A COMPARISON OF THE CARBOHYDRATE COMPOSITION OF LEGUME SEEDS: HORSEBEANS, PEAS, AND LUPINES

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ABSTRACT

Horsebeans, smooth peas, wrinkled peas, and lupines were analyzed for their starch, furfural generator, cellulose, and sugar contents. Horsebeans had an average starch content of 41%. Smooth peas were richer in starch (48%) than wrinkled peas (33%). Lupines contained only traces (0.4%) of starch but were rich in structural and cell-wall polysaccharides. Total furfural generators ranged from 4.8% for horsebeans and smooth peas to 6% in wrinkled peas and 10% in lupines. Most of the furfural generators appeared to be either water-soluble or soluble in dilute acid. Acid hydrolysis of hemicelluloses yielded xylose, smaller amounts of arabinose, glucose, and galactose, and traces of rhamnose. Small amounts of glucose-containing polymers, soluble in water and dilute acid, were present in horsebeans and peas; lupines contained only traces of this fraction. Wrinkled peas and lupines contained more (10%) ethanol-soluble sugars than horsebeans (6.5%) and smooth peas (8%). Thin-layer and column chromatography of the ethanol-soluble sugars showed the presence of varying amounts of sucrose, raffinose, stachyose, verbascose, and higher molecular-weight α-galactosides.

Legume seeds, such as horsebeans (*Vicia faba*), peas (*Pisum sativum*), and lupines (*Lupinus*), are important potential protein sources for both human consumption and animal feed. These seeds contain a considerable amount of carbohydrates which have received only limited attention. In recent studies, the carbohydrate composition of horsebean seeds (1,2) and horsebean flour (3) has been reported. All legume seeds are known to contain sugars that belong to the raffinose family of oligosaccharides (4–6), also called α-galactosides, but few precise analytical data are available on the quantitative amounts of these sugars present, particularly for peas and lupines. α-Galactosides are supposed to be important in animal nutrition; their role in flatulence activity has been extensively reviewed by Cristofaro *et al.* (7).

Lupine and wrinkled pea seeds are apparently richer in hemicelluloses (6,8) than horsebean seeds (1,2). Qualitative investigations of these polysaccharides revealed the presence of xylose, arabinose, and smaller amounts of galactose and uronic acids in horsebean (2,9), pea (10,11), and lupine (8,12,13) seeds. Glucose, rhamnose, and fucose have also been identified, except in peas.

The purpose of the present work was to study the carbohydrate composition of some legume seeds, and to make a more detailed investigation of the soluble sugars, *i.e.*, α-galactosides and the cell-wall constituents.

MATERIALS AND METHODS

Materials

Two varieties each of horsebeans (*V. faba*), peas (*P. sativum*), and lupines (*Lupinus*) from various geographical origins were grown in 1973 in the center of France (Table I). One horsebean variety (Mainteneur) had a low tannin content and one (Bianka) was without tannin. The seeds were ground in a laboratory mill in the presence of solid carbon dioxide to a particle size of less than 0.5 mm.

Methods

Starch was determined by the glucoamylase method (14). Total furfural generators were estimated by the aniline-acetate reaction (15) and expressed in terms of xylose. Total ethanol-soluble sugars, obtained by exhaustive extraction with hot 80% ethanol, were determined by the anthrone method (16) and arbitrarily expressed as glucose (17). Sucrose was estimated enzymatically with invertase (18). Individual sugars were identified and determined by thin-layer (19) and column (20,21) chromatography.

Cell-wall constituents were investigated, after elimination of sugars and starch, by acid hydrolysis. Details of the procedure have been published (2,22). Of particular interest is the fact that the method permits one to obtain simultaneously analytical data on hemicelluloses and on their chemical composition.

RESULTS AND DISCUSSION

Starch, Furfural Generators, Sugars

Starch, furfural generator, and ethanol-soluble sugar contents are shown in Table II. The two horsebean varieties had similar starch contents. Smooth peas were considerably richer in starch (47.9%) than wrinkled peas (32.9%). Lupines contained only traces of starch. The values for horsebeans were in the range (30–42%) previously reported (2). Smooth peas have been reported to be much

TABLE I
Origin, 100-Kernel Weight, and General Appearance of Horsebean, Pea, and Lupine Seeds

Seeds	Geographical Origin	Color	Dimension[a] mm	Appearance	100-Kernel Weight (g dry matter)
Bianka horsebeans	Holland	Beige-greenish	16 × 12 × 6	Partially wrinkled, flat	67.4
Mainteneur horsebeans	Germany	Reddish-beige	13 × 9 × 7	Smooth, flat	61.3
Lincoln wrinkled peas	Great Britain	Yellowish-beige	6 × 6 × 5	Completely wrinkled, cylindrical	15.6
Dicktrom smooth peas	Holland	Green	6 × 7 × 7	Very slightly wrinkled, ovoid	19.6
Weiko yellow lupines	Germany	Light beige	7 × 5 × 3	Smooth, flat	11.6
Blusa blue lupines	Germany	Maroon-beige and gray spots	7 × 5 × 5	Smooth, flat	12.2

[a]Length × width × thickness.

higher in starch than were wrinkled peas (23). Only small amounts of starch have been detected in lupine seeds (8).

Smooth peas and horsebeans had the lowest furfural generator content (4.7% average); that of wrinkled peas was 6.34%. Lupines were found to be much richer in furfural generators (9.3 and 10.5%) than the other seeds. Values ranging from 4.6 to 6.9% of furfural generators have been observed in various horsebean samples (2). To our knowledge, no previous information was available on the furfural generator contents of pea and lupine seeds. The total ethanol-soluble sugars content, expressed as glucose in horsebean seeds (5.9 and 7.1%) was in the range observed by Pritchard *et al.* (1) and Cerning *et al.* (2). Horsebean flour has been reported to contain 7.8% ethanol-soluble sugars (3). The ethanol-soluble sugars content in smooth peas (8.0%) was greater than the 5.41% reported by Täufel *et al.* (6), and that in lupines (9.5%) was also greater than the 7.2% indicated by Hove (8). These differences are probably due to the fact that different methods have been used and that the varieties were not the same.

Wrinkled pea seeds had the highest ethanol-soluble sugars content (10.2%). Kooistra (23) indicated that wrinkled peas always have a higher sugar content than smooth peas. Sucrose was only a small portion of the ethanol-soluble sugars, *i.e.*, it comprised an average of 24% of the total sugars (1.78 and 1.35%) in horsebeans, 30 and 38% of the total sugars in wrinkled and smooth peas (3.05 and 3.05%), respectively, and 10 and 30% of the total sugars in lupines (1.00 and 2.80%). The remaining ethanol-soluble sugars were essentially α-galactosides; their proportion, calculated on the basis of sucrose and total sugars content, varied from 65 to 90%.

Individual Sugars

α-Galactosides were reported to be involved in flatus formation (14). Therefore, ethanol extracts of the legume seeds were further investigated by thin-

TABLE II
Carbohydrate Composition (% Dry Matter) of Horsebean, Pea, and Lupine Seeds[a]

Sample	Starch	Furfural Generators	Ethanol-Soluble Sugars	Sucrose
Bianka horsebeans	41.2	4.72	7.1	1.78
Mainteneur horsebeans	41.3	4.63	5.9	1.35
Lincoln wrinkled peas	32.9	6.34	10.2	3.05
Dicktrom smooth peas	47.9	4.87	8.0	3.05
Weiko yellow lupines	0.3	10.5	9.5	1.00
Blusa blue lupines	0.5	9.3	9.5	2.80

[a]Mean value of three determinations.

layer and column chromatography. The two techniques are complementary for the following reasons: a) sugars of similar molecular weight cannot be separated and distinguished satisfactorily by column chromatography; *i.e.*, retention times for raffinose, stachyose, and verbascose are the same as for maltotriose, maltotetraose, and maltopentaose; b) it is not possible to distinguish clearly between hexoses, pentoses, and methylsugars; and c) thin-layer chromatography not only separates the different sugars, but also permits one to affirm the presence or absence of a particular sugar by specific detection with aniline-diphenylamine.

Thin-layer chromatography revealed the presence of sucrose, raffinose, stachyose, verbascose, and higher molecular-weight α-galactosides (remained at the origin) in the ethanol extracts of the three legume seeds. Only very small traces of glucose were present. Furthermore, the presence of four low-molecular-weight, ethanol-extractable compounds in horsebean extracts was observed. They had mobilities corresponding to those of fructose, arabinose, xylose, and rhamnose, but were not identical in color to the spots of those standards after development with aniline-diphenylamine. The presence of these compounds in horsebeans has been reported before (2), and further investigations are necessary to identify them.

Chromatographic separation of the sugars present in horsebeans on a column of Bio-Gel P-2 is illustrated in Fig. 1. Patterns from the two horsebean varieties investigated were similar. The separation of sugars from peas and lupines is shown in Figs. 2 and 3, respectively. The pattern of the sugars from smooth peas

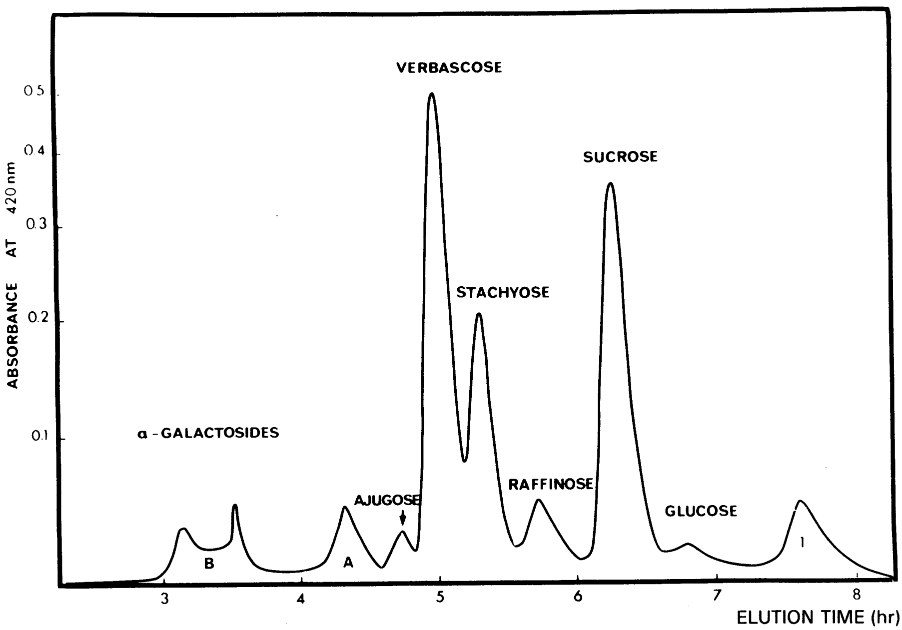

Fig. 1. Elution diagram of ethanol-soluble sugars from a column (1.5 × 100 cm) of Bio-Gel P-2 at 65°C, using water at a flow rate of 46.8 ml/hr. Mainteneur horsebeans.

was similar to that of the sugars from wrinkled peas. The elution diagrams confirm the results obtained with thin-layer chromatography and enzymatic analysis of sucrose, *i.e.*, sucrose was present in smaller amounts than α-galactosides (Figs. 1—3). Peaks A and B are believed to be higher molecular-weight α-galactosides, since they yielded galactose and smaller amounts of glucose and fructose after acid hydrolysis. An attempt was made to determine the molecular weight of these fractions from a semi-logarithmic plot of molecular weight *vs.* elution volume (21). This indicated a $\overline{DP}$ of 9 for peak A (Fig. 1), and a $\overline{DP}$ of 14 to 15 for peak B (Figs. 1—3). Oligosaccharides of this molecular weight have been identified in other plants (24,25) but not, to our knowledge, in legume seeds. We suppose that peak 1 (Fig. 1) corresponds to the low-molecular-weight ethanol-extractable compounds observed on thin-layer chromatograms.

Table III lists the sugar distribution (per cent of total) obtained by measuring the total and the individual peak areas on the column chromatograms, in the ethanol extracts. As mentioned before, legume seeds contained (other than 20 to 30% of sucrose) high amounts of α-galactosides. The raffinose content, except for wrinkled peas, was low. The proportion of verbascose was higher than that of stachyose in horsebeans and lupines; it was the same in wrinkled peas. Stachyose exists in greater amounts in smooth peas than verbascose. With the exception of one lupine variety, only small amounts of ajugose were present. Only horsebeans seemed to contain an oligosaccharide of $\overline{DP}$ 9, but the extracts of all three legume seeds contained an α-galactoside fraction of $\overline{DP}$ 14 to 15.

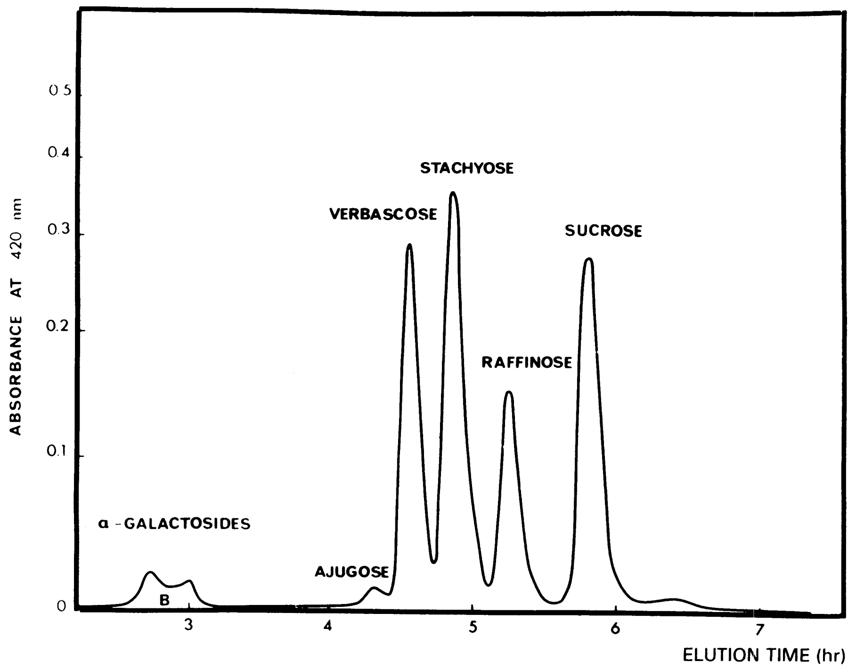

Fig. 2. Elution diagram of ethanol-soluble sugars from a column (1.5 × 100 cm) of Bio-Gel P-2 at 65°C, using water at a flow rate of 46.8 ml/hr. Lincoln wrinkled peas.

Cell-Wall Constituents

Qualitative Aspects. Thin-layer chromatograms indicated that mild acid hydrolysis of the three legume seeds (0.7*N* HCl) liberated xylose, glucose, galactose, and rhamnose. Arabinose appeared only in horsebeans and wrinkled peas. Xylose was predominant in every case, galactose and arabinose were

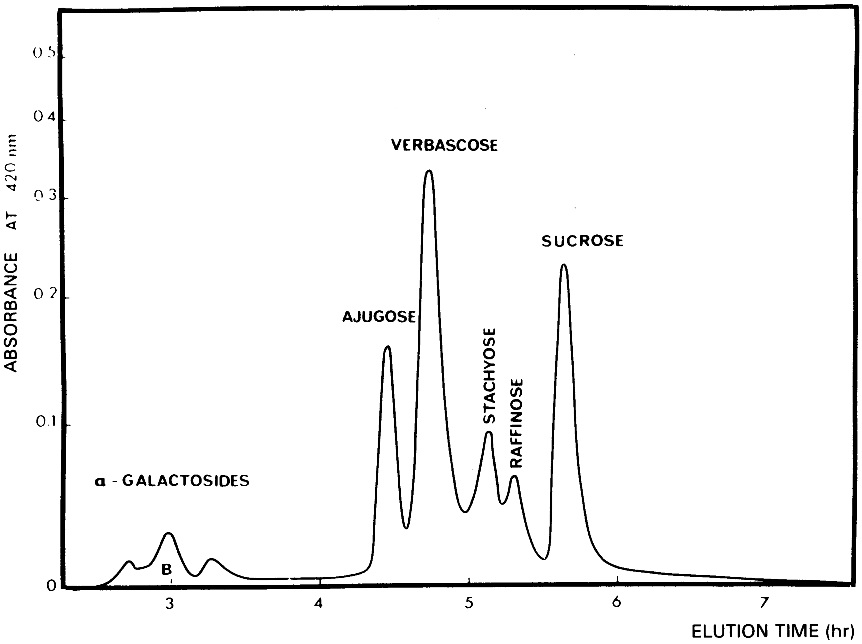

Fig. 3. Elution diagram of ethanol-soluble sugars from a column (1.5 × 100 cm) of Bio-Gel P-2 at 65°C, using water at a flow rate of 46.8 ml/hr. Blusa lupines.

TABLE III
Sugar Composition (% of Total) in Ethanol Extracts from Horsebean, Pea, and Lupine Seeds, after Separation on a Column of Bio-Gel P- 2[a]

Sugar	Bianka Horse-beans	Mainteneur Horsebeans	Lincoln Wrinkled Peas	Dicktrom Smooth Peas	Weiko Yellow Lupines	Blusa Blue Lupines
Peak 1	2.0	3.4	...	...	...	...
Glucose	1.2	1.4	...	...	traces	traces
Sucrose	26.1	25.7	22.6	30.3	10.6	27.2
Raffinose	7.7	3.4	15.8	11.9	10.1	4.9
Stachyose	23	17.8	28.0	29.3	35.2	9.0
Verbascose	30	35.1	28.0	21.7	36.1	30.9
Ajugose	2	3.1	1.3	0.8	2.7	21.5
Peak A ($\overline{DP}$ 9)	3.2	4.2	...	...	...	...
Peak B ($\overline{DP}$ 14–15)	4.6	6.3	4.2	6.1	5.4	6.5

[a]Mean value of two determinations.

present in small amounts, and traces of rhamnose existed. The color intensities of the components indicated traces of glucose in the hydrolysates of lupines and smooth peas, but higher amounts in wrinkled peas and horsebeans. This chemical composition of the hemicelluloses hydrolyzed by mild acid treatments is in general agreement with results reported for horsebeans (2,9), peas (10,11), and lupines (8,12,13). However, rhamnose and glucose have not been identified in pea hemicelluloses before.

In the hydrolysates obtained with stronger acid (72% H_2SO_4) glucose predominated as a degradation product of cellulose. Only traces of cellobiose were present. Sulfuric acid also liberated small quantities of pentoses, probably originating from hemicelluloses that were associated with cellulose. Xylose was present in every case and traces of rhamnose appeared in horsebeans and wrinkled peas.

Fucose [a minor constituent of *V. faba* hemicelluloses (9)], uronic acids (10,11,13), and mannose (1) could not be identified under our experimental conditions.

Furfural Generators, Hemicelluloses

Results obtained after acid hydrolysis of the legume seeds with hydrochloric acid (0.7*N*) sulfuric acid (72%) are given in Table IV. The total furfural generator contents and those determined in the filtrate after glucoamylase hydrolysis of starch (water-soluble) are indicated also. This permits one to sum up the pentosans that are soluble in water, and in mild and strong acid, to compare with the values for total furfural generators. Comparison of the first two columns of Table IV shows that agreement between the two methods was quite satisfactory.

The water-soluble hemicelluloses represent the highest amount of the total; *i.e.*, 2.22 and 2.30% water-soluble hemicelluloses in horsebean seed represent 50 and 55% of the total hemicelluloses. These values are in agreement with those reported previously (2). Water-soluble hemicelluloses (3.55 and 3.30%) represent 53.5 and 65% of the total hemicelluloses in wrinkled and smooth peas, respectively. Water-soluble hemicelluloses (4.59 and 4.85%) in lupine seeds represent 46.5 and 52% of the total.

Acid-soluble hemicelluloses were distributed differently depending on the variety. The amount of pentoses liberated by 0.7*N* hydrochloric acid was higher in wrinkled peas (1.93%) and lupines (1.82 and 1.78%) than in horsebeans (1.42 and 1.10%) and smooth peas (0.8%). The same trend was observed with the pentoses liberated by sulfuric acids ranging from 0.4 and 1.07% in horsebeans, and 0.96 and 1.16% in wrinkled and smooth peas, respectively, to 2.71 and 3.50% in lupines. These pentoses were probably associated originally with cellulose.

Glucose-Containing Polymers

In the glucose-containing polymer contents indicated in Table IV, only those liberated by sulfuric acid can be considered to originate from cellulose (2). Those determined after mild acid hydrolysis of the glucoamylase hydrolysate, as well as those determined after hydrolysis by 0.7*N* hydrochloric acid (2), may perhaps originate from noncellulosic glucans that have been found in field beans (1) and other legume seeds (26). The amount of water-soluble and acid-soluble glucose-containing polymers was considerably higher in horsebeans (0.93 and 1.64%) and wrinkled peas (2.03%) than in lupines (0.29 and 0.58%).

TABLE IV

Furfural Generators, Hemicelluloses, Glucose-Containing Polymers, and Crude Lignin (% Dry Matter) in Horsebean, Pea, and Lupine Seeds[a]

Sample	Total Furfural Generators	Hemicelluloses				Glucose-Containing Polymers			Crude Lignin
		Sum of hemi-celluloses	Water-soluble	Acid-sol. HCl	Acid-sol. H_2SO_4	Water-soluble	Acid-sol. HCl	Acid-sol. H_2SO_4	
Bianka horsebeans	4.14	4.04	2.22	1.42	0.40	0.6	0.33	5.02	1.08
Mainteneur horsebeans	4.63	4.56	2.30	1.10	1.07	1.28	0.36	5.72	0.97
Lincoln wrinkled peas	6.34	6.64	3.55	1.93	1.16	0.93	1.10	8.47	0.98
Dicktrom smooth peas	4.87	5.06	3.30	0.80	0.96	0.59	0.16	5.32	0.48
Weiko yellow lupines	10.50	9.91	4.59	1.82	3.50	0.14	0.15	14.71	0.75
Blusa blue lupines	9.33	9.31	4.85	1.78	2.71	0.37	0.21	14.20	0.67

[a]Mean value of two determinations.

Glucose in the sulfuric acid hydrolysate (originating from cellulose) was considerably higher in lupines (14.71 and 14.20%) and wrinked peas (8.47%) than in horsebeans (5.02 and 5.72%) and smooth peas (5.32%). The values are in the range of those reported for horsebeans (1,2) and peas (6).

The crude lignin content of horsebeans and wrinkled peas was about the same (1.0% average) in lupines (0.67 and 0.75%) and higher than in smooth peas (0.48%).

GENERAL DISCUSSION

The legume seeds had a total carbohydrate content ranging from 35% for lupines, to 60% for horsebeans and wrinkled peas, to 67% for smooth peas. The major constituent of these carbohydrates was starch, except for lupines, in which the structural and cell-wall polysaccharide contents were highest. Compared to horsebeans and smooth peas, wrinkled peas were richer in hemicelluloses and cellulose; these seeds also contained the highest amount of ethanol-soluble sugars.

The major constituents of the ethanol-soluble sugars were, in order of decreasing magnitude, verbascose, stachyose, sucrose, and raffinose. Varying amounts of these sugars, supposedly of importance in animal nutrition (7), have been reported previously in legume seeds (1,3,6,25).

Small amounts of one (peas and lupines) or two (horsebeans) higher molecular-weight α-galactosides were identified having $\overline{DP}$ values of 9 and 14–15. α-Galactosides of $\overline{DP}$ 8, 9, and 10 have been identified in other plant material (24,25), but not in legume seeds.

As in an earlier report (2), none of the horsebean and pea samples analyzed in this study showed the presence of sugars originating from starch degradation (maltotriose-tetraose, and -pentaose) or reversion products (isomaltose), as has been observed by Pritchard *et al.* (1). Considering that amylase activity in horsebeans is very low (27), and that no maltose appears upon germination of horsebean (28) and pea seeds (6), the absence of maltose and maltosaccharides is not surprising. The highest amount of hemicellulose seems to be either water-soluble or soluble in dilute acid, as has been observed before (2).

The presence of insoluble glucans has been reported in horsebeans (1,2), but not in peas. It is believed that they are essentially located in the hull (2).

In general, it may be concluded that the carbohydrate composition of horsebeans, particularly the composition of the ethanol-extractable sugars, is in agreement with earlier findings (1–3). However, caution should be exercised in drawing a general conclusion from the small number of pea and lupine samples analyzed.

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