Study of Soybean Seed Coat Components and Their Relationship to Water Absorption

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The occurrence of hard or "stone" seeds in shipments of food grade soybeans can cause serious problems in processing, particularly in the preparation of fermented soy products. Climatic conditions during the growing season and as the seed matures may trigger the production of hard seeds. Total water absorption of soybeans is also a significant parameter in assessing quality for export markets. The seed coats of six varieties of soybeans, covering a wide range of water absorption and stone seed content, were analyzed for ash and cations, protein, lignin, and complex carbohydrates. The water absorption characteristics and macrochemical constituents of the whole seed were also determined. The results indicated that there was no correlation between the concentration of any of the cations and the occurrence of hard seeds. The results from analysis of the complex carbohydrates indicated there were differences in hemicellulose content of seed coat fractions, particularly xylans, that correlated with the water uptake ratio and the occurrence of hard seeds.

Keywords: *Soybeans; carbohydrates; polysaccharides; xylan; water absorption*

INTRODUCTION

There is a potential problem in processing legume seeds when hard or "stone" seeds occur. These are seeds that do not readily absorb water and may account for 10% of the total in some extreme cases. Stone seeds are not a concern in processing soybeans for vegetable oil, but in processing into soy foods the stone seeds can result in adverse quality and cost factors. In the manufacture of traditional soy foods, such as soy milk, tofu, miso, natto, or tempeh, the soybeans are first washed in water to remove dirt and foreign matter. They are soaked at or below ambient temperature for 12−16 h and then cooked under slight pressure to eliminate endogenous enzyme activity and to soften the beans. Stone seeds do not absorb water or soften during the cooking phase. If there are significant amounts of stone seeds, they have to be removed by some means, usually after the soaking phase, or else the organoleptic qualities and, in particular, the texture of the product may be adversely affected. Stone seeds are more prone to formation if the crop has to endure a period of low moisture and/or high heat during the final maturation process before harvest. Some varieties are more prone to stone seed formation than others, but the mechanism is not clear.

A study of a Chinese variety of food grade soybeans (*1*) found that resistance to water absorption was consistent with a higher calcium content in the seed coat and with the micropyle being covered with palisade cells. An earlier paper (*2*) showed that there was a close relationship between calcium content in the seed coat and the hardness of the cooked bean. Yaklich et al. (*3*) studied the development of pores on the soybean seed coat and their relationship to permeability after soaking the seeds for only 1 h and found variation even within the seeds of each variety. In a study of several varieties of lentils (*4*) it was shown that there was a correlation between cell structure of the seed coat and hardness and also that phytate may influence the hardness of the cotyledon and cooking quality. There have been other studies on legume seed coat hardness (*5*), on seed imbibition (*6*, *7*), and on soybean hard seed traits (*8*). These studies were concerned with water absorption for germination, which takes place over several days, and do not relate directly to food processing, but breeding for "hard seed" varieties may complicate the problem of stone seeds in food grade variety development.

There have been relatively few studies of the complex carbohydrates of soybeans. Although the carbohydrate composition will certainly influence the functional properties of the cell structures, there has been more interest in protein and lipid contents. The only comprehensive study was a series of papers on fractionated soybean hulls and cotyledons of a single oilseed variety, which is no longer in common use (*9*−*15*). Not surprisingly, the data from these studies showed that there were major differences in carbohydrates of the seed coat compared to corresponding cotyledon fractions. Kikuchi et al. (*16*) have studied the polysaccharides of the whole soybean without differentiating between the seed coat and cotyledon. A paper on soybean polysaccharides and varietal differences by Mullin (*17*) led to a further study (*18*) on the fractionation and determination of complex carbohydrates in separated seed coat and cotyledons. Some significant intervarietal differences were found. These findings have prompted a more detailed examination of seed coat components and how they may be correlated with seed hardness or water absorption.

A total of six varieties of soybeans containing various amounts of stone seeds were selected for analysis. The lignin, protein, ash, and cations were determined in the seed coat as well as the water absorption characteristics and macroconstituents of the whole seed. The complex

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Table 1. Physical Characteristics of Soybeans[a]

variety	seeds/100 g	% stone seeds	water uptake ratio	% broken seed	% protein[b]	% oil[b]	total soluble sugars[b]
OX 951	640a	72.4a	1.54d	3.13d	46.5	19.4	10.0
Harosoy	540b	5.6b	2.58c	5.25d	41.2	20.7	10.7
Bobcat	485c	3.1bc	2.61c	13.75b	41.0	20.9	11.6
Harovinton	378d	0.0c	2.57c	6.17cd	48.4	18.4	9.9
AC Onrei	332e	0.5c	2.72b	10.05bc	47.3	18.2	10.4
Tachanagaha	275f	0.0c	2.84a	33.33a	43.8	18.3	12.2

[a] The same letter within a column indicates means are not significantly different ($p < 0.01$). [b] Measured by Grainspec using near infrared.

carbohydrates were extracted, fractionated, and acid hydrolyzed to determine the constituent monosaccharides and uronic acids. These data were analyzed to determine correlations between water absorption characteristics and seed coat structure.

MATERIALS AND METHODS

Plant Material. Soybeans for this study were grown in trial plots in 1998 at the Greenhouse and Processing Crops Research Centre, Harrow, ON, Canada, under normal agronomic conditions. The following varieties were chosen: Tachanagaha (a Japanese miso variety), AC Onrei (a Canadian variety bred from Japanese stock), Harovinton and Bobcat (Canadian food grade varieties), Harosoy (an older Canadian oilseed variety), and OX 951 (an experimental hard-seeded line). To ease the separation of the seed coat from the cotyledon, the soybeans were first plunged into liquid nitrogen until the boiling had subsided, drained, and dried overnight at 60 °C in an air oven. The soybeans were placed between sheets of paper and broken into small pieces with a hammer. The seed coat portion was separated from the cotyledon by hand and stored in screw-capped vials at 4 °C.

Water Uptake. Samples of 20 g of soybeans were weighed into 250 mL glass beakers, excess distilled water at 20 °C was added, and the beakers were placed into a temperature-controlled water bath at 20 °C. Every half hour, for the first 6 h, the beakers were removed from the water bath and the soybeans were recovered from the soaking water in a sieve, then patted dry with paper towels, and weighed. The samples were placed back in the soaking water and returned to the water bath. The same data were determined after soaking for 12 and 24 h, and after the final measurement, the stone seeds were counted and the remaining soybeans examined for any evidence of seed coat splitting that had occurred as a result of soaking.

Fractionation. The seed coat material was ground to a fine powder in a small coffee mill, 3 g was weighed into a 250 mL centrifuge bottle, and 150 mL of 1.5% w/w sodium dodecyl sulfate was added. After extraction in an ultrasonic bath for 5 min, the mixture was centrifuged at 24000*g* for 25 min and the supernatant discarded. The residue was re-extracted with 150 mL of 80% ethanol to remove simple sugars and centrifuged at 24000*g*; the supernatant was discarded. Soluble pectins were extracted from the residue with 100 mL of water at 95 °C for 2 h with stirring. After cooling to room temperature, the mixture was centrifuged (24000*g*), and the supernatant was retained and evaporated to a smaller volume, ~8 mL, under reduced pressure at 40 °C. The concentrate was evaporated to dryness (fraction 1) in a vacuum concentrator (Savant Instruments Inc., Farmingdale, NY). The residue was dispersed in 100 mL of a 0.5% w/w ammonium oxalate solution and heated to 95 °C for 2 h, cooled to ambient temperature, and centrifuged at 24000*g*. The supernatant was dialyzed against several changes of distilled water for 24 h, concentrated to 5 mL, and then evaporated to dryness in the vacuum concentrator (fraction 2). The residue was extracted with 100 mL of 2 M KOH at room temperature overnight, then sonicated for 15 min, and centrifuged (24000*g*), and the supernatant was dialyzed against several changes of distilled water for 24 h. A precipitate formed during the dialysis, which was recovered after centrifugation and dried at 50 °C (fraction 3a). The supernatant volume was measured and ethanol added to make it 80 vol %; a precipitate formed, which was centrifuged off and dried (fraction 3b). The remaining supernatant was evaporated to dryness in a rotary evaporator at 45 °C (fraction 3c). A second alkaline extraction with 4 M KOH from the residue resulted in a supernatant that was dialyzed against several changes of distilled water; a precipitate formed (fraction 4a), which was centrifuged off and dried in a forced-air oven at 50 °C overnight and the filtrate (fraction 4b) dried by rotary evaporation at 45 °C. The final residue was washed with water and 80% ethanol and then dried in air at 60 °C (fraction 5). All tubes in the final vacuum-drying process were tared before use and weighed after drying to give the yields. There were three replicates for each variety.

Hydrolysis for HPLC of Neutral Sugars. Samples of 50 mg were weighed into 25 mL screw-cap glass tubes; 3 mL of 1 M sulfuric acid was added, the contents were mixed well, and then the tubes were placed in an oven at 97 °C. The tubes were heated for 5 h and mixed every 30 min with a vortex mixer. After the tubes had cooled to room temperature, 0.5 mL was removed, diluted with 1.5 mL of water, passed through a 0.45 μm filter, and analyzed by HPLC. The conditions for HPLC analysis were the same as described by Mullin and Xu (*18*).

Proximate Analysis. Total nitrogen was determined by a semi-micro-Kjeldahl method and the protein calculated using a conversion factor of 6.25. The ash content of dried defatted seed coat and cotyledon material was determined according to standard methods (*19*). Cations and phosphorus in the ash were determined by atomic absorption spectrometry of the hydrochloric acid hydrolysate.

Complex Carbohydrates. The uronic acid content of the seed coat was determined according to the method of Englyst et al. (*20*). The pectins are the sum of fractions 1 and 2; the hemicelluloses are the sum of fractions 3a, 3b, 3c, 4a, and 4b. Cellulose was calculated from the difference between the glucose determined by 12 M and 1 M acid hydrolysis of fraction 5. The residue left from the 12 M sulfuric acid hydrolysis of fraction 5 was washed three times with distilled water, dried, and weighed to determine the Klason lignin content.

RESULTS AND DISCUSSION

The water adsorption characteristics of the six varieties are shown in Table 1, and it would appear that seed size is a factor in the incidence of stone seed. There has been anecdotal evidence to show that if stone seeds are present in a bulk sample, then the majority will be found among the small seeds. However, there are also small seed varieties, such as those used for natto production, that show zero incidence of stone seed formation. The water uptake ratio was highest in the Tachanagaha variety from Japan, which is greatly favored for miso making. The second highest, AC Onrei, was a Canadian variety bred from Enrei, which originated in Japan. The "broken seed" was a measurement of the number of seeds that had a ruptured seed coat after 24 h of soaking, which may be related to seed coat thickness; the thinner seed coat of Tachanagaha was

Table 2. Cations and Total Phosphorus Content of Cotyledons and Seed Coat, Oil- and Moisture-Free Basis[a]

	%					ppm					
variety	Ca	P	Mg	K	Na	Fe	Mn	Cu	Zn	B	Al
					Cotyledon						
OX 951	0.27	0.9	0.3	2.41	0.02	86.7	51.7	21	63	26.4	11.2
Harosoy	0.21	0.9	0.32	2.49	0.02	80.3	27.9	21.8	58.1	37.8	7.7
Bobcat	0.21	0.74	0.26	2.22	0.01	80.3	29.7	17.9	46	39.3	10.0
Harovinton	0.29	0.88	0.33	2.44	0.01	81.1	55.6	18.6	65.3	31.8	13.0
AC Onrei	0.21	0.87	0.3	2.46	0.01	83.8	44.2	20.4	62.9	29.1	4.8
Tachanagaha	0.11	0.82	0.24	2.55	0.01	76.9	30.3	15.6	57.4	31.4	5.0
overall mean	0.22	0.85	0.29	2.43	0.01	81.5	39.9	19.2	58.8	32.6	8.6
SE[b]	0.03	0.02	0.01	0.05	0.00	1.4	5.0	0.9	2.8	2.0	1.4
					Seed Coat						
OX 951	0.52	0.1	0.24	1.19	0.02	386.7	18.1	7.7	70.9	22.0	18.0
Harosoy	0.46	0.14	0.22	1.62	0.02	326.3	10.1	5.8	46.6	21.4	22.5
Bobcat	0.56	0.07	0.21	1.44	0.02	364.7	12.7	6.9	40.1	21.1	41.9
Harovinton	0.68	0.07	0.35	1.01	0.02	385.7	11.0	15.9	86.5	21.5	26.3
AC Onrei	0.63	0.08	0.28	1.10	0.02	394.3	11.6	15	86.0	23.9	33.4
Tachnagaha	0.53	0.12	0.25	1.48	0.02	433.3	16.1	7.3	79.0	22.4	17.7
overall mean	0.56	0.09	0.26	1.30	0.02	381.8	13.3	9.8	68.2	22.0	26.6
SE	0.03	0.01	0.02	0.10	0.00	14.4	1.3	1.8	8.2	0.4	3.9

[a] All data are means, $n = 3$ [b] Standard error.

Table 3. Recovery of Fractions as Percent Weight of Starting Material[a]

	fraction[b]								
variety	1	2	3a	3b	3c	4a	4b	5	total
OX 951	4.9d	4.8 e	9.4a	2.1c	4.2a	1.2c	3.2c	50.6	80.5
Harosoy	6.2a	5.8cd	6.2b	3.6a	3.2b	1.4b	3.5c	73.1a	102.9
Bobcat	5.4b	5.5d	5.8bc	3.5a	3.2b	1.6a	4.0a	67.2b	96.1
Harovinton	5.2bc	6.8a	5.2bc	2.7b	3.2b	1.4b	2.3c	60.5d	80.7
AC Onrei	5.2c	6.4b	4.8c	2.6b	2.8b	1.0d	3.8ab	64.0c	90.6
Tachanagaha	5.0d	5.8c	2.1d	2.6b	4.6a	1.0d	3.5b	63.2c	87.8

[a] The same letter a within column indicates means are not significantly different ($p < 0.01$). [b] For explanation of fractions see Figure 1.

Table 4. Percent Weight Recovery of Carbohydrates in Each Fraction by HPLC[a]

	fraction							
variety	1	2	3a	3b	3c	4a	4b	5
OX 951	32.58	15.54	71.29	56.14	5.26	82.74	44.96	13.18
Harosoy	43.33	21.55	85.31	55.97	4.97	86.13	47.49	12.78
Bobcat	43.07	15.34	80.95	53.47	4.87	86.55	65.34	15.51
Harovinton	29.47	21.34	74.8	68.41	7.19	89.5	ND[b]	10.20
AC Onrei	25.89	9.94	85.05	59.59	9.32	78	51	10.70
Tachanagaha	34.58	12.26	79.96	51.01	4.79	80.07	47.44	12.95

[a] Data are means, $n = 3$. [b] Not determined.

more prone to spontaneous splitting during soaking than that of OX 951 soybeans. This is different from what is commonly termed mechanical damage, which occurs during harvest operations and results in split seeds and lower quality.

Data from the analysis of the ash from both the seed coat and cotyledons are summarized in Table 2. Saio (*1*), using samples of soybeans selected from a bulk sample, found some evidence of higher calcium and potassium contents, and lower total phosphorus content, in hard seeds. In the data reported in our study there does not appear to be a correlation between the water absorption properties of any variety and the cation content, although calcium is acknowledged as an important factor in cell wall structure (*21*−*23*). There is no evidence to suggest that a relatively high or low concentration of the other elements found in the ash analysis would result in normal or hard seed coat or cotyledon.

Table 5. Macroconstituents of Soybean Seed Coat (Grams per 100 g of Dry Weight)[a]

variety	ash	uronic acid	lignin	pectin	hemi-cellulose	protein	cellu-lose
OX 951	3.83c	6.63e	2.45c	9.7d	20.1a	10.25c	14.14d
Harosoy	4.67a	8.24d	5.85a	12.0a	17.9b	11.94a	19.88b
Bobcat	4.60a	9.15c	4.44ab	10.9c	18.1b	9.12e	14.28d
Harovinton	4.20b	8.53d	3.98b	12.0a	14.8c	8.75e	15.86cd
AC Onrei	3.90c	10.46a	4.24ab	11.6b	15.0c	9.38d	24.50a
Tachanagaha	4.60a	9.84b	4.04b	10.8c	13.8d	11.50b	17.72bc

[a] The same letter within a column indicates means are not significantly different ($p < 0.01$).

The finely ground seed coat material was fractionated according to the scheme outlined in Figure 1. The weight of material recovered from each fraction was recorded; the percent recoveries are shown in Table 3. Apart from the protein, which was solubilized in the first step, and the ash, which may be irreversibly associated with some of the isolates (Table 5), these data show little, if any, sample loss during the fractionation process. The percent recovery weights of each fraction did not vary significantly between varieties ($p < 0.01$) except for fractions 3a and 5. Recoveries of fractions 1, 2, 3b, 3c, 4a, and 4b did not exhibit any particular correlation with the physical seed coat characteristics; they were similar for each variety, and the triplicate determinations were reproducible.

Each fraction was hydrolyzed with 1 M sulfuric acid and the monosaccharide content determined by HPLC. Recoveries of monosaccharides calculated as percent of the sample weight are shown in Table 4. The first two fractions are considered to contain the main bulk of the pectins and soluble dietary fiber (Table 3). The weights of residue recovered from the hot water extract, fraction 1, and the ammonium oxalate extract, fraction 2, were similar for all varieties. The neutral monosaccharides recovered from the fraction 1 acid hydrolysate, although variable, were consistently greater than neutral monosaccharides from fraction 2 (Table 4), part of the difference being due to the uronic acid content (unpublished data). In these fractions the monosaccharides resulting from the 1 M sulfuric acid hydrolysis were predominantly mannose and galactose, which was the result of similar fractionation by Aspinall et al. (*11*).

Table 6. Mole Percent, Neutral Monosaccharides from Each Fraction, Mean (n = 18), and Range of All Varieties

fraction	fucose	arabinose	rhamnose	galactose	glucose	xylose	mannose
1 (SEM)	0.49 (0.23)	8.21 (3.52)	2.41 (0.94)	20.88 (0.80)	2.85 (0.80)	0	64.77 (6.19)
range	0.28−1.09	3.88−16.87	1.33−4.06	16.05−24.76	1.57−4.41		49.59−74.31
2 (SEM)	3.27 (0.57)	26.36 (5.96)	9.84 (2.12)	21.32 (2.08)	1.46 (0.41)	6.75 (1.38)	31.02 (8.33)
range	1.88−3.63	12.84−29.50	4.96−11.10	19.01−24.94	0.40−1.79	3.16−8.03	27.75−50.71
3a (SEM)	0	0.69 (0.34)	0	0	0	99.19 (0.44)	0
range		0.29−1.32				98.72−99.71	
3b (SEM)	2.19 (0.39)	35.23 (4.81)	3.50 (0.97)	17.52 (2.14)	9.75 (1.91)	12.48 (3.45)	19.33 (7.43)
range	1.71−2.86	28.76−41.65	1.06−4.67	15.09−21.61	7.36−13.40	9.14−20.19	8.66−31.18
3c (SEM)	4.04 (1.07)	73.94 (6.40)	0	7.64 (1.78)	3.98 (1.48)	2.53 (1.13)	7.87 (3.99)
range	2.49−6.12	64.95−86.95		3.16−11.29	1.76−5.94	0.60−4.95	2.90−16.96
4a (SEM)	0	0.87 (0.39)	0	0	0	99.08 (0.50)	0
range		0.22−1.67				97.96−99.78	
4b (SEM)	3.31 (0.18)	45.76 (4.23)	5.71 (1.68)	17.32 (2.78)	11.61 (1.51)	12.73 (1.33)	3.56 (1.49)
range	3.05−3.73	38.83−53.85	4.05−9.41	12.16−20.93	8.98−14.86	10.34−14.86	1.51−6.55
5 (SEM)	2.10 (0.39)	36.78 (4.01)	5.23 (0.56)	10.19 (1.49)	17.01 (2.11)	13.88 (1.40)	14.80 (3.63)
range	1.57−2.88	31.62−43.16	4.11−6.12	7.24−12.83	13.62−20.54	10.84−16.67	8.93-21.16

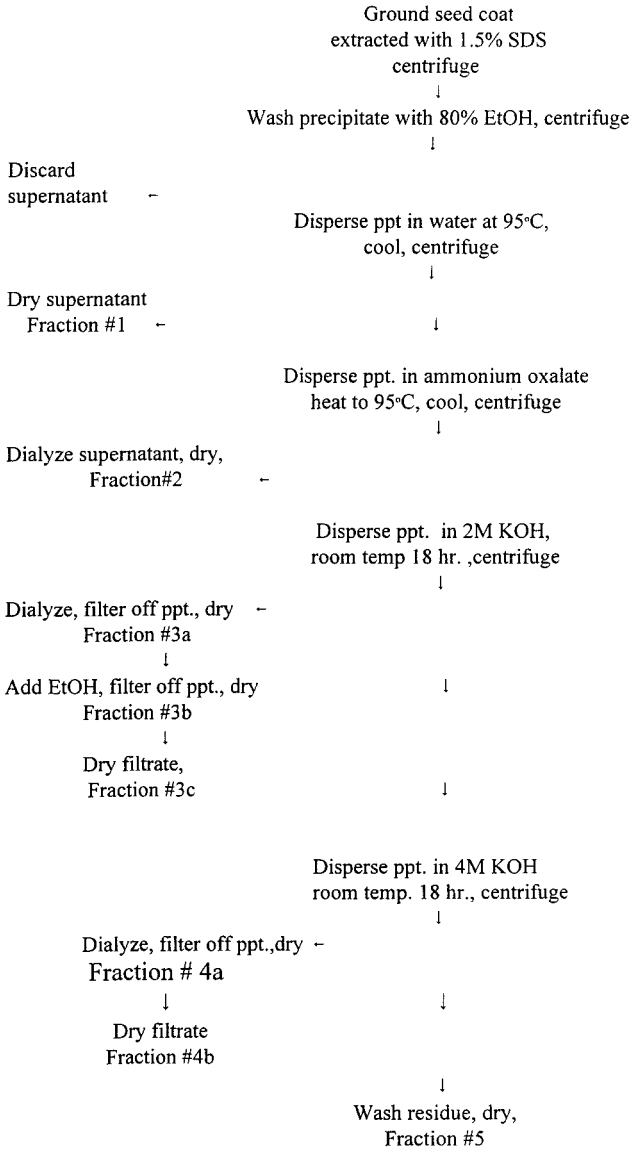

Figure 1. Simplified fractionation scheme used for finely ground seed coat material.

In the 2 M KOH fraction a precipitate formed as the dialysis progressed, and this was filtered off (fraction 3a) before the addition of ethanol to precipitate fraction 3b from the remaining soluble material, fraction 3c. A similar protocol was used for the 4 M KOH soluble fraction 4 except that the dialyzed supernatant was not divided into two subfractions. Fractions 3a and 4a were almost exclusively xylans as shown by the recovery of xylose from the acid hydrolysates (Table 6), which was in agreement with the results of Aspinall et al. (*10*). The acid hydrolysates of the more water soluble fractions 3b, 3c, and 4b were predominantly arabinose with significant amounts of other sugars (Table 6). These alkali soluble fractions are mostly hemicelluloses, with the xylans being water insoluble, and may be part of the key to the water adsorption properties of the seed coat. The percent recovery of fraction 3a (Table 3) decreased in reverse order to the water uptake ratio.

Fraction 3a is predominantly xylan hemicellulose, and being almost insoluble at neutral pH may be an important factor in the hydrophobic character of the seed coat. Fraction 5 is the residue after all other solubles have been removed and is essentially a concentration of cellulose and lignin, which would remain insoluble through all of the fractionation procedures. It was low in 1 M acid hydrolyzable polysaccharides, but the 12 M acid hydrolysate resulted in a high proportion of glucose from the cellulose. The highest recovery of this residue was from Harosoy and the lowest from OX 951 and Harovinton.

Variety OX 951, exhibiting the greatest stone seed content, contained the lowest concentration of uronic acid, lignin, pectin, and cellulose and the highest concentration of hemicellulose in the seed coat (Table 5). It has been shown that there is a relationship between the seed coat lignin content and resistance to mechanical damage (*24*), but the low lignin content of OX 951 does not appear to be a cause for increased susceptibility to mechanical damage. The combination of lower pectin content and higher hemicellulose, which is essentially composed of xylans, and overall low uronic acid would tend to provide reduced hydrophilicity in the seed coat and result in increased stone seed production.

This study has shown that there are differences between varieties which potentially could affect cell wall structure and intracellular composition. The key to the occurrence of stone seeds may be found in a combination of factors rather than any specific one. The functional

properties of polysaccharides are dependent on the order and abundance of basic monosaccharides and their production during the development of the seed. The data in Table 6 summarize the HPLC analysis of the 1 M acid hydrolysates and show the complete absence of certain monosaccharide residues in some of the fractions. Arabinose, galactose, mannose, and xylose are the overall predominant residues, but apart from xylose and the total hemicellulose there was no other correlation with water absorption. Whereas the occurrence of a higher concentration of xylan in the OX 951 variety than in the others appears to be a plausible factor in stone seed development, there are certain to be more structures involved. Further analysis of structural components of the soybean will be required before the mechanism for the formation of stone seeds is understood.

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