NON—STARCH POLYSACCHARIDES OF SEEDS OF SOYBEAN [*GLYCINE MAX.* (L)]

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Abstract: Mature soybean seeds (variety FFR 559) were analysed for the non-starch polysaccharide composition. Non-starch polysaccharide (NSP) fractions were extracted by two methods, namely, chemical (trichloroacetic acid-TCA) and enzymatic methods. Yields of TCA- soluble, enzyme-soluble, TCA-insoluble and enzyme-insoluble NSP were 1.4, 13.1, 24.9 and 28.8% of the defatted flour, respectively. The differences in yields between the two methods were primarily due to contamination with non-carbohydrate materials (protein, ash and lignin) to the extent of 18.4-30.8%. The results showed the soluble NSP to be an arabinogalactan type of polysaccharide containing arabinose and galactose in approximately 2:1 ratio, and a uronic acid content of 30.4%. Cellulose associated with arabinogalactan, xylan and pectic polymers predominate the insoluble NSP. Mature soybean seeds were found to be free of starch.

1. Introduction

Carbohydrates of legumes have been extensively studied for their starch constituents.[17] Cell walls, essentially constituting of non-starch polysaccharides, which together with lignin are now referred to as the dietary fibre,[5] have received comparatively little attention because they are highly insoluble and therefore lack the functional properties exhibited by starch. They are indigestible by the human digestive system and consequently believed to contribute little nutritionally.[18] However, there is current evidence that this indigestible fraction could be nutritionally beneficial in lowering blood cholesterol levels and various intestinal disorders.[18]

The significance of these legume seed components also stems from the fact that they are important in many technological processes. One example is the arabinogalactan of black gram. It has been shown that in the preparation of 'idli', arabignogalactan is essential for stabilization of the texture created by the protein network.[20]

Some researchers have attempted to characterise the complex polysaccharide in various legume seeds without any special interest in dietary fibre. Morita,[11] and Aspinall and Cottrell[3] have extracted a crude hot water soluble polysaccharide using NaOH and phenol:acetic acid:water, respectively to solubilise proteins. In these studies, components representing only a small proportion of the total polysaccharide were isolated and characterised. This approach tends to yield highly purified fractions which can be chemically well characterised, but are not representative of the total polysaccharide. The objective of this study was to isolate, identify and quantify the constituent monosaccharides in the non-starch polysaccharide (NSP) of mature soybean seeds. In addition, the starch content was also determined.

2. Materials and Methods

2.1 Sample Preparation

Mature soybean seeds (variety FFR 559) were obtained from Kentucky Seed Improvement Association, U.S.A. They were ground in a Wiley laboratory mill to pass through a 60 mesh sieve and defatted with hexane for 16 h. Moisture determination was carried out by heating samples at 130^0C for 1 h. All yields and composition were calculated on a moisture - free basis. All reagents used were of analytical grade.

2.2 Isolation of Non-Starch Polysaccharides

Non-starch polysaccharides were isolated by two different methods. The first method[20] involved extraction of 10g flour with 60 ml 10% (w/v) trichloro-acetic acid (TCA) for 6 h at 4^0C with continuous stirring and then centrifuging for 30 min at 10,000 x g (4^0C). The extraction was repeated thrice. The supernatants were combined, filtered and precipitated with 3 volumes of 75% (v/v) acetone. The precipitate was redissolved in 10 ml TCA and reprecipitated with 3 volumes of acetone. The white precipitate that resulted was thoroughly washed with acetone, dissolved in water, dialysed (72 h) and freeze-dried to obtain the soluble polysaccharide (TCA—S).

Since the residue after TCA extraction contained both the insoluble polysaccharide and the protein precipitated with TCA, the residue was treated (1 h) with 0.2 N NaOH until the pH was 11.5, to solubilise the proteins. The residue after deproteinization was the insoluble polysaccharide (TCA—I).

In the second method, the soluble and insoluble polysaccharides were extracted by the procedures of Hellendoorn,[9] as modified by Schweizer and Wurch.[19] This method involved stepwise degradation by pepsin followed by pancreatin, a preparation which has proteolytic, lipolytic and amylolytic activities and precipitation of soluble polysaccharide (ENZ — S) with 4 volumes of ethanol. This residue forms the insoluble polysaccharide (ENZ—I). Since our analysis showed soybean to be free of starch, autoclaving and the use of glucoamylase were omitted from the original procedure. However aqueous sample suspension was boiled at 95^0C for 20 min in order to inactivate the protease inhibitors.

2.3 Analytical Methods

The polysaccharide fractions were hydrolysed by a modified Saeman's procedure as reported earlier.[13] Preliminary experiments showed that a 96% recovery of standard sugars is possible by this procedure.

Preliminary identification of sugars in the deionized and filtered

hydrolysates was obtained by Thin Layer Chromatography.[7] Neutral sugars present in the soluble and insoluble polysaccharides were determined by High Performance Liquid Chromatography (HPLC) as described by Ravindran and Palmer.[13] Uronic acid in the polysaccharide fractions was determined by the method of Ahmed and Labavitch[1] using a galacturonic acid standard (Sigma Chemical Co., St. Louis, MO).

Protein and lignin were determined by the micro-Kjeldahl procedure of Robinson[14] and the permanganate oxidation method of Goering and Van Soest,[8] respectively. The ash in the polysaccharide fractions was determined by weighing the residue after heating at 525^0C for 5 h.

Starch in the defatted flour was determined by perchloric acid method[12] and the results of which was checked by an enzymatic assay.[16]

3. Results and Discussion

There is no generally accepted approach to the extraction and/or analysis of non-starch polysaccharides of plants. In the present study, two methods were employed to extract the NSP fractions from defatted soybean flour. The determination of NSP by purely chemical methods offers some advantages. The major one is their effectiveness in deproteinising the materials. The TCA—S extracted in our study contained only 1% protein. But the chemical methods are obviously less 'biologically' relevant than the use of enzyme(s) *in vitro*[22,23,] which simulates normal human digestion. ENZ—S and ENZ—I obtained by this method however, contained 9 and 25% protein, respectively. These high levels may have arisen as a result of incomplete digestion of proteins by the enzymes. The indigestible fraction from some plant sources is known to represent considerable percentage of the total protein.[6] Whether this indigestible protein is of structural cell wall nature or merely refractory to digestion requires clarification. There is however, ample evidence that it is not wholly of a cell wall nature in all cases.

Soluble NSP fractions extracted by both methods contained appreciable amounts of ash (Table 1). Decreased solubility of chelated minerals in alcohol and acetone may have contributed to the presence of such levels of minerals in the soluble fractions. In the ENZ—S, however, since the method involved incubation in relatively high concentration of sodium phosphate buffer, it seems likely that most of the ash came from precipitation of buffer salts. ENZ—S also contained 9.3% unhydrolysed residue and is probably attributable to ligno-cellulose complex. In addition to ash and protein, the insoluble fractions also contained lignin (3.3%).

Yields of TCA—S, ENZ—S, TCA—I and ENZ—I fractions were 1.4, 13.1, 24.9, and 28.8% of the defatted soybean meal, respectively. As noted earlier, the differences in yields between the two methods, were primarily due to contamination with protein and ash to varying extents. The TCA

procedure includes an extensive dialysis of the soluble fraction and it is possible that some losses of low molecular weight material may have occurred during dialysis. Another possible reason for the observed differences may have been the incomplete precipitation of the soluble fraction with acetone.

TCA and enzyme soluble extracts contained 47.4 and 49.5% neutral sugars and 24.6 and 20.8% acidic polysaccharides, respectively (Table 1). The insoluble polysaccharide fractions contained less uronic acid, 58.3-65.1% neutral polysaccharides and 7.8-9.5% acidic polysaccharides. The data was corrected for the presence of non-carbohydrate material (28.4-30.2% in soluble and 18.4-30.8% in insoluble polysaccharide fractions) and expressed as a percentage of polysaccharides in Table 2. Expressed either as percent of defatted meal (Table 1) or of polysaccharides, the data for the sugar and uronic acid composition of soybean NSP was found to be considerably consistent.

Table 1. Composition of Soluble(S) and Insoluble(I) polysaccharide Fractions of Defatted Soybean Flour (% of dry matter)[a]

Composition	TCA–S	ENZ–S	TCA–I	ENZ–I
Yield	1.4	13.1	24.9	28.8
Galactose[b]	26.2±0.8	26.6±2.1	19.9±0.9	18.0±1.7
Arabinose[b]	14.3±0.4	13.7±0.7	10.5±1.3	9.8±1.0
Xylose[b]	3.9±0.6	4.2±0.8	6.1±1.2	5.7±0.3
Glucose[b]	1.5±0.1	2.9±0.1	28.6±2.2	24.8±1.1
Mannose[b]	1.5±0.1	2.1±0.9		
Rhamnose[b]	trace	trace	trace	trace
Uronic Acid[b]	24.6±0.5	20.8±0.3	7.8±0.3	9.5±0.4
(Total neutral + acidic sugars)[b]	(72.0±2.4)	(70.3±4.9)	(72.9±5.9)	(67.8±4.5)
Protein	1.0±0.0	8.9±0.8	12.3±1.8	24.9±0.9
Ash	29.2±0.3	12.2±0.5	2.9±0.1	2.5±0.1
Lignin		7.3±0.6[c]	3.2±0.9	3.4±0.6
Recovery	102.2±2.7	98.7±7.8	91.3±8.8	98.6±7.4

[a] Each value represents the mean of three determinations ± standard deviation.
[b] Neutral and acidic sugars are expressed as polysaccharides.
[c] Unhydrolysed residue.

Galactose (36-39%) and to a lesser extent arabinose (19-20%) are the major neutral sugar constituents. The galactose : arabinose ratio was approximately 2 : 1, suggesting that much of the soluble NSP of soybean may be the arabinogalactan characterised by Morita[11] and Aspinall.[3] Lupins are also reported to have gal/ara ratio of 2.5 : 1 in the cold water soluble polysaccharide.[10] Galacturonic acid (27-34%) was the accompanying acidic sugar. Xylose, glucose and mannose were also present in the soluble NSP fractions in smaller quantities, generally in the range of 2-6% (Table 2). Mannose is reported to be present as a side chain sugar in water soluble polysaccharides[21] and might have given rise to the mannose determined in our samples. Glucose might have arisen from the fraction in the residue. Rhamnose was observed in trace amounts. The presence of rhamnose and galacturonic acid suggests that rhamnogalacturonans might occur as cell wall constituents. Moreover, the predominance of arabinose and galactose which are the major pectic neutral sugars indicates that soybean NSP are mainly of the pectic type.

Table 2.   Distribution of Neutral and Acidic Sugars as % of Polysaccharide.

	TCA–S	ENZ–S	TCA–I	ENZ–I
Yield	1.0	9.2	18.2	19.5
Galactose	36.4	37.8	27.3	26.5
Arabinose	19.9	19.5	14.4	14.5
Xylose	5.4	5.9	8.4	8.4
Glucose	2.1	4.1	39.2	36.6
Mannose	2.1	3.0	-	-
Uronic acid	34.2	26.7	10.7	14.0
Galactose: arabinose ratio	1.8:1	1.9:1	1.9:1	1.8:1

Glucose (37-39%) was the predominant sugar in both insoluble NSP fractions and was probably derived from cellulose. The composition of the non-cellulosic polysaccharides in the insoluble fractions was calculated, based on the assumption that all the glucose in the hydrolysates was derived from cellulose. The mean composition of the non-cellulosic polysaccharide in the two fractions was 43.4% galactose, 23.3% arabinose, 13.5% xylose and 19.8% uronic acid. Apparently galactose predominates the insoluble NSP of soybean as they do in the soluble NSP. Xylans and pectic substances are also prominent and the proportion of galactose : arabinose is same as the soluble NSP. Hull polysaccharides of legumes are reported to be mainly arabino- and glucuronoxylans.[4] Hence the high xylose content in the insoluble NSP could be due to the fact that whole seeds were used in the present study.

The results of the present study indicate that there is no sharp borderline between the soluble and insoluble NSP fractions. Polysaccharides of very

related composition, arabinogalactan for example, are found both as soluble and insoluble components.

Starch determination on defatted soybean flour by the enzymatic and perchloric acid procedures showed the mature soybean to be free of starch, confirming the report of Altschul.[2] Several other legume seeds, sunflower,[4] winged beans[13] and lupins[10] are also reported to be nearly starch-free.

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