AIR CLASSIFICATION OF FIELD PEAS AND HORSEBEAN FLOURS: CHEMICAL STUDIES OF STARCH AND PROTEIN FRACTIONS[1]

J. R. VOSE, M. J. BASTERRECHEA[2], P. A. J. GORIN, A. J. FINLAYSON, and C. G. YOUNGS, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan S7N 0W9

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ABSTRACT

Smooth field peas and horsebeans were each pin-milled and the flours air-classified to yield a protein concentrate (60–70% protein) and a crude starch fraction. Dehulling was not essential to this process, as the fiber is dense and is collected with the starch fraction, resulting in a low-fiber protein concentrate. The chemical nature of the pea and bean flour components was studied and the effects of air classification on the partition of the protein, starch, lipid, phytic acid, and oligosaccharides of the sucrose family into two markedly different fractions in a proportion of between 1:2 and 1:3 were evaluated. The less dense fraction contained 60–70% protein with an adequate lysine content and low methionine—a useful complement to protein derived from cereal grain. The larger fraction contains up to 80% of a 32–34% amylose starch. The application of air-classification techniques to grain legume processing has relatively low capital requirements and obviates the need for costly effluent disposal operations.

There is a growing interest in the production of grain legumes in the Prairie Provinces to meet industrial demands, primarily for protein feed (1). The development of satisfactory processing techniques is of considerable importance to the future development of these crops.

Pin-milling of field pea and horsebean seeds, either whole or dehulled, yield flours that contain two distinct populations of particles based on both their size and density. It was observed (2) that this phenomenon could be exploited, using air-classification separation techniques, to produce a protein concentrate (the light fraction) and a starchy flour (the heavy fraction). The chemical composition of the peas and beans used in this work and of the fractions obtained by air classification of their flours is presented in this paper.

MATERIALS AND METHODS

Processing

Smooth field peas (*Pisum sativum* L. var. Trapper) were provided by Newfield Seeds Ltd., Nipawin, Saskatchewan, and the horsebeans (*Vicia faba equina* L. var. Diana) by Northern Sales Ltd. of Winnipeg, Manitoba.

Clean seeds were equilibrated to a moisture content of 8% prior to processing. When necessary, seeds were effectively dehulled in a Currier plate mill followed by air aspiration. Whole or dehulled seeds were pin-milled, and yielded flours of less than 325 mesh in an Alpine Pin Mill Model 250CW. The flours were then fractionated into "protein" and "starch" concentrates using an Alpine Air Classifier Type 132 MP. Physical characteristics of the flours were observed by use of a scanning electron microscope (Cambridge Stereoscan Mark II).

[1]NRCC No. 15407.

[2]UNESCO Fellow, 1971–72. Present address: School of Chemistry, University of Havana, Havana, Cuba.

Analytical Methods

Protein. The nitrogen content of the flours was determined by standard AOAC Kjeldahl methods (3), and also by use of a Hewlett Packard 185B CHN Analyzer.

To determine the amino acid content of the flours, samples of vacuum oven-dried meal (40–50 mg) were hydrolyzed in a sealed tube under a nitrogen atmosphere with 5.7*N* hydrochloric acid for 20 hr at 110° C. The hydrolysates were evaporated to dryness *in vacuo* and made up to the appropriate volume in 0.01*N* hydrochloric acid. Aliquots of these solutions were analyzed using a Model 120C Beckman amino acid analyzer.

Nitrogen extraction profiles were determined by the standard AACC method (4).

Starch. The starch was assayed by a gas-liquid chromatographic quantitation of glucose in acid-hydrolyzed flours. Following extraction of a 1-g sample with refluxing methanol containing 20% v/v water, the residue was obtained by filtration and dried in a vacuum oven at 100° C. Dried extract (25 mg) was mixed with an equal weight of internal standard (myoinositol) and hydrolyzed overnight on a steam bath in 1*N* sulfuric acid. The solution was then cooled, neutralized with $BaCO_3$, filtered, and evaporated under reduced pressure to yield a syrup. Samples were trimethylsilylated (5) at room temperature and quantitated on a Model 402 Hewlett-Packard gas-liquid chromatograph (glc) with twin hydrogen flame detectors. The columns were twin 2 ft × 3/16-in. stainless steel, and contained 3% Dexsil-300 on DMCS-treated Chromosorb W (80–100 mesh). Peak areas of the TMS derivatives of α- and β-glucose were added and compared with that of myoinositol.

The starch content of these flours was confirmed by the dual-enzyme semi-micro method of Banks *et al.* (6).

Iodine affinities of the starches were determined by potentiometric titration (7).

Polysaccharide fractionation techniques used for assay of the pea and bean hulls were based on those used by Aspinall *et al.* (8) for the isolation of polysaccharides from soybean hulls.

Oligosaccharides. Samples of flour (1.0 g) were refluxed with methanol containing 20% v/v water for 2 hr. An aliquot of the extract was trimethylsilylated (5) at 60° C for several hours for complete dissolution of the sugars. Internal standard was 2-O-β-D-glucopyranosyl-D-erythritol. A sample was injected onto the glc equipped with twin stainless steel columns (2 ft × 1/8 in.) packed with 3% Dexsil-300 on DMCS-treated Chromosorb W (80–100 mesh). Initial temperature was 160° C and was programmed up to 370° C at a rate of 5° C/min. Retention times of these sugars are shown in Table I. Fractions were also examined using paper chromatography (solvent, butanol:ethanol:water 1:1:1 v/v/v; spray: ammoniacal silver nitrate). Sucrose, raffinose, stachyose, verbascose and ajugose were characterized by comparison of their proton magnetic resonance (pmr) spectra with those of authentic samples (gift from J. E. Courtois, Paris, France).

Lipids. The neutral lipids were extracted from oven-dried flours for 8 hr with hexane in a Soxhlet apparatus; polar lipids were similarly extracted from dried hexane-extracted flours with chloroform:methanol (2:1 v/v). Fatty-acid determinations on the lipid fractions were made by gas-liquid chromatography

of the methyl esters of the hydrolyzed fats (9).

Phytic Acid. Legume flours were refluxed three times with 0.5*N* hydrochloric acid; the extracts were pooled and the phytic acid precipitated as Fe III phytate by addition of 0.25% w/v $FeCl_3$ in 1*N* HCl (0.2 ml $FeCl_3$/ml test solution. The precipitate was washed twice with cold 0.2*N* HCl and then dissolved in 0.2*N* NaOH. Ferric hydroxide was removed by centrifugation (10). The resultant phytic acid was hydrolyzed by digestion with 70% perchloric acid under reflux. Inorganic phosphate was assayed by the Fiske and Subbarow procedure (11).

Moisture, ash, and crude fiber were determined by standard AOAC procedures (3). All data are presented on dry weight basis.

RESULTS AND DISCUSSION

The proximate analyses for both whole and dehulled seed of field peas and horsebeans are presented in Table II, and indicate the similarity in overall composition between these two grain legumes. The peas are, however, considerably smaller in seed size than the beans. They also have a lower protein content and correspondingly higher starch content than the beans.

TABLE I
Retention Temperatures and Retention Times at these Temperatures of the TMS Derivatives of Five Free Sugars on a 2 ft × 1/8-in. Column of 3% Dexsil-300, Injected at 160°C and with a Program of 5°C/min up to 370°C

Sugar	Retention Temperature °C	Retention Times at these Temperatures min
Inositol	170	2
2-O-β-D-glucopyranosyl-D-erythritol	190	6
Sucrose	200	8
Raffinose	245	13
Stachyose	290	26
Verbascose	322	32
Ajugose	340	43

TABLE II
Proximate Analysis of Whole and Hand-Dehulled Seeds of Field Peas and Horsebeans

	Whole Seed		Dehulled Seed	
	Peas	Beans	Peas	Beans
1000-Seed weight (g)	123	342	...	...
% Protein (% N × 6.25)	25.7	27.9	29.1	34.1
% Starch	43.7	41.2	46.6	43.5
% Lipid—neutral	0.9	1.0	0.9	1.2
% Lipid—polar	1.5	1.0	1.3	1.3
% Ash	2.7	3.2	2.9	3.5
% Crude fiber	6.8	7.2	1.4	1.6
% Phytic acid	...	...	0.74	1.80

The starch content of the dehulled pea flour was 46.6%, while the starch content of dehulled horsebean flour was 43.5%, indicating a predominance of starch as the major polysaccharide in field peas and horsebeans. Glucans (other than starch) that are hydrolyzed in 1 *N* H_2SO_4 under reflux for 12 hr appear to be absent in these grain legumes.

Paper chromatography of neutralized ($BaCO_3$) acid hydrolysates of 80% methanol-extracted flours showed glucose as the major sugar, with small amounts of arabinose. The presence of a nonproteinaceous material (3–4%) that was not solubilized by refluxing in 8 *N* H_2SO_4, but was soluble in 5% KOH, was observed in these flours. Paper chromatograms indicate that glucose and arabinose are also the major sugars of this material.

The iodine affinities obtained by potentiometric titration of water-washed starches were 6.8 for pea starch and 6.4 for horsebean starch, and indicate the presence of 34% amylose in pea starch and 32% amylose in horsebean starch (assuming "pure" amylose has an iodine-binding capacity of 20.0%).

The oligosaccharides present in 80% methanol extracts of the pea and horsebean flours are detailed in Table III. In accord with other work (12,13) su-

TABLE III
Carbohydrates in 80% Methanol Extracts from Dehulled Pea and Horsebean Flours

	% Composition	
	Pea flour	**Horsebean flour**
Sucrose	2.2	2.9
Raffinose	0.8	0.2
Stachyose	2.0	0.2
Verbascose	2.3	2.0
Total	7.4	5.6

TABLE IV
Amino Acid Compositions of Pea (PPC) and Bean (BPC) Protein Concentrates (g Amino Acid/16 g Nitrogen)

	PPC	**BPC**
Aspartic acid	11.47	10.72
Threonine	3.75	3.28
Serine	5.26	6.06
Glutamic acid	17.55	16.01
Proline	4.88	4.52
Glycine	4.32	4.61
Alanine	4.21	4.23
Valine	4.23	4.01
Cystine	1.15	1.15
Methionine	1.08	0.84
Isoleucine	3.75	3.59
Leucine	7.77	7.80
Tyrosine	3.75	3.61
Phenylalanine	5.04	4.42
Lysine	7.77	7.30
Histidine	2.26	2.47
Arginine	8.79	9.58

crose is a major low-molecular-weight carbohydrate in the horsebean flour. In addition, both flours contain substantial amounts of verbascose; stachyose was present in significant amounts in the field pea flour. A small peak corresponding to ajugose was detected in some of the pea extracts.

The amino acid profile of the pea and bean proteins (Table IV) demonstrates the relatively high lysine content and low methionine content characteristic of grain legume proteins. The nitrogen solubility index of these proteins is shown in Fig. 1 and indicates an isoelectric point at pH 4.0–4.2. Greater than 80% solubility of these proteins was observed at pH values above 7.0. Approximately 85% of the total nitrogen was recovered as amino acids.

Total lipid content of these legume flours is low (2–3%). Pea flour lipid contains a predominance of linoleic acid (47.8%), with oleic acid (18.4%), linolenic acid (15.3%), and palmitic acid (10.3%) as the other major fatty acids

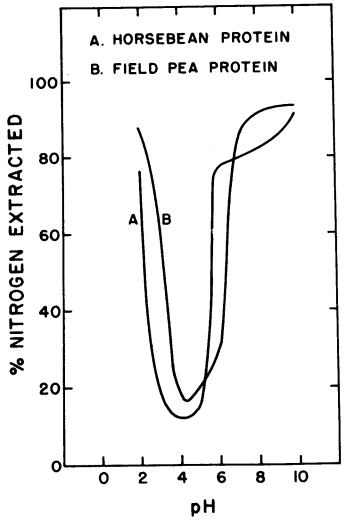


Fig. 1. The nitrogen solubility index of field pea protein and horsebean protein.

TABLE V
Field Pea and Horsebean Hulls—% Composition

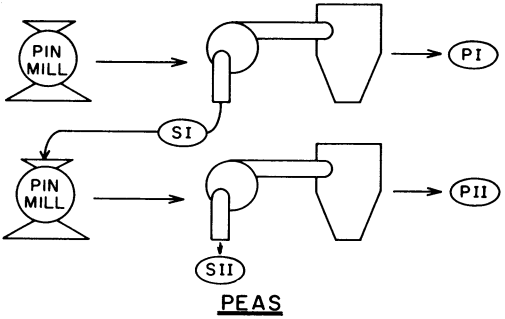
	Peas	Horsebeans
% Hull in seed	8.2	13.0
Water-soluble polysaccharides	8.9	16.7
Pectin-type polysaccharides	8.2	9.0
Lignin	1.9	11.3
Hemicellulose	23.1	13.0
Cellulose	55.2	48.9
Nitrogen	0.6	0.8
Neutral lipid	0.6	0.4
Ash	2.4	2.6

obtained upon hydrolysis. The levels of linoleic acid (50.3%) and oleic acid (21.7%) were higher in the lipid of horsebean flour, and the linolenic acid (5.1%) was lower.

The composition of the isolated hull fraction was examined, and these data are presented in Table V. The horsebeans have a significantly higher hull content than peas, and their hulls appear to contain a greater proportion of lignin than found in pea hulls. Both pea and horsebean hulls contain about 50% cellulose by weight. The sugars formed by aqueous acid hydrolysis of these hulls indicated the presence of xylose and arabinose, smaller quantities of glucose, galactose, and rhamnose, and traces of fucose and uronic acid. This hydrolysis pattern is suggestive of hemicellulosic material. Traces of sucrose, raffinose, stachyose, and verbascose were also observed in these hulls (total 1%), possibly by contamination from the cotyledon fraction.

Fractionation by Air Classification

The techniques of fine grinding and air classification have been applied to a number of cereal grains and legumes (14,15,16). When pin-milled grain legumes such as field peas and horsebeans were air-classified, we observed a significantly greater protein shift than has been reported for cereals. Pin-milled legume flours were air-classified in a spiral air stream with a cut point of about 800 mesh (or 15 μ) between the fine and coarse particles. The fractionation of these flours by this procedure is illustrated in Fig. 2.



PEAS

	WHOLE SEED		DEHULLED SEED
FLOUR	100 lbs	(25.7% protein)	100 lbs (28.8% protein)
SI	75 lbs "STARCH"	(13.3% protein)	69 lbs (14.5% protein)
PI	25 lbs "PROTEIN"	(63.5% protein)	31 lbs (60.5% protein)
SII	56 lbs "STARCH"	(5.6% protein)	52 lbs (4.8% protein)
PII	19 lbs "PROTEIN"	(48.3% protein)	17 lbs (40.0% protein)

HORSE BEANS

	WHOLE SEED		DEHULLED SEED
FLOUR	100 lbs	(27.9% protein)	100 lbs (31.9% protein)
SI	72 lbs	(15.1% protein)	70 lbs (16.5% protein)
PI	28 lbs	(66.1% protein)	30 lbs (69.0% protein)
SII	58 lbs	(5.2% protein)	52 lbs (4.2% protein)
PII	14 lbs	(51.1% protein)	18 lbs (49.6% protein)

Fig. 2. Schematic flowsheet for dry processing of field peas and horsebeans.

In these experiments, a 25% yield of protein concentrate (10.2% N) and a 28% yield of protein concentrate (10.6% N) were obtained from air-classified whole field peas and horsebeans, respectively. Combining the two protein fractions obtained from two passes through the air-classifier produced a 44% yield of a protein meal (9.1% N) from peas (Table VI) and a 42% yield of a protein meal (9.8% N) from horsebeans. Dehulled flours yielded 48% of a protein flour (8.5% N) from peas, and 48% of protein flour (9.9% N) from horsebeans.

The composition of the two protein fractions and the two starch fractions obtained by two passes through the pin mill and air-classifier was studied, and the results are presented in Tables VI and VII. Air classification did not remove all nitrogen from the "heavy" fraction even after two classifications.

The starch granules from both the smooth field peas and horsebeans consisted of simple oval granules, often fissured, and 25–40 μ in diameter. Starches prepared by classification of pin-milled flours, equilibrated to 8% moisture or less, were found to have retained a layer of proteinaceous material on their

TABLE VI
Percentage Composition of the "Light" Fractions from Air-Classified Peas and Beans

	Field Peas				Horsebeans			
	Whole		Dehulled		Whole		Dehulled	
	PI	PII	PI	PII	PI	PII	PI	PII
Protein (% N × 6.25)	63.4	48.3	60.5	40.0	66.1	51.1	69.0	49.6
Starch	7.6	17.4	7.8	27.6	7.3	18.0	4.2	22.1
Oil (a) neutral	2.2	1.4	1.8	1.4	2.1	1.9	2.5	1.8
Oil (b) polar	2.7	1.7	2.3	2.6	2.2	1.4	2.2	1.7
Ash	5.8	4.5	5.6	4.4	7.0	5.3	6.7	5.2
Sugars	...	...	11.2	9.7	...	...	5.8	5.5
Crude fiber	2.1	3.2	2.2	2.6	2.6	3.8	2.0	2.3
Phytic acid	...	...	1.9	...	...	...	4.2	...

TABLE VII
Percentage Composition of the "Heavy" Fractions from Air-Classified Peas and Beans

	Field Peas				Horsebeans			
	Whole		Dehulled		Whole		Dehulled	
	SI	SII	SI	SII	SI	SII	SI	SII
Protein (% N × 6.25)	13.3	5.6	14.5	4.8	15.1	5.2	16.5	4.2
Starch	60.3	73.6	63.0	78.0	58.5	69.3	61.2	76.6
Oil (a) neutral	0.48	0.14	0.46	0.17	0.7	0.4	0.6	0.2
Oil (b) polar	0.77	0.55	0.96	0.37	0.9	0.4	0.9	0.4
Ash	1.6	1.0	1.6	0.8	2.0	1.0	2.0	0.7
Sugars	...	...	5.3	3.8	...	...	5.5	4.5
Crude fiber	8.4	8.4	1.1	0.5	10.2	11.4	1.1	0.7
Phytic acid	...	...	0.2	...	...	...	0.9	...

surface. This layer is presumably derived from the granal membrane within which the starch granule developed (R. Reichert, unpublished data), and can be removed to yield low protein starches by repeated water washings (Fig. 3). This effect is particularly apparent in the case of the field peas, whereby starch containing less than 0.05% nitrogen could be readily achieved by water-washing.

The presence or absence of the hulls in these legumes had little effect on the crude fiber content of the high-protein, low-density fraction. Milled hulls were classified along with the denser starch fraction; this was particularly evident with the field peas.

The oil moiety was concentrated along with the light protein fraction in both peas and beans. The soluble sugars were also largely classified with the light fraction in the field peas (Table VIII) but no significant overall partition was observed with the soluble sugars of the horsebeans. However, in both peas and beans, the verbascose and stachyose were mainly fractionated into the high-protein flour.

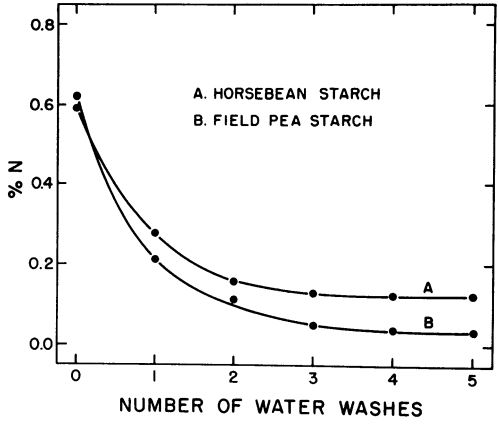


Fig. 3. The effect of water-washing on the protein content of legume starches prepared by air classification.

TABLE VIII
Partition of Soluble Sugars between the Different Fractions Obtained by Air Classification of Dehulled, Pin-Milled Legume Flour

	% Composition							
	Field Peas				Horsebeans			
	PI	PII	SI	SII	PI	PII	SI	SII
Inositol	0.04	0.05	0.12	0.12	0.02	0.02	0.03	0.03
Sucrose	2.2	2.1	1.9	1.9	1.6	2.2	3.4	3.5
Raffinose	1.1	0.9	0.6	0.5	0.2	0.2	0.2	0.2
Stachyose	4.0	3.4	1.3	0.7	0.8	0.7	0.4	0.2
Verbascose	3.9	3.3	1.4	0.6	3.2	2.5	1.5	0.6
Total	11.2	9.7	5.3	3.8	5.8	5.6	5.5	4.5

The phytic acid content of the legume flours was 0.7 and 1.8% in dehulled field peas and horsebeans, respectively. This material was concentrated by air classification along with the light protein fraction to give a content of 4.2% in the bean protein, and 1.9% in the pea protein. Elemental analysis by atomic absorption spectroscopy also indicated a partition of phosphorus and zinc, and to a lesser extent, calcium, into the light fraction. This high-protein flour from dehulled peas contained 0.86% phosphorus, 0.04% calcium, and 82 ppm zinc, while the starch fraction contained 0.09% phosphorus, 0.02% calcium, and 15 ppm zinc. Elemental iron behaved in the opposite manner, and was largely found in the starchy moiety (186 ppm in the starch and 76 ppm in the protein fraction).

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