Carbohydrates of Legume Flours Compared With Wheat Flour. III. Nonstarchy Polysaccharides[1]

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ABSTRACT

The yield of total water extractable material and recovery of crude water-soluble nonstarchy polysaccharides (WSNP) from legume flours was higher than that obtained from hard red spring (HRS) wheat flour. The yield of amylase-treated WSNP from legume flours, however, was lower than values reported for HRS wheat flour. The yield and sugar composition of the diethylaminoethyl-cellulose fractions obtained for each purified WSNP from the various legume flours were different than those obtained for wheat flour. The navy and pinto bean WSNP were composed mainly of arabinose and some xylose, glucose, and galactose. The faba bean WSNP were composed primarily of glucose. Mannose was present only in lentil WSNP that also contained the sugars, arabinose, xylose, galactose, and a trace amount of glucose. The mung bean WSNP were composed of polymers containing glucose, arabinose, and xylose. Paper and gas-liquid chromatography of the various hydrolyzed legume water-insoluble nonstarchy polysaccharides (WINP) revealed that pinto and navy bean WINP were composed of the same sugars as those in WSNP, whereas faba bean, lentil, and mung bean showed a different sugar composition between WINP and WSNP. Faba bean, lentil, and mung bean papain-treated WINP contained primarily glucose and only trace amounts of arabinose and xylose.

Peterson and Churchill (1921) analyzed the carbohydrate composition of navy bean seeds by using various solvents. They found pentosans, galactans, dextrins, and hemicelluloses made up the nonstarchy polysaccharide fraction. Anderson (1949) used a hot water extraction method to isolate the soluble endosperm mucilages of a large number of legume seeds that were mainly galactomannans.

Pritchard et al (1973) studied the carbohydrates of spring and winter field beans (*Vicia faba* L.). Of the unavailable carbohydrate fractions, the water-soluble polysaccharides and hemicelluloses showed the largest intervarietal differences. The former were composed of several different sugars, but were particularly rich in uronic acids and glucose. A high percentage of glucose was found in the hemicellulose fraction and it is suggested that this may be present as a glucan with a linkage resistant to digestion by takadiastase. The spring-sown beans contained more glucose than the winter varieties did.

Cerning et al (1975) gave results on the furfurgal generators, hemicelluloses, and glucose-containing polymers in cotyledons (including germ), hulls, and whole horsebean (*Vicia faba*) seeds obtained after successive acid hydrolysis. Composition of cell-wall constituents was examined after acid hydrolysis. The monosaccharides resulting after acid hydrolysis of hemicelluloses were essentially xylose, smaller amounts of arabinose, and traces of galactose and rhamnose. Most of the furfural generators appeared to be either water-soluble or soluble in dilute acid. Small amounts of glucose-containing polymers soluble in dilute acid were present in the cotyledons, with greater amounts in the hulls.

Cerning-Beroard and Filiatre (1976) compared the carbohydrate composition in horsebeans, peas, and lupines. The study included the furfural generators, hemicelluloses, glucose-containing polymers, and crude lignin. The structural and cell-wall polysaccharides were the major carbohydrate constituents of lupines. Wrinkled peas were richer in hemicelluloses and cellulose than horsebeans and smooth peas.

The purposes of this study were to isolate the nonstarchy polysaccharides (water-soluble and water-insoluble) from legume flours, investigate their chemical composition, and compare the information obtained with data on the nonstarchy polysaccharides found in wheat flour. Such knowledge would be useful to better understand the use of legume flours in bread baking.

MATERIALS AND METHODS

The various legume flours were described previously (Naivikul and D'Appolonia 1978). Figure 1 shows the schematic diagram used for the purification of both the water-soluble and water-insoluble nonstarchy polysaccharides from legume flours.

Water-Soluble Nonstarchy Polysaccharides

The method of isolation of water-soluble nonstarchy polysaccharides (WSNP) was similar to that used by Medcalf et al (1968). α-Amylase was used to remove the soluble starch from the isolated crude WSNP according to the method of Kundig et al (1961). The purified α-amylase treated WSNP from the legume flours was separated into five fractions by stepwise elution from a 2.4×30-cm column of diethylaminoethyl (DEAE-cellulose in the borate form according to Neukom and Kundig, 1965).

The protein content of the total water-solubles and of the crude WSNP was determined by the Kjeldahl procedure, AACC Method 46-10, final approval 4-13-61.

The method of Folin-Ciocalteu as modified by Lowry et al (1951) was used to measure the protein content of the α-amylase treated WSNP and DEAE-cellulose fractions.

Paper and gas-liquid chromatography were used to identify the sugar composition and the ratio of component sugars of the WSNP fractions from the various legume flours. The sample (10 mg) was hydrolyzed with 1*N* sulfuric acid for 4 hr at 100°C and neutralized with barium carbonate according to the procedure of Medcalf et al (1968).

Water-Insoluble Nonstarchy Polysaccharides

The procedure used to obtain the sludge was identical to that given for the isolation of water-soluble polysaccharides, except the sludge layer was removed and saved. The isolated sludge was resuspended in distilled water and passed through a No. 400-sieve to eliminate much of the starch contamination. The material remaining on top of the sieve was freeze-dried and represented the purified sludge (Fig. 1). This material was extracted and purified by papain treatment according to D'Appolonia and Gilles (1971) and modified by Kim and D'Appolonia (1976).

The protein content in the crude sludge material was determined by the Kjeldahl procedure, AACC Method 46-11, first approval 4-13-61. The micro-Kjeldahl procedure, AACC Method 46-13, first approval 4-13-61, was used to determine protein content of the isolated crude and purified water-insoluble nonstarchy polysaccharides (WINP).

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Paper chromatography was used to qualitatively determine the sugars present in the hydrolyzed crude and purified WINP.

The relative percentage of component sugars was determined by gas-liquid chromatography.

RESULTS AND DISCUSSION

Water-soluble Nonstarchy Polysaccharides

The yield of the water extractable material from the legume flours is shown in Table I. The yield of total water-solubles from the legume flours ranged from 33.6 to 42.2%. Lentil flour contained the least amount of crude WSNP (2.23%); mung bean flour had the highest content (8.08%). After treatment with α-amylase, the yield of purified WSNP was very low and ranged from 0.15% for lentil flour to 0.54% for pinto bean flour. The total water-soluble material extracted from hard red spring (HRS) wheat flour was about 10 times lower than that obtained from the legume flours (D'Appolonia et al 1970). Recovery of crude pentosans from HRS wheat flour was also lower (1.4%) than the crude WSNP obtained from the legume flours; however, the yield of amylase-treated pentosans from wheat flour (D'Appolonia and MacArthur 1975a) was higher (0.7%) than values obtained for the legume flours. These results indicate that although the water-solubles of HRS wheat flour contain an appreciable amount of pentosan material, only a small amount of nonstarchy polysaccharide material was extracted from the legume flours.

TABLE I
Yield of Water-Soluble Extractable Material From Legume Flours[a]

Flour Source	Total Water-Solubles (%)	Crude WSNP[b] (%)	Amylase-Treated WSNP[b] (%)
Navy bean	38.9	2.65	0.18
Pinto bean	35.6	3.67	0.54
Faba bean	42.2	7.27	0.23
Lentil	33.6	2.23	0.15
Mung bean	39.2	8.08	0.51

[a]Values reported are an average of two determinations based on flour weight and reported on a moisture-free basis.
[b]WSNP = Water-soluble nonstarchy polysaccharides.

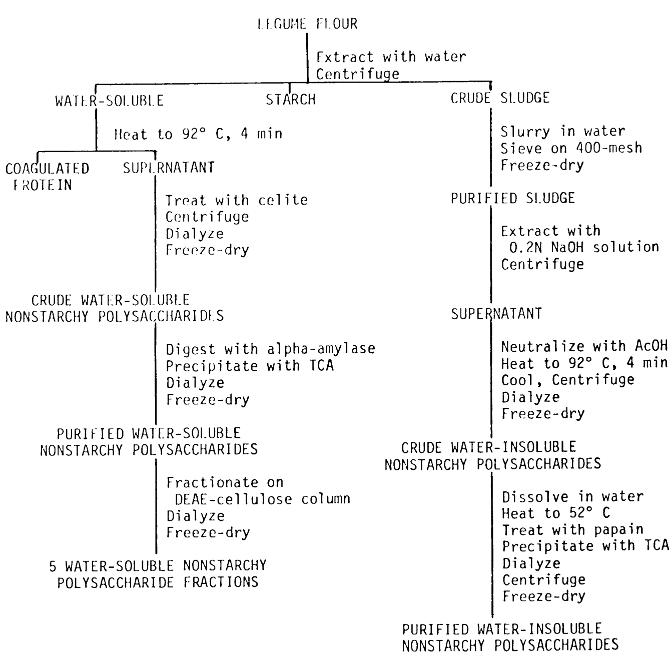

Fig. 1. Schematic diagram for the purification of nonstarchy polysaccharide material from legume flours.

Protein content of the total water-solubles and crude and amylase-treated nonstarchy polysaccharides isolated from the various legumes is shown in Table II. The total water-soluble material extracted from navy and pinto bean flours contained about 50% protein, whereas the total water-solubles of faba, lentil, and mung bean flours were mostly protein (87.1–93.8%). These data indicate that the amount of extractable carbohydrate material was quite low compared to wheat flour water-solubles that contain about 30% protein (D'Appolonia et al 1970).

The crude WSNP, which represented the material after heating the extract to coagulate protein followed by treatment with celite to precipitate further protein, showed lower values for protein content than that present in the total water-solubles. Values were still high when compared to wheat flour crude pentosans that contain about 25% protein (D'Appolonia et al 1970). Likewise after α-amylase treatment and dialysis, the freeze-dried purified WSNP for each legume contained lower amounts of protein than the crude WSNP, but the amounts were relatively high. For example, faba bean, lentil, and mung bean amylase-treated WSNP still contained over 50% protein. In comparison, the protein content of amylase-treated pentosans of HRS wheat flour was 23.0% (D'Appolonia et al 1970).

The purified WSNP of all legume flours were subjected to DEAE-cellulose column chromatography, using five eluents. The yields of the DEAE-cellulose fractions for each purified WSNP obtained from the different legume flours are reported in Table III. The yield of fractions 1, 2, and 3 (F_1, F_2, and F_3) from the purified WSNP was low in all cases compared to the amount of material obtained in fractions 4 and 5 (F_4 and F_5). F_4 for all of the purified legume WSNP gave the highest yield. These results are totally different from those obtained with wheat flour. In general, the yield of DEAE-cellulose F_1 of α-amylase treated water-soluble pentosans of wheat is the highest (30.0%) (D'Appolonia and MacArthur 1975a).

TABLE II
Protein Content of Total Water-Solubles and Crude and Amylase-Treated Water-Soluble Nonstarchy Polysaccharides[a]

Flour Source	Total Water-Solubles (%)	Crude WSNP[b] (%)	Amylase-Treated WSNP[b] (%)
Navy bean	51.7	43.9	24.3
Pinto bean	50.2	28.0	13.0
Faba bean	93.8	71.9	58.0
Lentil	87.1	67.7	63.6
Mung bean	93.0	58.9	55.3

[a]Values reported are an average of two determinations and expressed on a moisture-free basis, using 6.25 to convert nitrogen to protein.
[b]WSNP = Water-soluble nonstarchy polysaccharides.

TABLE III
Yield of Water-Soluble Nonstarchy Polysaccharide (WSNP) Fractions Obtained by Diethylaminoethyl-Cellulose Column Chromatography[a]

Source of Amylase-Treated WSNP	DEAE-Cellulose Fraction				
	F_1 (%)	F_2 (%)	F_3 (%)	F_4 (%)	F_5 (%)
Navy bean	0.93	16.48[b]	...	30.8	27.0
Pinto bean	8.57	9.97[b]	...	27.0	18.8
Faba bean	1.60	tr[c]	3.70	21.8	21.1
Lentil	1.48	tr[c]	6.19	24.3	12.2
Mung bean	2.53	1.28	6.06	27.1	10.0

[a]Yield values are based on the amount of material applied to the column.
[b]Represents yield of fractions 2 and 3 combined.
[c]tr = Trace amount.

Since the yield of DEAE-cellulose fractions 1, 2, and 3 was very low, there was insufficient material for protein analysis, with the exception of fractions 2 and 3 combined for the navy bean WSNP that contained about 9% protein.

F_5 for all of the legume WSNP had a higher protein content than F_4. F_5 of the mung bean WSNP was not determined due to insufficient material (Table IV). In the amylase-treated fractions from wheat flour, fractions 1 and 2 were essentially arabinoxylans with only trace amounts of protein, whereas fractions 3, 4, and 5 were glycoproteins (D'Appolonia et al 1970).

Paper chromatography was used to analyze the component sugars present in the legume WSNP and the DEAE-cellulose fractions (Fig. 2).

TABLE IV
Protein Content of Diethylaminoethyl-Cellulose Fractions 4 and 5[a]

Flour Source	DEAE-Cellulose Fraction F_4 (%)	F_5 (%)
Navy bean	25.9	58.4
Pinto bean	14.3	44.3
Faba bean	52.1	75.0
Lentil	53.2	76.5
Mung bean	55.0	...

[a]Values reported are expressed on a moisture-free basis, using 6.25 to convert nitrogen to protein.

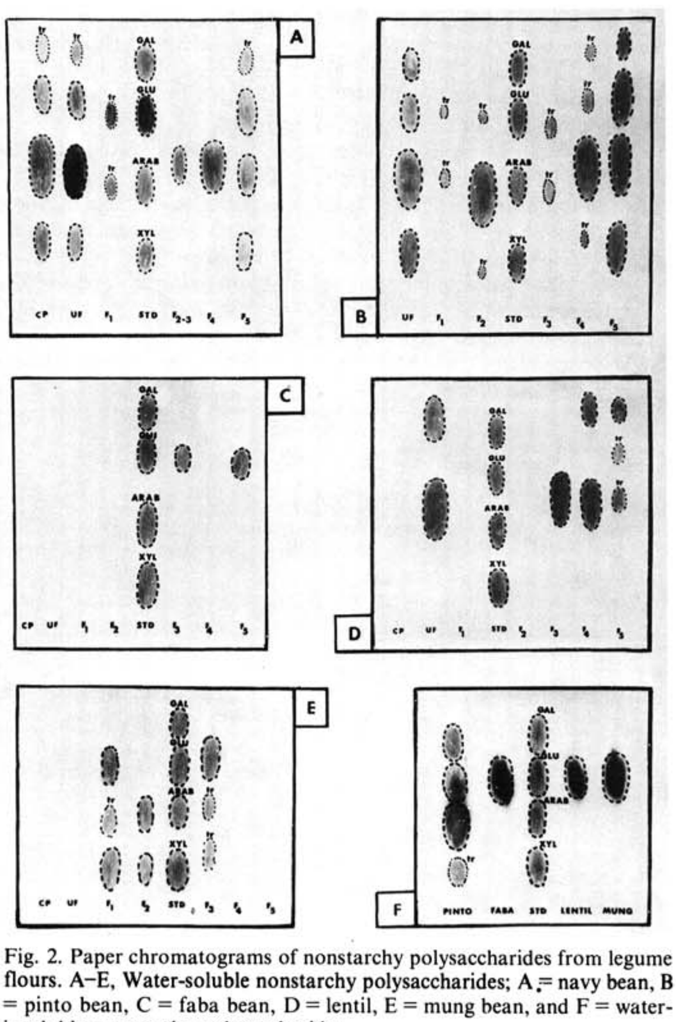

Fig. 2. Paper chromatograms of nonstarchy polysaccharides from legume flours. A–E, Water-soluble nonstarchy polysaccharides; A = navy bean, B = pinto bean, C = faba bean, D = lentil, E = mung bean, and F = water-insoluble nonstarchy polysaccharides.

Figure 2A shows the paper chromatogram of the navy bean WSNP and its DEAE-cellulose fractions. The crude WSNP (CP) and the unfractionated (UF) or α-amylase treated WSNP were similar in component sugars. Arabinose was the predominant sugar followed by xylose, glucose, and a trace of galactose. F_1 contained arabinose and glucose. Fractions 2 and 3 combined and F_4 contained only arabinose. F_5 contained the same sugars found in the crude and amylase-treated WSNP.

In general, the component sugars found in the pinto bean WSNP were similar to those found in the navy bean WSNP (Fig. 2B). In this instance, F_2 and F_3 were not combined as was done with the navy bean. F_2 was predominantly arabinose with traces of xylose and glucose; F_3 showed a trace amount of arabinose and glucose. F_4 of pinto bean and F_4 of navy bean showed traces of xylose, glucose, and galactose and a high amount of arabinose. F_5 of pinto bean contained the same sugars as found in the corresponding fraction of the navy bean.

The paper chromatogram of hydrolyzed faba bean WSNP is shown in Fig. 2C. This chromatogram indicated that the only sugar that could be detected was glucose (F_3 and F_5). No sugar components were visualized in CP, UF, F_1, F_2, and F_4, which indicates that the material hydrolyzed was predominantly protein or material other than carbohydrate.

Figure 2D shows the paper chromatogram of hydrolyzed lentil WSNP. Arabinose was the predominant sugar and appeared in high amounts in the UF, F_3, and F_4, followed by galactose that was present in the UF, F_4, and F_5 fractions. Glucose and arabinose also were present in F_5. Sugars were not detected in the hydrolyzed CP, F_1, and F_2, again probably due to their high protein content. Because of the solvent system used to separate the sugars, mannose was not detected as a component sugar; however, it was later detected by gas-liquid chromatography.

The paper chromatogram for hydrolyzed mung bean WSNP is shown in Fig. 2E. F_1 contained a high amount of xylose and glucose and some arabinose, F_2 contained arabinose and xylose, and F_3 contained predominantly glucose and some arabinose and xylose. No sugar components were found in the CP, UF, F_4, and F_5 fractions. Apparently CP and UF were very high in protein so that the sugars were not detectable.

A paper chromatogram of unfractionated and fractionated water-soluble pentosans from HRS wheat flour showed a totally different sugar profile (D'Appolonia 1968). Arabinose and xylose were the main components in fractions 1 and 2. Galactose was present in the unfractionated material and F_4 with a trace amount in fractions 3 and 5. No glucose was detected in any fraction (D'Appolonia 1968). Thus, the water-soluble carbohydrates present in wheat flour are composed primarily of pentose sugars (arabinoxylans), which is not true for the legume flours. Arabinogalactans have also been reported in a water extract from wheat flour (Neukom 1976).

The relative amounts of the different sugars present in the unfractionated and DEAE-cellulose fractions of WSNP from the various legumes were determined by gas-liquid chromatography. Mannitol was used as an internal standard, except for the lentil WSNP. The latter legume contained mannose, so fucitol was used as internal standard.

The main sugar present in all fractions was arabinose. Xylose and glucose were present in the unfractionated material and in F_5. Galactose was detected in trace amounts.

The arabinose content of both pinto bean and navy bean WSNP was high. Xylose, galactose, and glucose also were present in certain fractions.

Glucose was the main sugar present in faba bean WSNP, and appeared predominately in F_2, F_3, and F_5. Traces of arabinose, xylose, and galactose were detected by gas chromatography that were not observed with paper chromatography (Fig. 2C).

Mannose was present only in lentil WSNP and appeared in the unfractionated material and fractions 4 and 5. Arabinose was predominant in the unfractionated material and fractions 3, 4, and 5. Galactose, in addition to being in the unfractionated material, was found in fractions 4 and 5.

Xylose and glucose were present in only small amounts. Glucose was the predominant sugar present, followed by arabinose and xylose. Galactose was present in only trace amounts in F_2.

A direct comparison between the results obtained by paper and gas-liquid chromatography was not possible due to the variation in amount of sample spotted on the paper. The sensitivity of the gas chromatographic procedure would be greater than paper chromatography.

Based on results obtained by paper and gas-liquid chromatography, the amylase-treated WSNP of legume flours had a different sugar composition than that of wheat flour WSNP. In wheat flour, the carbohydrate material was predominant in fractions 1 and 2 and was composed mainly of arabinose and xylose (arabinoxylans). The legume flours contained carbohydrate material mainly in fractions 4 and 5 (primarily heteropolysaccharides).

Among the various legume flours, the sugar composition of the WSNP differed. The navy and pinto bean WSNP were composed mainly of arabinose and some xylose, glucose, and galactose. The faba bean WSNP were composed of glucan as the main polysaccharide. The lentil WSNP were probably mixtures of several heteropolysaccharides, and the mung bean WSNP were composed of polymers containing glucose, arabinose, and xylose.

The data in Tables II and IV indicate that the bulk of the WSNP was protein in nature, with only small amounts of carbohydrate material. High amounts of protein could interfere with analysis of the component sugars by paper and gas-liquid chromatography.

The role of water-soluble pentosans of wheat flour in baking and the rate of bread staling have been investigated (D'Appolonia 1973, D'Appolonia et al 1970, Jelaca and Hlynka 1972). Recently, Kim and D'Appolonia (1977) studied the effect of pentosans on dough, bread, and bread staling rate. They found that the water-soluble pentosans affected the absorption and absorbed 4.4 times their weight. Addition of water-soluble pentosans did not effect loaf volume. The bread staling rate was slightly decreased by incorporating water-soluble pentosans due to the reduction of starch components available for crystallization. The possible effect of WSNP from various legume flours in baking and on rate of bread staling warrants further studies.

Water-Insoluble Nonstarchy Polysaccharides

The sludge material obtained from the water extracts of the various legume flours was purified as shown in Fig. 1.

Table V shows the yield of total sludge material, crude WINP, and papain-treated or purified WINP for each lelgume flour. The mung bean flour had the highest yield for total sludge and crude and papain-treated WINP. The faba bean flour gave the lowest yield for total sludge material; the pinto bean flour gave the lowest yield for both crude and papain-treated WINP. In general, the yield decreased from total sludge recovery to crude and papain-treated WINP.

The protein content for the WINP extracted from the various legume flours is shown in Table VI. Lentil flour had the highest protein content in the WINP, and the faba bean showed the lowest values. In general, when the total sludge material was treated, the protein content decreased. The papain-treated WINP had the lowest protein values.

Figure 2F shows a paper chromatogram of the sugar components present in the papain-treated WINP for the various legumes after hydrolysis. Although only the sugars present in the pinto bean WINP are shown, the same sugars were present in the navy bean WINP. Xylose, arabinose, glucose, and galactose were present. Faba, lentil, and mung bean papain-treated WINP contained only glucose.

Comparison of the paper chromatograms of the purified WINP and unfractionated (or α-amylase-treated) WSNP for the corresponding legume flour revealed that pinto and navy bean WINP were composed of the same sugars found in WSNP, whereas faba bean, lentil, and mung bean showed a somewhat different sugar composition between the WINP and WSNP.

Nonstarchy polysaccharides from wheat sludge are composed mainly of arabinose and xylose (D'Appolonia and MacArthur 1975b), which is different from the sugars found in the nonstarchy polysaccharide from the sludge of legumes.

Gas-liquid chromatography was used to determine the relative percentage of the sugar components present in the papain-treated WINP. Arabinose was predominant in the navy bean papain-treated WINP (74%), followed by galactose (10%), xylose (10%), and glucose (8%).

The pinto bean papain-treated WINP contained a sugar distribution somewhat similar to that of navy bean. Faba bean, lentil, and mung bean papain-treated WINP contained primarily glucose and only trace amounts of arabinose and xylose.

Comparison of the sugar components in the WSNP and WINP of each legume indicated that navy and pinto beans had a similar sugar composition for both fractions. Faba bean contained primarily glucose for both the WSNP and WINP fractions. Kawamura and Narasaki (1958) found a high glucose content and a smaller amount of xylose, galactose, and fucose in the hydrolysate of hemicellulose B_1, prepared from dehulled seeds of broad beans (*Vicia faba*). The WSNP of lentil contained arabinose, mannose, and galactose in addition to glucose, which was the only sugar detected in the WINP fraction. Mung bean contained a high amount of glucose in both the WSNP and WINP fractions. The WSNP also contained xylose and arabinose.

The effect of wheat water-insoluble pentosans on baking and staling rate has been investigated (Bechtel and Meisner 1954, D'Appolonia and Gilles 1971, Kulp and Bechtel 1963). Kim and D'Appolonia (1977) found that the water-insoluble pentosans absorbed 9.9 times their weight of water. Bread loaf volume was slightly decreased with incorporation of water-insoluble pentosans. A more pronounced decrease in the bread staling rate was obtained with water-insoluble than water-soluble pentosans.

The possible role of WINP from various legume flours remains to be elucidated.

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TABLE V
Yield of Water-Insoluble Nonstarchy Polysaccharides (WINP) Extracted From Legume Flours[a]

Flour Source	Total Sludge[b] (%)	Crude WINP (%)	Papain-Treated WINP (%)
Navy bean	12.8	2.24	1.12
Pinto bean	13.5	1.62	0.65
Faba bean	11.4	3.53	1.41
Lentil	17.3	3.79	1.52
Mung bean	24.4	11.47	4.59

[a]Values reported are expressed on flour weight and on a moisture-free basis.
[b]Sludge recovered after washing through a No. 400-mesh sieve.

TABLE VI
Protein Content of Water-Insoluble Nonstarchy Polysaccharides (WINP) Extracted From Legume Flours[a]

Flour Source	Total Sludge (%)	Crude WINP (%)	Papain-Treated WINP (%)
Navy bean	9.2	3.3	3.1
Pinto bean	6.4	5.3	4.2
Faba bean	4.0	4.0	2.6
Lentil	11.7	9.8	5.0
Mung bean	5.6	4.8	4.0

[a]Values are expressed on a moisture-free basis, using the factor 6.25 to convert nitrogen to protein.

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