghum contained 0.90 and 0.16% water-soluble pentosans and 0.42 and 0.09% alkali-soluble pentosans, respectively. The nonstarchy polysaccharide content of some cereals was compared by Kamath and Belvady (1980). They found that rice had the least nonstarchy polysaccharide content and pearl millet the highest, whereas sorghum, wheat, and ragi had intermediate values.

The objective of this study was to investigate the composition of sorghum endosperm cell walls and to correlate differences in cell wall composition in different varieties as well as between the corneous and floury endosperm from within kernels.

MATERIALS AND METHODS

Eight sorghum varieties (SPV 104, M-35-1, SPV 475, SPV 351, SPV 386, IS 12611, SPV 354, and E-35-1) grown in the Rabi season with a range of endosperm textures, from almost completely floury (soft) to predominantly corneous (hard), were obtained from the International Crop Research Institute for Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. The crushing strength (hardness) of whole and pearled grain was determined by using the Kiya hardness tester (Kiya Seisakusho Ltd., Tokyo, Japan) as described by MacRitchie (1979). Endosperm texture was rated soft, intermediate, or hard. Thousand-kernel weight was determined by counting using a Numigral grain counter (Tecator, Sweden) and weighing. Sorghum grain was decorticated using a Satake grain testing mill (Satake Engineering Co., Ltd, Tokyo, Japan) to yield 90–95% pearled grain. The germ in these grains was removed manually.

The pearled and degermed grains were ground into flour in a Udy cyclone sample mill (Udy Corp., Ft. Collins, CO). The flour was passed through a 0.175-μm sieve, and the overs were reground to pass through the same sieve. The flour was kept at 65% rh at 37°C for seven days before use to bring the flour samples to 14% moisture content.

Hard and Soft Endosperms

Varieties M-35-1, IS 12611, and SPV 354 were pearled and degermed as described above. The hard and soft portions were separated manually. The hard endosperm was ground in a Udy mill, and the soft endosperm was ground in a mortar and pestle. In both cases, the flour was passed through a 0.175-μm sieve and placed in a humidity chamber as described above. The relative proportions by weight of corneous and floury endosperm were 66.9 and. 29.9% in M-35-1, 70.0 and 25.8% in IS 12611, and 75.3 and 21.8% in SPV 354, respectively.

Estimation of Nonstarchy Polysaccharides

A modified Southgate procedure (Southgate 1981) was used to estimate the nonstarchy polysaccharide content of flour. Pearled degermed flour was treated with bacterial α-amylase (Sigma Chemical Co., St. Louis, MO) and then with amyloglucosidase (Koch-Light Laboratories Ltd., Colnbrook Bucks, England) to remove starch. The removal of starch was monitored using a microscope and iodine staining. The gruel was washed repeatedly with 70% ethanol by centrifuging to remove mono- and oligosaccharides. The water-soluble fraction was extracted from the alcohol-washed residue. The residue insoluble in water was hydrolyzed with 2*N* sulfuric acid to obtain the water-insoluble noncellulosic fraction. The residue that resisted hydrolysis was treated with 72% (w/v) sulfuric acid and left overnight at 4°C to hydrolyze cellulose. Total sugars, pentose, and pectin (galacturonic acid) were estimated in water-soluble, water-insoluble, and cellulosic fractions. Total sugars were estimated by the phenol-sulfuric acid method (Dubois et al 1956), pentose was estimated by the phloroglucinol method (Douglas 1981), and pectin was estimated by the sulfamate-borate method (Katan and Bovenkamp 1981).

The water-insoluble fractions after acid hydrolysis were derivatized to alditol acetates, and the composition of sugars was analyzed by gas liquid chromatography (Sawardekar et al 1965).

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Statistical Analysis

Correlation coefficients between grain hardness and all other parameters were calculated. All of the r values reported are at the 1% level of significance except where mentioned otherwise. Estimations were carried out in quadruplicates. The data were analyzed by using the two-way analysis of variance and t test (Sunderaraj et al 1972).

RESULTS

Crushing Strength of Grain

The crushing strength, visual hardness score, and 1,000-kernel weight for eight sorghum varieties are presented in Table I. The crushing strength for the eight varieties varied from 4.0 to 13.4 kg/cm^2 with the whole grain and from 1.1 to 11.8 kg/cm^2 after polishing. In general, the trend of grain hardness was maintained even after polishing ($r = 0.98$), indicating the importance of the endosperm in grain hardness. The proportion of corneous endosperm estimated visually followed the trend in Kiya crushing strength values as shown by Sullins and Rooney (1974).

TABLE I
Crushing Strength, Visual Hardness Score, and 1,000-Kernel Weight for Eight Sorghum Varieties

Variety	Crushing Strength (kg/cm^2) Unpolished	Polished	Visual Score	1,000-Kernel Weight (g)
SPV 104	4.0	1.1	Soft	38.8
M-35-1	5.8	2.3	Soft	36.7
SPV 475	6.5	4.0	Intermediate	29.4
SPV 351	6.6	3.3	Intermediate	25.4
SPV 386	7.1	3.4	Intermediate	28.0
IS 12611	9.0	4.6	Hard	25.9
SPV 354	13.3	10.8	Hard	24.7
E-35-1	13.4	11.8	Hard	26.2

Nonstarchy Polysaccharide Content in Varieties Varying in Hardness

Nonstarchy polysaccharide content and composition of the endosperms of eight varieties was determined (Table II). The water-soluble, nonstarchy polysaccharides varied from 0.04 to 0.12%, whereas the water-insoluble, nonstarchy polysaccharides varied from 0.45 to 0.58% and the cellulose content ranged from 0.05 to 0.13% in the different varieties. The total nonstarchy polysaccharide content varied from 0.62 to 0.75%.

Soft endosperm varieties had significantly more water-soluble, nonstarchy polysaccharide content than did the hard endosperm varieties ($r = -0.73$), whereas hard endosperm varieties contained less water-insoluble, nonstarchy polysaccharides ($r = -0.87$, $\alpha = 0.05$). Hard endosperm varieties had significantly more cellulose than did soft endosperm varieties ($r = 0.79$).

The pentose content in the water-soluble fraction was negligible (Table II). Pentose content in the water-insoluble fraction varied from 0.23 to 0.35% and was negatively related to grain hardness ($r = -0.94$). Pectin content was not significantly related to grain hardness in this study. Hexose values obtained by difference between the total water-insoluble, nonstarchy polysaccharide content and that of the pentose and pectin varied from 0.17 to 0.23% (Table II). The pentose to hexose ratio ranged from 1.1 to 1.7 and was lowest in the hard endosperm varieties ($r = -0.92$).

The predominant monosaccharides present in the water-insoluble fraction were arabinose, xylose, galactose, glucose, and mannose (Table III). Rhamnose was present in trace amounts. The soft endosperm varieties had higher content of pentose than did hard endosperm varieties (Table III). The arabinose to xylose ratios decreased with increasing hardness from 2.6 to 1.4 and agreed with the values reported by Karim and Rooney (1972b) for alkali-soluble pentosans. The pentose to hexose ratios

TABLE II
Water-Soluble, Insoluble, and Cellulosic Nonstarchy Polysaccharide in Endosperms of Sorghums Varying in Grain Hardness and Pentose, Pectin, Hexose, and Pentose to Hexose Ratio in the Water-Insoluble Fractions

	Nonstarchy Polysaccharide (g %)[a]				Water-Insoluble Fraction (g %)[a]			
Variety	Water-Soluble	Water-Insoluble	Cellulose	Total	Pentoses	Pectin[b]	Hexose[c]	Pentose/Hexose
SPV 104	0.12 a[d]	0.58 a	0.05 a	0.75 a	0.35 a	0.01 a	0.22	1.6
M-35-1	0.12 a	0.54 b	0.06 ab	0.72 ab	0.34 ab	0.01 a	0.19	1.7
SPV 475	0.08 b	0.55 b	0.08 d	0.71 b	0.34 a–c	0.01 a	0.20	1.8
SPV 351	0.05 c	0.57 a	0.10 e	0.72 ab	0.33 b–d	0.01 a	0.23	1.5
SPV 386	0.04 c	0.58 a	0.06 b	0.68 b	0.32 cd	0.02 a	0.23	1.4
IS 12611	0.05 c	0.46 c	0.13 c	0.64 c	0.27 e	0.01 a	0.17	1.6
SPV 354	0.04 c	0.45 c	0.12 c	0.61 c	0.24 f	0.01 a	0.19	1.2
E-35-1	0.04 c	0.46 c	0.12 c	0.62 c	0.23 f	0.01 a	0.22	1.1
SEM[e]	0.002	0.002	0.0023	0.0056	0.0056	0.003		

[a] Dry-weight basis.
[b] Pectin was estimated as galacturonic acid.
[c] The hexose content was estimated by difference of water-insoluble nonstarchy polysaccharide content and the sum of pentose and pectin.
[d] Values within columns with the same letter are not significantly different at $P < 0.01$ using the t test.
[e] Standard error of mean derived by using the two-way analysis of variance.

TABLE III
Relative Proportion (%) of Various Monosaccharides[a] in the Water-Insoluble Fractions of Eight Varieties of Sorghum

Variety	Arabinose	Xylose	Mannose	Galactose	Glucose	Pentose/Hexose	Arabinose/Xylose
SPV 104	51	20	2	11	13	2.5	2.6
M-35-1	42	23	4	12	18	2.0	1.8
SPV 475	41	26	1	14	17	2.0	1.6
SPV 351	38	26	4	11	19	1.7	1.4
SPV 386	41	24	1	18	15	1.9	1.7
IS 12611	34	27	2	13	23	1.6	1.3
SPV 354	32	29	3	16	18	1.6	1.1
E-35-1	32	22	2	18	23	1.3	1.4

[a] Rhamnose was found in traces.

decreased significantly from 2.5 in soft endosperm varieties to 1.3 in hard endosperm varieties ($r = -0.93$). The pentose to hexose values reported here did not agree with the values reported by Karim and Rooney (1972a) since the proportion of pentose reported here is larger.

Nonstarchy Polysaccharide Content in Different Parts of the Endosperm

The floury endosperm had about 2.5 times more total nonstarchy polysaccharide than did the corneous endosperm. Cell walls of floury endosperm contained more water-soluble and insoluble nonstarchy polysaccharide and less cellulose than did the cell walls of the corneous endosperm (Table IV).

The pentose content in the water-soluble fraction was almost negligible. The amount of water-insoluble pentoses in relation to the water-insoluble nonstarchy polysaccharides was more in the soft (67%) than in the hard (36%) endospermal fraction (Table IV). The amount of pectin was the same in both endosperm fractions. The pentose to hexose ratios were estimated to be 1.0–1.1 in hard and 2.1–2.2 in soft endosperm (Table IV).

Water-insoluble nonstarchy polysaccharides of the soft endosperm contained more arabinose and pentose, whereas that from the hard endosperm had more xylose and hexose. The pentose to hexose ratio ranged from 1.7 to 1.8 in the nonstarchy polysaccharides from the hard endosperm and from 2.0 to 2.7 from the soft endosperm (Table V).

DISCUSSION

Karim and Rooney (1972b) observed that the total nonstarchy polysaccharide content in sorghum endosperm was only 0.25%, whereas Nyman et al (1984) reported a value of 2.3% at 63% extraction of flour. Our values lie in between these two estimates. Karim and Rooney (1972b) reported a higher content of water-soluble nonstarchy polysaccharides than water-insoluble nonstarchy polysaccharides in sorghum. Kamath and Belvady (1980), Woolard (1976), and Nyman et al (1984) reported more water-insoluble than water-soluble nonstarchy polysaccharide as in this study.

Kernel hardness was positively related to pentosan content of grain within the "normal endosperm group" of sorghums (Karim and Rooney 1972b). Elder et al (1953), Weswig et al (1963), and Medcalf et al (1968) showed that in hard endosperm kernels, the level of acid-soluble pentosan was higher. A significant correlation was observed by Hong et al (1989) between pentosans and kernel hardness in wheat. Hard wheats had significantly higher levels of water-soluble pentosans than did soft or club wheats. All findings are in marked contrast to that reported in this study that cell walls of endosperm from hard endosperm varieties have lower amounts of pentoses.

Our study represents an attempt to correlate the content and composition of different nonstarchy polysaccharides with grain hardness, whereas other reports have concentrated mainly on the pentose fraction. Thus, it was possible for us to study the interrelationship between the various nonstarchy polysaccharides. Hard grains contained less water-soluble and insoluble, nonstarchy polysaccharides and more cellulose than did soft grains. Hard grains also contained more xylose and hexose than did soft grains. These differences appear even larger when expressed as a percentage of the total nonstarchy polysaccharide content rather than a percentage of flour basis. Similar variations between hard and soft portions of three sorghum varieties have been noticed. The cell wall compositional differences observed between hard and soft grains may, therefore, simply reflect those seen between hard and soft portions within kernels.

The lower amounts of nonstarchy polysaccharide in the hard endosperm may be a consequence of increased protein content, whereas decreases in arabinose to xylose ratios and pentose con-

TABLE IV
Water-Soluble, Water-Insoluble, and Cellulose Nonstarchy Polysaccharide in Isolated Hard and Soft Endosperms of Three Varieties of Sorghums and Pentose, Pectin, Hexose, and Pentose to Hexose Ratio in Water-Insoluble Fractions

Variety and Endosperm Type	Nonstarchy Polysaccharide (g %)[a]				Water-Insoluble Fraction (g %)[a]			
	Water-Soluble	Water-Insoluble	Cellulose	Total	Pentoses	Pectin[b]	Hexose[c]	Pentose/Hexose
M-35-1								
Soft	0.45 a[d]	1.06 a	0.03 a	1.54 a	0.72 a	0.02 a	0.33	2.1
Hard	0.08 b	0.46 b	0.07 ab	0.61 b	0.24 b	0.01 a	0.23	1.0
IS 12611								
Soft	0.39 c	1.02 c	0.04 ac	1.45 c	0.69 c	0.02 a	0.31	2.2
Hard	0.05 d	0.48 b	0.06 abd	0.59 b	0.25 b	0.02 a	0.21	1.2
SPV 354								
Soft	0.28 e	1.02 c	0.03 ac	1.33 d	0.65 d	0.02 a	0.36	2.1
Hard	0.06 d	0.39 d	0.08 abd	0.53 e	0.20 e	0.01 a	0.17	1.2

[a] Dry-weight basis.
[b] Pectin was estimated as galacturonic acid.
[c] The hexose content was estimated by difference of water-insoluble, nonstarchy polysaccharide content and the sum of pentose and pectin.
[d] Values within columns with same letter are not significantly different at $P < 0.01$ using the t test.

TABLE V
Relative Proportion (%) of Various Monosaccharides[a] in the Water-Insoluble Fractions of Isolated Hard and Soft Endosperms of Three Varieties of Sorghum

Variety and Endosperm Type	Arabinose	Xylose	Mannose	Galactose	Glucose	Pentose/Hexose	Arabinose/Xylose
M-35-1							
Soft	51	20	2	10	16	2.7	2.5
Hard	35	27	2	9	25	1.8	1.2
IS 12611							
Soft	41	22	4	15	17	2.1	1.8
Hard	39	25	3	8	24	1.7	1.6
SPV 354							
Soft	44	21	2	12	18	2.0	2.1
Hard	35	30	2	10	23	1.8	1.2

[a] Rhamnose was found in traces.

tent seem to reflect biochemical alterations in the direction of synthesis of the various nonstarchy polysaccharides. Linear polysaccharides are known to form rigid connections among themselves (Mares and Stone 1973a,b). Decreased arabinose and increased xylose levels would result in a less branched and more linear polysaccharides, whereas increased cellulose in the cell wall may increase rigidity of the cell wall in hard grains. Lee and Stenvert (1973) and Stenvert and Kingswood (1977) reported that water penetration was lower in hard grains and in grains with a higher arabinose to xylose ratio. The effect of these compositional differences of cell wall in water uptake rates in hard and soft endosperm of sorghum remains to be investigated.

CONCLUSIONS

The biochemical reasons for the differences between hard and soft portions of grains and between hard and soft grains is not clear. Hard grains contain more prolamin (Chandrashekar and Kirleis 1988). Alterations in the amylose content between hard and soft endosperm starches also have been reported (Cagampang and Kirleis 1985). Hard grains contained more glycolipids than soft grains (Aravind 1990). We have reported significant differences in cell wall composition between grains and between hard and soft endosperm within grains. A unified explanation for grain hardness is still awaited.

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