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CARBOHYDRATE COMPOSITION OF HORSE BEANS (*VICIA FABEA*) OF DIFFERENT ORIGINS¹

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ABSTRACT

A series of 16 horse bean samples (*Vicia faba*) from various geographical origins (Europe, North Africa, Asia) were grown in different areas of France. Analysis for their starch, furfural generators, and sugar content showed noticeable variations in carbohydrate composition. The samples had a total carbohydrate content of 51 to 66%, starch ranged from 30 to 42.3% and was inversely related to protein content. The total furfural generator content of most of the samples was in the range of 5 to 5.7%, the average value for ethanol-soluble sugars was 5.7%. Thin-layer and column chromatography of the ethanol-soluble sugars showed the presence of varying amounts of verbascose, stachyose, raffinose, sucrose, and, in some cases, traces of glucose and fructose. Quantitative analysis reveals an

average of 25% sucrose in the total sugars, the major part of which is composed of α -galactosides. Separate analysis of the cotyledons (including the germ) and hulls showed that the latter contain 78.9% total carbohydrates, most of which were structural polysaccharides. The composition of the 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 (0.5%) are present in the cotyledons, but increase to 3.6% in the hulls.

The increasing need for protein in the world has stimulated scientists and agronomists to search for new sources of protein. Horse beans (*Vicia faba*), also called field beans or broad beans, have attracted attention as a possible home-grown protein source and successive selection has made it possible to introduce higher-yielding varieties. Botanically, horse beans are dicotyledons and belong to the Leguminosae family. Their contents of amino acids, fatty acids, minerals, and vitamins have been reported (1), but relatively little is known concerning the precise carbohydrate composition of these leguminous seeds.

The ethanol-soluble sugars of broad beans (*Vicia faba*) (2,3) and other leguminous seeds, such as soybeans, peas, and French beans (4), are known to contain α -galactosides of sucrose, that is raffinose, stachyose, verbascose, and ajugose. However, precise analytical data on the quantitative amounts of these oligosaccharides are not available for horse beans (*Vicia faba*).

Pritchard et al. (5) indicate, in a recent study on field beans, that the composition of the ethanol-soluble fraction varies considerably between

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varieties, the main components being oligosaccharides and sucrose or its hydrolysis products. The same authors report that field beans contain over 60% total carbohydrates on a dry matter basis, and that winter-sown varieties provide more available carbohydrates (46 to 48%), that is starch and Taka-diestase-digestible polysaccharides, than the spring-sown varieties (30 to 42%).

In this paper we wish to report the results of investigations on the soluble and insoluble carbohydrates of horse beans from different geographical origins and to outline tentatively their distribution within the different parts of the seed.

MATERIALS AND METHODS

Materials

A series of 16 horse bean samples (*Vicia faba*) from various geographical origins were grown in 1972 in different areas of France (Table I). The samples were quite heterogeneous in color, size, and form. 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. Great difficulties were encountered in obtaining the desired particle size for some of the samples; additional grinding in a ball mill was necessary.

For one horse bean sample of British origin, uniform in morphological aspects, the hulls were separated from the cotyledons (including the germ) and the two different parts of the seed were analyzed separately.

Methods

Starch was determined by the glucoamylase method (6). Total furfural generators were estimated by the aniline-acetate reaction (7) and expressed in terms of xylose. Crude fiber was determined by the Scharrer-Kürschner procedure (8), which consists of weighing the residue remaining after treatment of the sample with a mixture of acetic, nitric, and trichloroacetic acids. The total soluble sugars obtained by exhaustive extraction with hot 80% ethanol were determined by the anthrone method (9), and arbitrarily expressed as glucose. Sucrose was estimated enzymatically with invertase (9). The invertase used was free of α -galactosidase activity, and the presence of high amounts of α -galactosides did not inhibit the enzyme². It was found, however, that horse beans have high α -galactosidase activity (4) which persists even after repeated hot-ethanol extractions. As a matter of fact, the sucrose content of the ethanol extracts (kept at -20°C .) increased considerably after only 3 weeks' storage. This was due to enzymatic degradation of α -galactosides. Therefore, long storage of the ethanol extracts, even at -20°C . which is without any risk with cereals, is not possible with horse beans.

Individual sugars were identified and determined by paper (10), thin-layer (11), and column (12) chromatography.

In the second part of our investigation, the residue remaining after extraction of sugar and elimination of starch was submitted to successive acid hydrolysis (13) to study the cell wall constituents using the scheme diagrammed in Fig. 1.

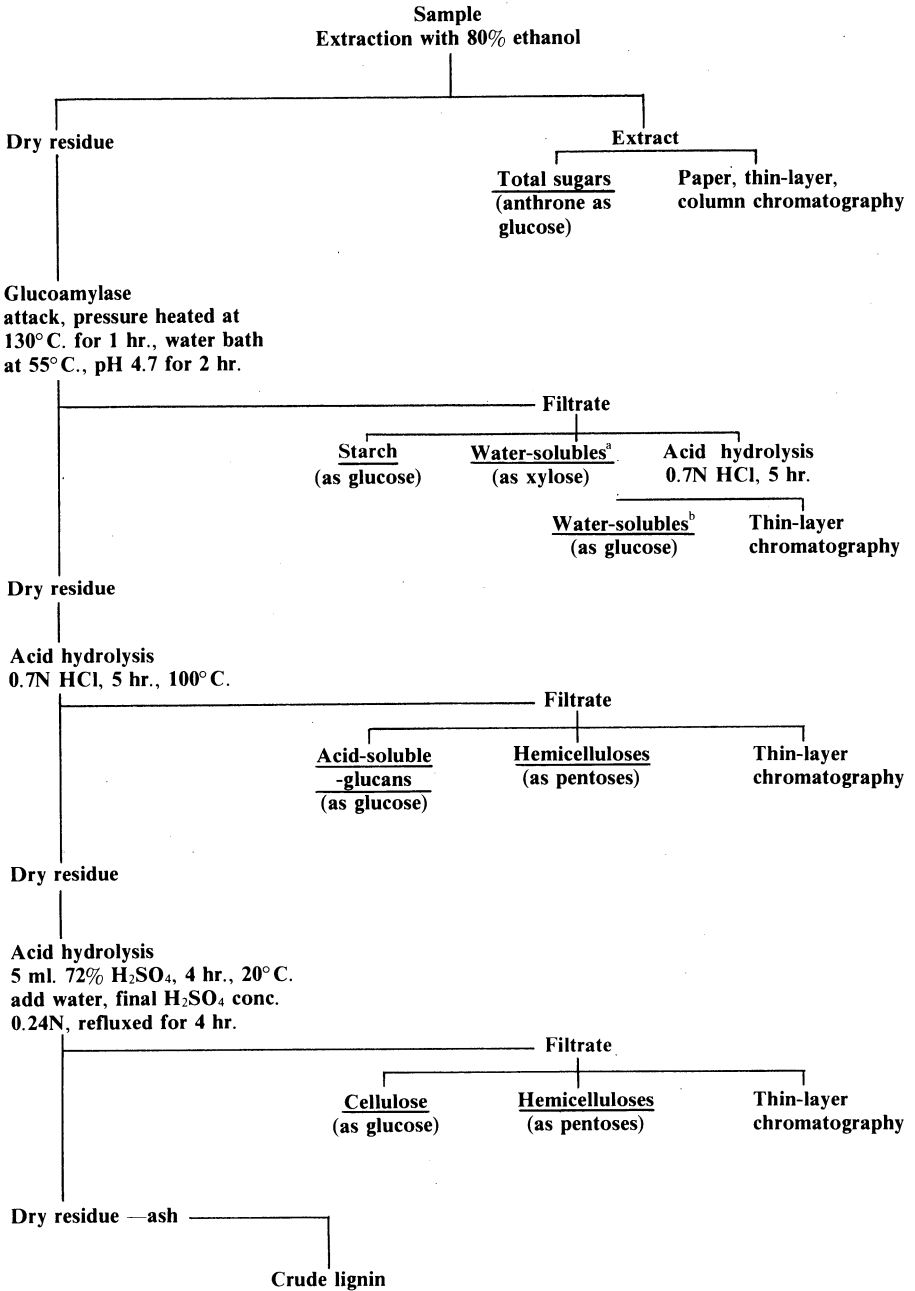
Acid hydrolysis of hemicelluloses was accomplished with 0.7N hydrochloric acid for 5 hr. in a boiling-water bath. The hydrolysate, after neutralization with Duolite A102D resin, was analyzed for reducing sugars (14) and glucose content (6).

²J. Cerning, unpublished results.

TABLE I. ORIGIN, GROWING AREA, AND GENERAL APPEARANCE OF HORSE BEAN SEEDS (*Vicia faba*)

Geographical Origin	Growing area in France	Color	Dimension ¹ mm.	Appearance
Poland	East (Colmar)	Brown-ochre	8 × 10	Partially wrinkled, ovoid
Poland	East (Mirecourt)	Black-violet	7 × 10	Smooth, ovoid
Poland	East (Mirecourt)	Brown-haematite	9 × 15	Smooth, ovoid
Agudadulce	Center (Clermont-Ferrand)	Brown-haematite	10 × 20	Smooth, flat
Holland	North (Mons en Chaussée)	Yellowish	10 × 16	Smooth, flat
Holland	North (Mons en Chaussée)	Beige	8 × 10	Smooth, ovoid
England	East (Mirecourt)	Ochre	10 × 15	Smooth, ovoid
England	North-West (Rennes)	Reddish-brown	10 × 15	Smooth, ovoid
France	North-West (Rennes)	Brown	7 × 10	Smooth, ovoid
Germany	East (Colmar)	Brown-haematite	8 × 12	Partially wrinkled, flat
Tunisia	West (Lusignan)	Brown-haematite	10 × 15	Smooth, flat
Israel	Parisian area	Brown-ochre	7 × 10	Partially wrinkled, ovoid
Afghanistan	Center (Clermont-Ferrand)	Dark-maroon	5 × 8	Partially wrinkled, ovoid
India	West (Lusignan)	Black	5 × 8	Smooth, ovoid
Tadjikistan	Center (Clermont-Ferrand)	Light-maroon	5 × 7	Partially wrinkled, ovoid
Maurice	Parisian area	Brown-haematite	7 × 10	Partially wrinkled, ovoid

¹Length presented first, then width.



^aMeasured as furfural generators.

^bMeasured as glucose-containing polymers.

Fig. 1. Scheme of separation and analysis.

In Somogyi's method, different monosaccharides usually undergo the same reactions when boiling time is 10 min., but the intensity of the reactions varies. However, when a boiling period of 30 min. is employed, differences in reducing power between glucose, xylose, and arabinose are minimized³.

Since glucose, xylose, and arabinose were the principal sugars liberated by acid hydrolysis, differences between the values for reducing sugars and glucose were considered to be due to the presence of pentoses.

To the dry residue from the preceding hydrolysis (13), 5 ml. of 72% sulfuric acid was added and the mixture was kept at 20° C., for 4 hr. with occasional stirring. Water (170 ml.) was added, the solution was refluxed for 4 hr., and the hydrolysate was analyzed for glucose and pentose content. This treatment liberates essentially glucose originating from cellulose. The residue remaining after the two successive hydrolyses was considered to be crude lignin (13).

RESULTS AND DISCUSSION

Starch, Furfural Generators, Sugars

Starch, furfural generator, ethanol-soluble sugars, and sucrose contents of 16 horse bean samples are summarized in Fig. 2. Starch content ranged from 30 to 42% dry matter and was inversely related to protein content. Six samples were in the average range of 35 to 37%, five samples were below and five were above the mean value.

The lowest furfural generator content, expressed in terms of xylose, was 4.6%, the highest 6.9%, but more than half of the samples were in the range of 5.3 to 5.7%.

Values for total ethanol-soluble sugars, expressed as glucose, ranged from 4.9 to 7.2%, but half of the samples analyzed had an average content of 5.6%. It should be mentioned that expressing total ethanol-soluble sugars in terms of glucose is arbitrary, particularly when the ethanol extract is predominantly composed of α -galactosides, as is the case in horse beans. Care is therefore advised in using the results, other than for comparative purposes².

Variations of sucrose content were considerable; the lowest value was 0.55%, the highest 2.2%. When the proportion of sucrose, as percent of the total sugars, is calculated, it appears that 2.2% sucrose in one sample accounts for 30% of the total, while 0.55% in another sample accounts for only about 10% of the total.

The rest of the sugars are essentially composed of α -galactosides, and their proportion, calculated on the basis of sucrose and total sugar content, varied from 70 to 85%.

Individual Sugars

α -Galactosides are supposed to be important in animal nutrition (15,16), that is, they appear to have an effect on flatulence activity. Therefore, some samples were subjected to quantitative paper chromatography, so that the sugar distribution of horse beans could be obtained. Figure 3 shows the results obtained as compared to those of Täufel (4) for soybeans (*Glycine hispida*), French beans (*Phaseolus vulgaris*), and peas (*Pisum sativum*).

It is important to underline that our results on horse beans, as indicated in Fig.

³M. T. Toller, unpublished results.

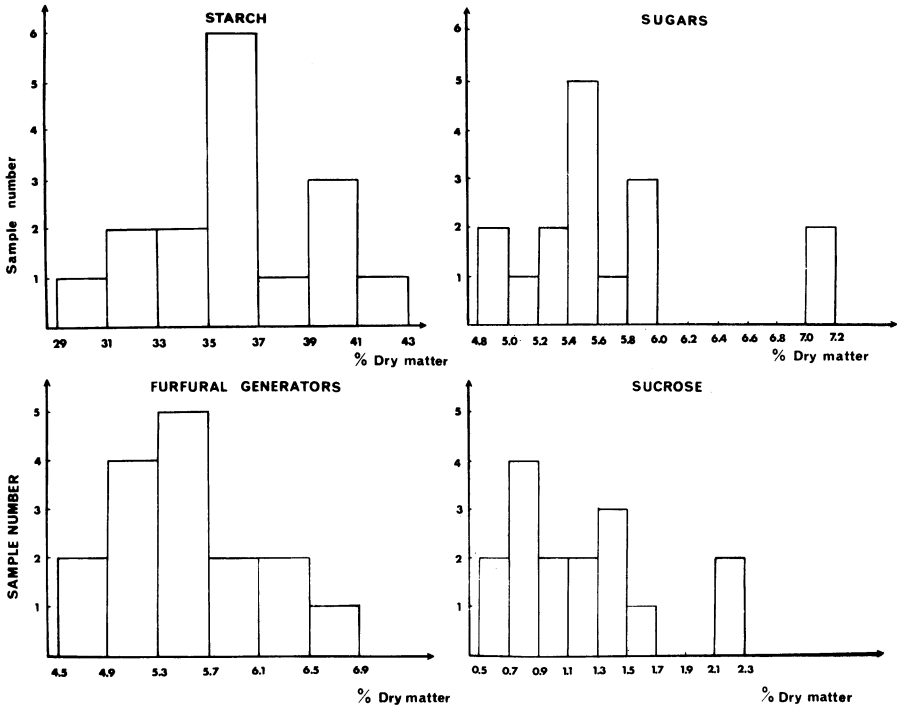


Fig. 2. Starch, furfural generators, ethanol-soluble sugars, and sucrose content (% dry matter) of 16 horse bean (*Vicia faba*) samples.

3, have been obtained using calibration curves of the individual sugars (sucrose, raffinose, and stachyose) with the exception of verbascose. For this oligosaccharide, a theoretical calibration curve was drawn by extrapolating the values for raffinose and stachyose.²

Sucrose predominates in soybeans and peas (Fig. 3); French beans and horse beans contain less of this disaccharide. The amount of raffinose is small in all cases while stachyose content appears to be high in the leguminous seeds. However, horse beans show the highest proportion of verbascose compared to the other samples. In some cases, glucose and fructose were present, but their amounts were too low (less than 0.02%) to be determined quantitatively. Figure 3 is based on an average sugar content of 16 horse bean samples; that for soybeans, French beans, and peas is apparently related to values obtained from one sample of each legume only (4). Since the sucrose content showed great variations (Fig. 2), it should be born in mind that the proposed sugar distribution is to be considered as a tentative indication only.

It is not possible to decide with any degree of certainty whether observed variations in carbohydrate content of the samples analyzed are due to varietal factors or to environmental conditions. Further research is necessary to determine more precisely the different factors that may be responsible for variation in carbohydrate composition.

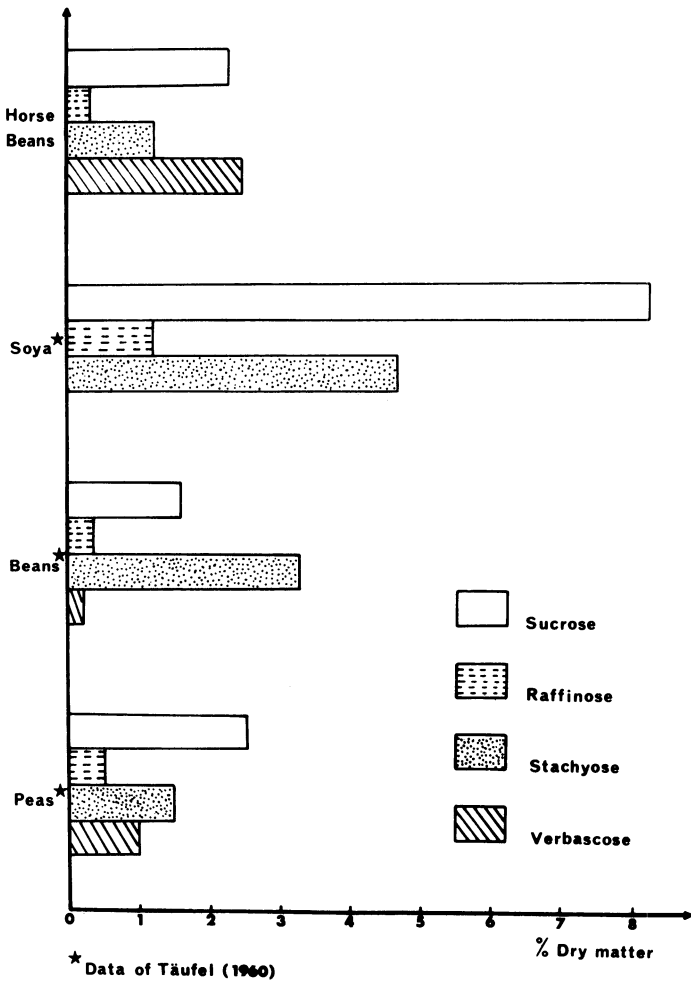


Fig. 3. Sugar distribution in horse beans (*Vicia faba*) as compared to the data of Täufel *et al.* (4) for soybeans, French beans, and peas.

Chemical Composition and Carbohydrate Constituents in Different Parts of the Seed

Chemical Composition. In a second stage of our study, cotyledons (including the germ), hulls, and whole horse bean seeds were analyzed for their chemical composition (Table II). The weight of hulls originating from 100 seeds was 4.8 g., that of cotyledons including the germ was 31.8 g.; thus the hulls represented 13% of the whole seed. With these values the analytical results could be checked by calculation of the balance, that is, the sum of the proportion of each constituent determined in the hulls and cotyledons had to agree with that found in the whole seed. In fact, when the two last columns of Table II are compared it becomes evident that the agreement is fairly satisfactory.

TABLE II. CHEMICAL COMPOSITION OF COTYLEDONS (INCLUDING GERM), HULLS, AND WHOLE HORSE BEAN (*Vicia faba*) SEEDS

	Hulls (13% of the seed)		Cotyledons (87% of the seed)		Whole Seed		Balance (Hulls + Cotyledons)
	% dry matter	Amount (g.) in 4.8 g.	% dry matter	Amount (g.) in 31.8 g.	% dry matter	Amount (g.) in 36.6 g.	
Starch	0.4	0.02	47.0	14.9	41.4	15.2	14.92
Furfural generators	13.5	0.65	3.5	1.12	5.6	2.05	1.77
Crude fiber	53.4	2.62	2.4	0.76	8.0	2.93	3.38
Sugars	1.6	0.08	5.5	1.75	4.5	1.65	1.83
Proteins (N × 6.25)	6.7	0.32	34.5	11.0	32.5	11.90	11.32
Ash	2.55	0.12	3.31	0.99	3.45	1.26	1.11
Lipids	0.42	0.02	1.85	0.59	1.63	0.60	0.61
Crude lignin	5.5	0.26	0.94	0.3	1.5	0.55	0.56
Total	84.1	4.09	99.0	31.41	98.60	36.14	35.50

TABLE III. ETHANOL-SOLUBLE SUGARS FROM COTYLEDONS (INCLUDING GERM), HULLS, AND WHOLE HORSE BEAN (*Vicia faba*) SEEDS DETERMINED BY PAPER CHROMATOGRAPHY (% dry matter)

Samples	Verbascose ¹	Stachyose	Raffinose	Sucrose	Total
Whole seed	2.57	0.83	0.45	1.60	5.45
Cotyledons	2.61	1.06	0.67	2.36	6.70
Hulls	0.75	0.29	traces	0.54	1.58

¹Includes higher-molecular-weight α -galactosides.

The hulls are extremely rich in carbohydrates; most of them are structural or cell wall polysaccharides, while starch is only present in traces. They also contain a considerable amount of nondigestible compounds such as crude lignin, but their sugar, protein, ash, and lipid content is low. From a nutritional viewpoint, it is interesting to note that when the hulls are eliminated, the nondigestible constituents, such as crude fiber and lignin, drop considerably in the remaining cotyledons. The total of the compounds determined is close to 100% in the cotyledons and the whole seed, while it is only 85% in the hulls. It will be seen later that the hulls contain a certain amount of water-soluble (3.0%) and acid-soluble (3.6%) noncellulosic glucose-containing polymers and that the total hemicelluloses compared to total furfural generators are 5% higher when the value is corrected according to the complexity of the cell wall constituents. This brings the total of the determined constituents close to 95%.

Individual Sugars. Table III shows data on the ethanol-soluble sugars determined by paper chromatography. The hulls contain less sugars than the

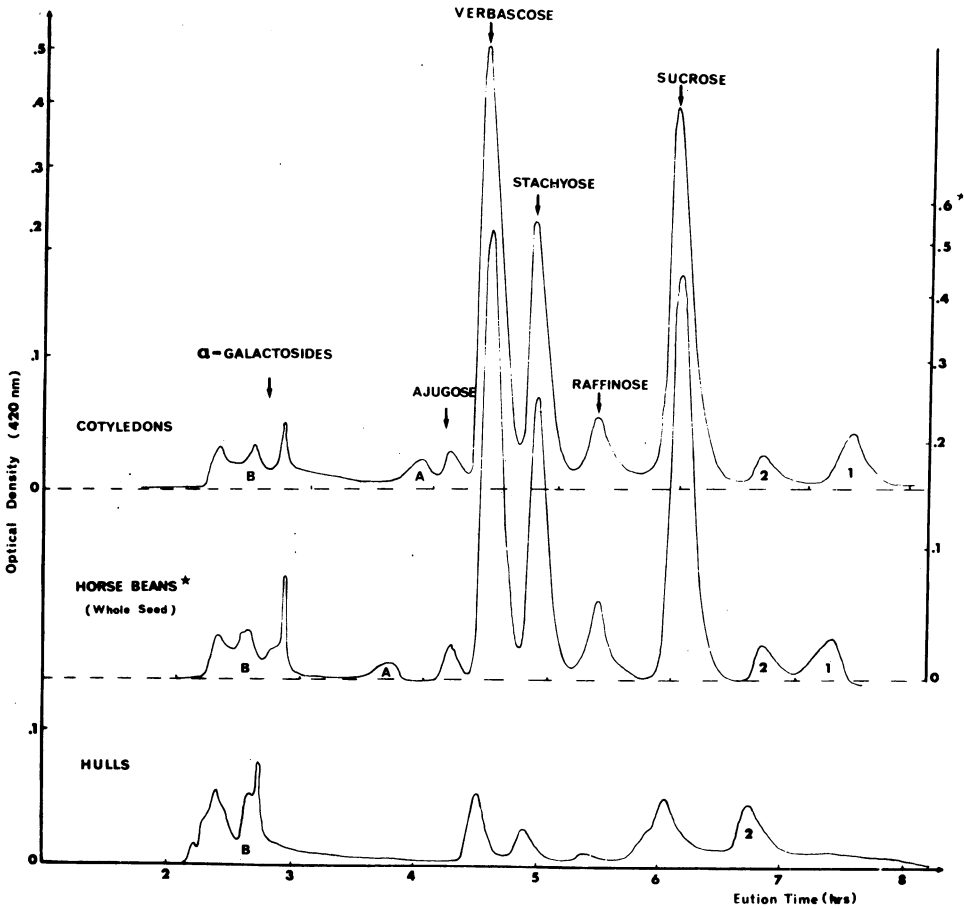


Fig. 4. Elution diagram of ethanol-soluble sugars from a column (1.5×100 cm.) of Bio-Gel P-2 at 65°C ., using water at a flow rate of 46.8 ml./hr. Left ordinate, absorbance (420 nm.) for cotyledons and hulls; right ordinate, absorbance for the whole seed.

cotyledons; only traces of raffinose are present, the sucrose content is 0.54% and the amount of verbascose (including higher-molecular-weight α -galactosides) is 0.75%. The percentage of verbascose is practically the same in the whole seed and the cotyledon, but the latter is richer in stachyose, raffinose, and sucrose.

The ethanol-soluble sugars of the same samples were separated by column chromatography (Fig. 4) on Bio-Gel P-2. The elution diagram confirms essentially the results obtained by paper chromatography, that is, sucrose is present in smaller amounts compared to the α -galactosides. The proportion of raffinose is low in the cotyledons and it is noticeable that the hulls contain only traces of this trisaccharide. The separation of ajugose and high-molecular-weight α -galactosides, which was not possible by paper chromatography, permits one to note that ajugose is absent in the hulls and that the amount of α -galactosides (peaks A and B) is the largest in this part of the seed. Peaks 1 and 2 correspond to

low-molecular-weight ethanol-extractable compounds, which have been observed also on thin-layer chromatograms. They have mobilities corresponding to those of pentoses, methyl sugars, and deoxysugars, but they are not identical with the mentioned standards. This can be affirmed by the color of the spot after development with aniline-diphenylamine. Further identification was not attempted.

Table IV lists the sugar distribution (percent of total) in the ethanol extracts, obtained by measuring the total and individual peak surface of the column chromatograms. As mentioned before, horse beans contain, other than 20 to 30% of sucrose, a high amount of α -galactosides. The values for stachyose and verbascose are 16% and 27%, respectively, in the cotyledons, whereas it is 6.7 and 9.5%, respectively, in the hulls. Peaks A and B represent roughly 13% of the total sugars in the cotyledons and it is interesting to see that they amount to 37% in the hulls.

Cell Wall Constituents

Furfural Generators, Hemicelluloses. Results obtained after successive acid hydrolysis of hulls, cotyledons, and whole seeds are given in Table V. The values for total furfural generators and those determined in the filtrate after glucoamylase hydrolysis of starch ("water-soluble") are indicated also. This in effect permits one to sum the water-soluble pentosans, those that are soluble in dilute acid, and those which are only hydrolyzed after stronger acid treatment; the latter appear to remain more closely associated to cellulose. The sum of the pentoses determined in the three fractions is in close agreement with the total furfural generators determined in whole seeds (total furfural generators 5.6%, sum of "water-solubles" and acid-solubles 6.0%) and cotyledons (total furfural generators, 3.5%; sum of "water-solubles" and acid-solubles, 3.6%). This is not true for hulls. As a matter of fact, the sum of hemicelluloses determined in the three fractions from hulls is 18.1%, whereas the total furfural generator content is 13.5%. The reason for this difference has not yet been satisfactorily explained. One possible explanation could be the fact that the cell walls of hulls contain a certain amount of rhamnose and fucose. These methyl sugars do not react with aniline-acetate and are therefore not measured by the method for total furfural generators (6). However, their reducing group is likely to react in the Somogyi-Nelson method and consequently they are included in the values for pentoses. Thus the sum of "water-soluble" and acid-soluble hemicelluloses (18.1%) probably comes closer to reality than the value (13.5%) for total furfural generators. Further studies are required to complete our knowledge in this field.

Sixty percent of the total pentosans in the cotyledons and almost 30% of those from the hulls are "water-soluble". It seems relevant to indicate that preparative paper chromatography showed that no free pentoses were present in the glucoamylase hydrolysate. Hence the enzyme preparation used for starch degradation was free of hemicellulase activity.

The pentoses liberated by 0.7N hydrochloric acid range from 0.9% in cotyledons to 11.1% in hulls. Sulfuric acid liberates small quantities of pentoses, which are probably originally associated with cellulose.

Insoluble Hemicelluloses and Cellulose. The glucose liberated by 0.7N hydrochloric acid is only 0.23% in the cotyledons but goes up to 3.62% in the hulls (Table V). Since acid hydrolysis was carried out on ethanol-extracted

TABLE IV. SUGAR COMPOSITION (% of total) IN THE ETHANOL EXTRACTS FROM COTYLEDONS (INCLUDING GERM), HULLS, AND WHOLE HORSE BEAN (*Vicia faba*) SEEDS AFTER SEPARATION ON A COLUMN OF BIO-GEL P-2

	Horse Beans	Cotyledons	Hulls
Peak 1	5.2	4.9	...
Peak 2	3.6	2.6	20.2
Sucrose	29.2	27.8	19.4
Raffinose	6.7	6.0	2.4
Stachyose	16.6	16.1	6.7
Verbascose	25.3	27.2	9.5
Ajugose	2.0	2.1	...
Peak A	1.8	3.1	...
Peak B	9.7	10.1	37.2

TABLE V. FURFURAL GENERATORS, HEMICELLOSES, AND GLUCOSE-CONTAINING POLYMERS IN COTYLEDONS (INCLUDING GERM), HULLS, AND WHOLE HORSE BEAN (*Vicia faba*) SEEDS (% dry matter)

Sample	Total Furfural Generators	Hemicelluloses				Glucose-Containing Polymers		
		Sum of hemi-celluloses	Water-soluble	Acid-sol. HCl	Acid-sol. H ₂ SO ₄	Sum of acid-soluble	Acid-sol. HCl	Acid-sol. H ₂ SO ₄
Whole seed	5.6	5.97	2.8	1.32	1.85	5.3	0.5	4.8
Cotyledons	3.5	3.60	2.2	0.90	0.5	2.58	0.23	2.35
Hulls	13.5	18.10	5.0	11.10	2.0	48.8	3.62	45.2

material which had been treated with glucoamylase, thus eliminating all existing α -1-4 and α -1-6 linkages, we suppose that the glucose liberated originates from insoluble noncellulosic glucose-containing polymers. This acid treatment (0.7N hydrochloric acid) is not likely to liberate glucose from cellulose and the fact that no cellobiose was identified by thin-layer chromatography in the hydrolysate as a degradation product of cellulose seems to support this hypothesis. That glucose is due to incomplete starch degradation is also excluded, since the hulls contain only traces of starch, but 3.62% of glucans.

Evidence for the possible presence of "water-soluble" glucose-containing polymers in the hulls is demonstrated by the fact that mild acid hydrolysis of the glucoamylase filtrate (Fig. 1) liberates glucose that amounts to almost 3% of the dry matter.

Qualitative thin-layer chromatography of the hydrochloric acid hydrolysate of the three samples reveals the presence of xylose, smaller amounts of arabinose, traces of galactose and rhamnose. The amount of rhamnose appears to be higher in hulls than in cotyledons. It may be presumed that the hemicellulose of *Vicia faba* is of type B (17). Fucose which is a minor constituent of *Vicia faba* hemicelluloses (5,17), as well as mannose and uronic acids, could not be identified with certainty under our experimental conditions.

The glucose determined in the sulfuric acid hydrolysate was 2.35% for the cotyledons, 4.8% for the whole seed, and ranged to 45.2% in the hulls (Table V). This glucose originates from cellulose and the data confirm the general trend observed for crude fiber (Table II), in such a way roughly half of the hulls are composed of cellulosic polysaccharides. However, these values should not be related closely to crude fiber content. As a matter of fact, a limitation is always imposed involving the definition of the term "crude fiber" and "cellulose." Cellulose is a polymer which, except for the molecular size, is supposed to be a uniform chemical substance. However, it cannot be determined as such, and one usually refers to cellulose as crude fiber which is the residue of essentially nondigestible constituents (cellulose and varying amounts of associated polysaccharides) remaining after a given acid or alkali treatment (8,13).

On the other hand, cell wall polysaccharides can be divided into fractions of various solubilities. What one measures then by extraction is susceptibility to degradation. In this manner, information can be obtained on the chemical composition, and the sugars of which a given fraction is composed, as well as on the possible association of similar fractions, such as hemicellulose and cellulose. Thus, thin-layer chromatography of the sulfuric acid hydrolysate reveals essentially the presence of glucose in the three samples; small traces of cellobiose appear as a result of incomplete cellulose hydrolysis. In addition, xylose is present in smaller amounts and traces of galactose and rhamnose can be detected. These sugars probably originate from hemicelluloses that are not readily hydrolyzed by dilute acid. It is possible that these hemicelluloses are physically entrapped in a cellulose matrix, as has been suggested for wheat (18).

GENERAL DISCUSSION

The 16 varieties of horse beans (*Vicia faba*) had a total carbohydrate content ranging from 51 to 66%. Starch content ranged from 30 to 42.3% and was inversely related to protein content.

Further studies on the same varieties are required to make it possible to determine whether variations in the carbohydrate composition are due to varietal or climatic factors.

The major constituents of the ethanol-soluble sugars are verbascose, stachyose, and higher-molecular-weight α -galactosides, which appear to be important in animal feeding (15,16). None of the samples analyzed showed the presence of sugars originating from starch degradation (maltotriose, -tetraose, and -pentaose) or reversion products (isomaltose) as has been reported by Pritchard *et al.* (5).

The highest amount of hemicellulose seems to be either water-soluble or soluble in dilute acid.

The presence of insoluble glucans has been reported from other plant sources, but only recently in legumes such as field beans (5). We feel that there is a great deal of evidence that horse beans contain a certain amount of water-soluble and acid-soluble glucose-containing polymers, which are essentially located in the hulls. Quantitative estimation of these glucans in the hulls, as well as determination of the sum of hemicelluloses, partially explained the unsatisfactory value (Table II) of the total constituents that had been determined in the hulls. In fact, when 3.0% water-soluble and 3.6% acid-soluble glucose-containing polymers are added and the total furfural generators content of 13.5% is tentatively corrected by the sum of hemicelluloses to 18.1%, the total of the constituents determined is about 95%.

Our study on cell wall polysaccharides using successive acid hydrolysis has to be considered an initial investigation. Further studies using more specific methods are necessary to provide more information, not only concerning the quantitative composition but also concerning structural aspects.

Acknowledgment

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Literature Cited

1. CLARKE, H. E. Evaluation of *Vicia faba* (broad bean) in animal nutrition. *Proc. Nutr. Soc.* 29: 64 (1970).
2. PRIDHAM, J. B. Occurrence and metabolism of oligosaccharides in the broad bean (*Vicia faba*). *Nature* (London) 182: 1687 (1958).
3. COURTOIS, J. E., and PERCHERON, F. Distribution des monosaccharides, oligosaccharides et polyols. In: *Chemotaxonomy of the leguminosae*, ed. by J. B. Harborne, D. Boulter, and B. L. Turner, chap. 5. Academic Press: London and New York (1971).
4. TÄUFEL, K., STEINBACH, K. J., and VOGEL, E. Mono- und Oligosaccharide einiger Leguminosensamen sowie ihr Verhalten bei Lagerung und Keimung. *Z. Lebensm. Unters. Forsch.* 112: 31 (1960).
5. PRITCHARD, P. J., DRYBURGH, E. A., and WILSON, B. J. Carbohydrates of spring and winter field beans (*Vicia faba* L.). *J. Sci. Food. Agr.* 24: 663 (1973).
6. THIVEND, P., MERCIER, C., and GUILBOT, A. Determination of starch with glucoamylase. VI. General carbohydrate methods. In: *Methods in carbohydrate chemistry*, ed. by R. L. Whistler and J. N. BeMiller. Academic Press: New York (1972).
7. CERNING, J., and GUILBOT, A. A specific method for the determination of pentosans in cereals and cereal products. *Cereal Chem.* 50: 220 (1973).
8. ROTHE, M., TUNGER, L., and SIBERT, H. G. Standardisierung der Rohfaserbestimmung bei Getreide und Getreideprodukten durch die Internationale Gesellschaft für Getreidechemie. *Ernährungsforschung* 13: 635 (1968).

9. CERNING, J. Contribution à l'étude de l'évolution de la composition glucidique des grains de céréales au cours de leur maturation: maïs, blé, orge. Thèse de Doctorat d'Université, mention Sciences, Lille, France (1970).
10. DRAPRON, R., and GUILBOT, A. Contribution à l'étude des réactions enzymatiques dans les milieux biologiques peu hydratés. *Ann. Technol. Agr.* 11: 175 (1962).
11. DE STEFANIS, V. A., and PONTE, J. G. Separation of sugars by thin-layer chromatography. *J. Chromatogr.* 34: 116 (1968).
12. TRENEL, G., JOHN, M., and DELLWEG, H. Gel chromatographic separation of oligosaccharides at elevated temperatures. *FEBS Letters* 2: 74 (1968).
13. SALO, M.-L. Determination of carbohydrate fractions in animal foods and faeces. *Suom. Maataloustieteellisen Seuran Julka.* 105. *Acta Agr. Fenn.* (1965).
14. MARAIS, J. P., DE WIT, J. L., and QUICKE, G. V. A critical examination of the Nelson-Somogyi method for the determination of reducing sugars. *Anal. Biochem.* 15: 373 (1966).
15. CRISTOFARO, E., MOTTU, F., and WUHRMANN, J. J. Study of the effect of stachyose and raffinose on the flatulence activity of soy-milk. *Int. Cong. Food Sci. Technol.*, 3rd, Washington, D. C. (1970).
16. CRISTOFARO, E., MOTTU, F., and WUHRMANN, J. J. Study of the effect on flatulence of leguminous seeds oligosaccharides. (Abstr) *Cereal Sci. Today* 17: 274 (1972).
17. KAWAMURA, S., and NARASAKI, T. Fucose as a constituent of hemicellulose B₁ from broad-bean seeds. *Bull. Agr. Chem. Soc. Jap.* 22: 436 (1958).
18. D'APPOLONIA, B. L., GILLES, K. A., OSMAN, E. M., and POMERANZ, Y. Carbohydrates. In: *Wheat: Chemistry and technology*, ed. by Y. Pomeranz; chap. 7. *Amer. Ass. Cereal Chem.*: St. Paul, Minn. (1971).

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