STARCHES AND LOW-MOLECULAR-WEIGHT CARBOHYDRATES FROM CHICK PEA AND HORSE BEAN FLOURS[1]

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ABSTRACT

Starches were isolated from chick pea (*Cicer arietinum* L.) and horse bean (*Vicia faba* L.) flours. Gelatinization temperature ranges were 63.5° – 65° – 69°C for chick pea and 61° – 63.5° – 70°C for horse bean starches. Iodine affinity values were 6.08 and 6.28%, respectively. Both starches presented single-stage swelling and solubility patterns. They also gave stabilized Brabender hot-paste viscosities similar to those of cross-bonded starches. Amylose and amylopectin, obtained by fractionating the starches, were estimated by iodine absorption. The amylose content of horse bean starch was approximately 30%; of chick pea starch, 20 or 30% depending on the analytical method used. Chick pea and horse bean flours contained 8.35 and 7.78% (w/w) carbohydrate soluble in 70% (w/v) ethanol. Rhamnose, xylose, fructose, glucose, sucrose, raffinose, stachyose, and two unidentified oligosaccharides were present in both flours.

Investigations have been made in this department on the use of chick pea (*Cicer arietinum* L.) and horse bean (*Vicia faba* L.) flours to supplement wheat flour for preparing baked or cooked products (cous cous, chapatties, roti) for use where diets are protein-deficient. Relatively few studies on the starches and low-molecular-weight carbohydrates in legume flours have been reported. Some

[1]Contribution No. 845, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan 66506. Presented at the 58th Annual Meeting, St. Louis, Nov. 1973.

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physicochemical properties of chick pea (1–4) and horse bean (5) starch have been reported for starches isolated from the seeds. Until recently, the identities and quantities of low-molecular-weight carbohydrates in chick pea and horse bean seeds have received only limited attention (6,7); very recently two studies of carbohydrate compositions of horse beans of different origins were reported (8,9).

This study was designed to obtain information on the nature of the starches from chick pea and horse bean flours and the identities and quantities of low-molecular-weight carbohydrates in these flours.

MATERIALS AND METHODS

Chick pea and horse bean seeds, obtained from Country Store, Kansas City, Kans., were decorticated and milled to pass 10XX sieves in a roller mill in the Department of Grain Science and Industry, Kansas State University. Flours ranged from 68 to 72% extraction. Carboxymethyl cellulose (CMC), obtained from Hercules Inc., Oak Brook, Ill., was type 7HF.

Starch Isolation

Starches were isolated from chick pea and horse bean flours by the method of Schoch and Maywald (2) with certain modifications. Legume flour (1000 g) was soaked overnight in distilled water (3 l.) in the cold. The mixture was screened (60-mesh sieve) and the pulp was washed with distilled water, then hand-squeezed dry. The residual pulp was again washed and rescreened. The combined starch suspensions were screened (270-mesh sieve) and sedimented three additional times in distilled water until the supernatant layer was substantially free of color and suspended haze. After the starch was centrifuged (2000 × *g*, 20 min), the supernatant was decanted and the brown upper layer of starch tailing removed with a spatula. Prime starch, air-dried in a thin layer at room temperature, was powdered to pass through a 60-mesh sieve.

Microscope

A Leitz Orthomat Research microscope was used to view and photograph starch granules in aqueous suspension.

Scanning Electron Microscopy (SEM)

Starch was sprinkled onto double-backed Scotch tape attached to specimen stubs and coated with a 150 Å gold-palladium layer. The specimens were viewed and photographed using an ETEC Autoscan scanning electron microscope.

Proximate Analysis

Standard AOAC methods (10) were used for moisture, ash, nitrogen, and fat determinations.

Gelatinization Temperature Range

Measurements were made, using a Kofler hot stage on a polarizing microscope, according to Schoch and Maywald (11). Temperatures were recorded that corresponded to loss of birefringence by 2, 50, and 98% of the

starch granules observed in the field, designating them initial, midpoint, and end point values, respectively.

Swelling and Solubility Characteristics

Swelling and solubility patterns were determined, using 5 g of starch, by the method of Leach *et al.* (12).

Brabender Viscosity Curves

Brabender curves were determined, using a Brabender Viscoamylograph, and analyzed by the procedure described by Mazurs *et al.* (13); five different concentrations (5, 6, 7, 8, and 9%) of each starch were used.

Brabender Pasting Temperatures

The pasting temperature range for each starch was determined by the CMC-amylograph technique described by Sandstedt and Abbott (14). Medcalf and Gilles (15) give a complete description of the procedure used.

Starch Fractionation

The starches were fractionated into amylose and amylopectin according to the procedure of Montgomery and Senti (16). Amylose was recrystallized three times from 1-butanol, isolated by precipitation with acetone, and vacuum-dried at 40°C (17). Amylopectin was isolated from the gel after extraction of amylose (17).

Iodine Affinity

Iodine affinities were determined by potentiometric titration (18) using samples which had been defatted (24 hr, 95% ethanol) and dried (air oven, 40°C).

Amylose Determination

Amylose content in starch was determined by a colorimetric procedure described by McCready and Hassid (19). Absorbance values were measured at 625 nm. Standard curves were made for each starch by mixing known quantities of amylose and amylopectin isolated from that particular starch.

The amylose content in starch was also calculated by assuming that pure amylose absorbs 200 mg iodine/g of amylose (16).

Isolation of Low-Molecular-Weight Carbohydrates from Flour

Low-molecular-weight carbohydrates were isolated from flour by the method described by Saunders and Walker (20) with certain modifications. Flour (50 g) was refluxed for 1 hr with 400 ml aqueous 70% ethanol. The mixture was cooled and filtered. The residue was again treated under the same conditions. The combined filtrates were concentrated to about 100 ml using a rotary evaporator. The aqueous solution was twice extracted with 200 ml of chloroform, then concentrated to about 50 ml.

Identification and Quantitative Determination of the Ethanol-Soluble Carbohydrates Present in Flour

Carbohydrates in the 70% ethanol extracts were identified by paper chromatography, accomplished on Whatman No. 1 or 3MM paper in a

descending system, in two solvents: A) 1-butanol:pyridine:water (5:3:2 v/v/v); and B) 1-butanol:acetic acid:water (4:1:2 v/v/v). The carbohydrates present were identified by comparing their chromatographic mobility with those of known carbohydrates chromatographed on the same chromatogram. Carbohydrates were detected on the dried papers with silver nitrate dip reagent (21).

Carbohydrate quantities were determined by chromatographically separating the components of the mixture, using Whatman 3MM paper and solvent A. After separation, the carbohydrates were eluted with water, and the carbohydrate content of the eluates was determined by the phenol-sulfuric acid method (22). A standard curve was prepared using 10 to 90 γ of glucose (amounts of total and individual carbohydrates are expressed as glucose).

Hydrolysis of Oligosaccharides

Two oligosaccharides observed in the mixtures, with chromatographic mobilities less and greater than raffinose (unknowns I and II, respectively), were eluted from the preparative paper chromatograms; the solutions were freeze-dried. The dried samples were hydrolyzed in screw-capped vials with 2 ml 1 *M* hydrochloric acid for 6 hr in boiling water. After hydrolysis the solution was transferred to a small beaker and neutralized to pH 5.0 with AG 3-X4 (OH^- form) resin. The resin, removed by filtration, was washed several times with distilled water. The filtrate and washings were combined and freeze-dried. The carbohydrates present in the hydrolyzed samples were then determined by paper chromatography of an aqueous solution on Whatman No. 1 paper in solvent A.

RESULTS AND DISCUSSION

Granule Size and Microscopic Appearance

Chick pea starch ranges from large oval-shaped (17 to 29 μ) to small spherical (6 to 7 μ) granules. Viewed under the light microscope, the starch granules possess fissures (Fig. 1A). However, seen with SEM, the granule surfaces are quite smooth (Fig. 1B) and show no evidence of fissures. Packing within the seed probably caused the irregularities on the surface of a number of granules. Srivastava *et al.* (1) reported that chenna (*Cicer arientinum*, chick pea) starch has "round" granules (3 to 4 μ, with a centric hilum) which were sometimes fissured. No mention was made of the presence of large granules although some large spherical granules appeared to be present in a photomicrograph of chenna starch.

Horse bean starch ranges from small spherical (6 μ) to large oval- or irregularly shaped (17 to 31 μ) granules. Some, viewed with the light microscope, possess fissures (Fig. 2A). However, as with chick pea starch, when viewed with the scanning electron microscope (Fig. 2B), the granules have relatively smooth surfaces, showing no evidence of fissures. In general, horse bean starch granules are more irregularly shaped than chick pea starch granules. Kawamura (5) reported that broad bean (*Vicia faba*, horse bean) starch had irregular, elliptical granules (shorter diameter, 11 to 35 μ; long diameter, 15 to 45 μ) with a centric hilum and a few fissures. The presence of small spherical granules was not reported although a few appeared to be present in a photomicrograph of broad bean starch.

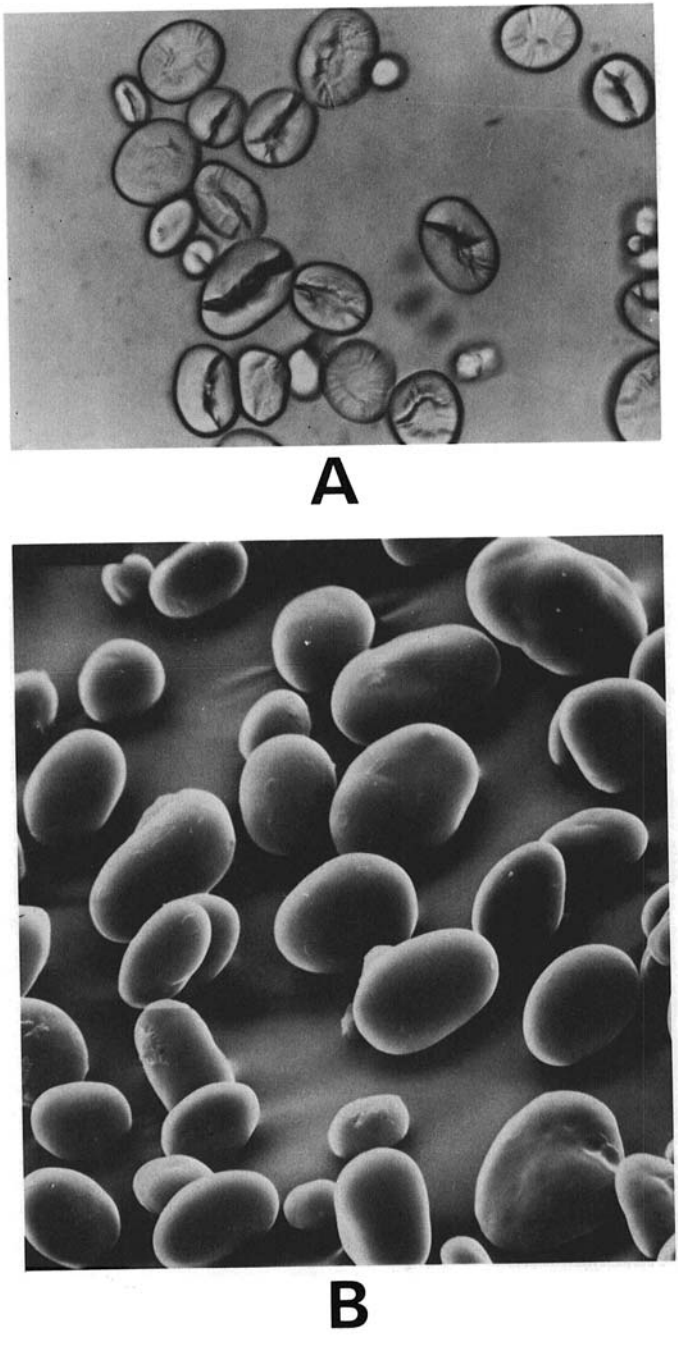
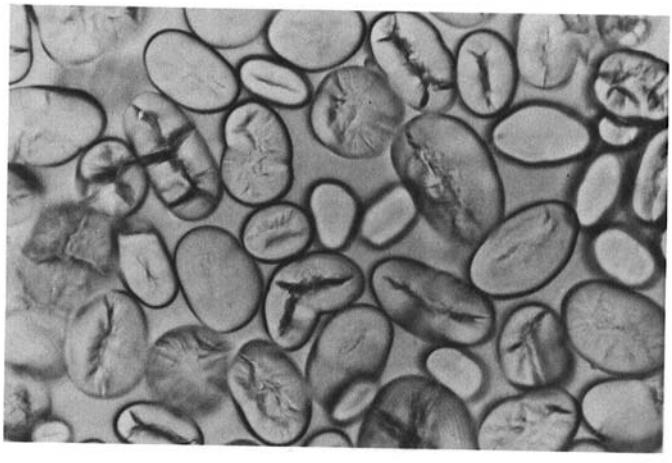


Fig. 1. Photomicrographs of chick pea starch: (A) Light micrograph (400×) and (B) scanning electron micrograph (800×).



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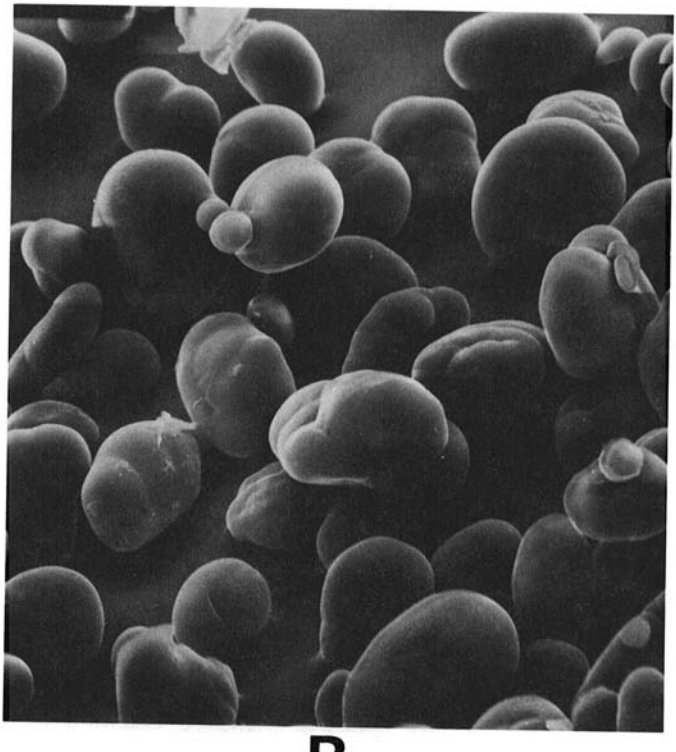

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Fig. 2. Photomicrographs of horse bean starch: (A) Light micrograph (400×) and (B) scanning electron micrograph (800×).

TABLE I
Analyses and Properties of Legume Starches and Flours[a]

Sample	Yield	Ash	Protein[b]	Fat	Iodine Affinity	Gelatinization Temperature Range[c]
	%	%	%	%	%	°C
Starch						
Chick pea	40	0.09	0.94	0.06	6.08	63.5–65–69
Horse bean	37	0.07	0.81	0.06	6.28	61–63.5–70
Flour						
Chick pea	...	2.75	20.4	6.05	...	...
Horse bean	...	3.56	32.4	1.33	...	...

[a]All data reported on dry basis.
[b]%N × 6.25.
[c]Values represent the initial, midpoint, and end point of starch gelatinization.

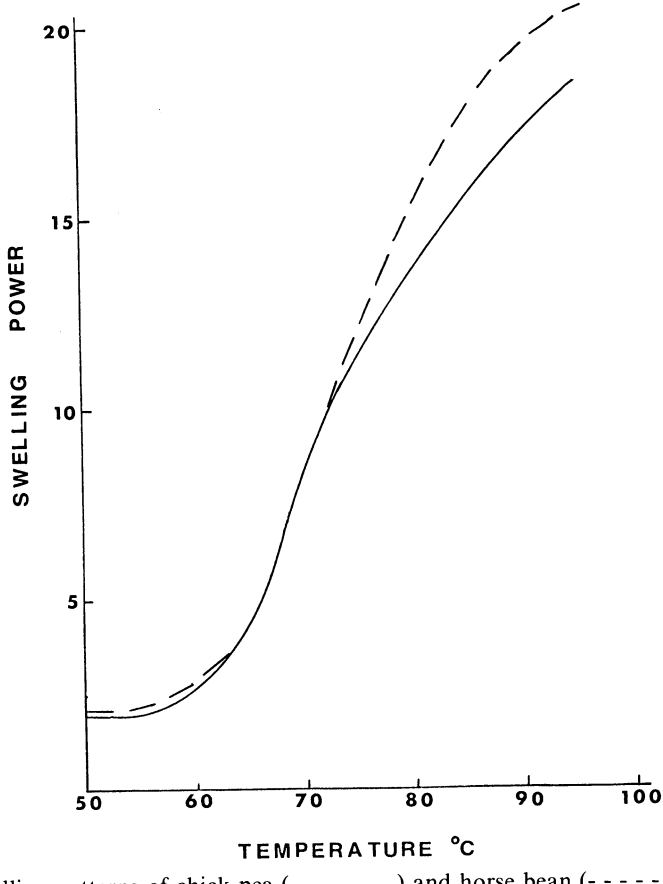

Fig. 3. Swelling patterns of chick pea (_____) and horse bean (- - - - -) starches.

Proximate Analysis and Gelatinization Temperature Ranges

The starches were white. Analyses of the isolated starches and the flours are given in Table I. The yield of chick pea starch from flour was nearly identical to that reported by Schoch and Maywald (2) from seed, but was lower than that (62%) of Srivastava *et al.* (1). Ash and nitrogen contents were higher than those obtained by previous investigators (1–3). No analytical data have been found for horse bean starch. Kawamura (5) obtained a 23 to 25% yield of starch from mature horse beans.

The gelatinization temperature ranges, determined using a microscope equipped with a Kofler hot stage, were 63.5° – 65° – 69°C for chick pea and 61° – 63.5° – 70°C for horse bean starch. These values agree with values previously reported: 62.5° to 68°C for chick pea (2), and 64° to 67°C for horse bean starch (23). Srivastava *et al.* (2) reported a higher birefringence end point temperature range (71° to 74°C) for chick pea starch.

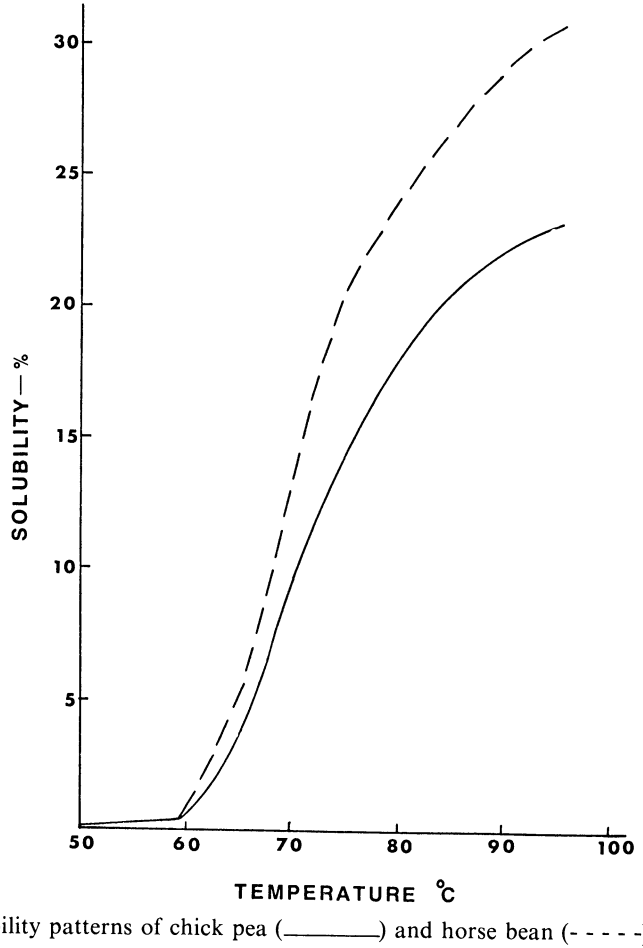

Fig. 4. Solubility patterns of chick pea (_____) and horse bean (- - - - -) starches.

Physical Characterization of Starches

The swelling and solubility patterns for chick pea and horse bean starches are shown in Figs. 3 and 4, respectively. The single-stage swelling pattern obtained for chick pea starch is similar to that obtained by Schoch and Maywald (2) and by Correa *et al.* (4); Srivastava *et al.* (1) reported a two-stage swelling for chenna (chick pea) starch. The swelling pattern of horse bean starch is similar to that of chick pea starch but swelling was slightly greater at higher temperatures. The swelling power of both legume starches at 95°C is somewhat restricted (*i.e.*, in the range 16 to 20) as Schoch and Maywald (2) observed for chick pea and three other legume starches. The restricted swelling, plus the single-stage swelling pattern, have been interpreted to indicate the presence in the granule of strong binding forces which relax over one temperature range and not at different temperature ranges as occurs in maize and milo (4). The solubilities of chick pea starch observed in this study are higher than those reported previously (1,2,4). Horse bean starch is more soluble than chick pea starch over the range of temperatures studied.

The Brabender hot-paste viscosity patterns for chick pea starch at concentrations of 5, 6, 7, 8, and 9% are similar to those reported by Schoch and Maywald (2), although the viscosities at each concentration are lower, and are also similar to those reported by Tolmasquim *et al.* (3) and Rosenthal *et al.* (24). But they differ from the pattern reported by Srivastava *et al.* (1) for a 5% concentration of chenna starch in which a low peak viscosity was observed; we observed no peak. Possibly the presence of an enzyme (absorbed in the granules and not eliminated during purification) lowered the viscosity of our chick pea starch compared to that of Schoch and Maywald's sample (2); such behavior has been verified for lablab bean (*Dolichos lablab*) and chick pea starches (24). Or possibly there were undetected impurities in the starch sample. The Brabender hot-paste viscosity patterns for horse bean starch (Fig. 5) are virtually identical to those obtained for chick pea starch at the same concentrations. The patterns for 6 and 9% horse bean starch differed considerably from those reported by Kawamura (5); those he noted had the same general shape but much lower viscosity at a cooking temperature of 92.5°C. The two legume starches, which give Brabender curves characteristic of restricted-swelling starches, contained no pasting peak and had rather constant viscosity during cooking at 95°C. Both starches showed some tendency to retrograde during cooling and had relatively constant cold-paste viscosity during 1 hr of agitation at 50°C, indicating that the cold paste is relatively stable to shear. Legume starches apparently produce Brabender curves of this type (2,3,24), indicating that the granules are very resistant to swelling and fragmentation. These curves are very similar to those of chemically cross-linked starches. It is still not apparent why a native starch produces a viscosity curve similar to that of a chemically cross-linked starch (2,24).

The pasting characteristics of the two starches are shown in Fig. 6. Using the CMC-amylograph technique (4,5), we observed for both starches a single-step gelatinization. The initial pasting temperatures, 68.5°C for chick pea and 67°C for horse bean starch, coincide with the upper limits of the gelatinization temperature range measured by loss of birefringence, emphasizing that loss of birefringence occurs prior to any appreciable increase in viscosity measurable by the CMC-amylograph method. The pasting temperatures for both starches are

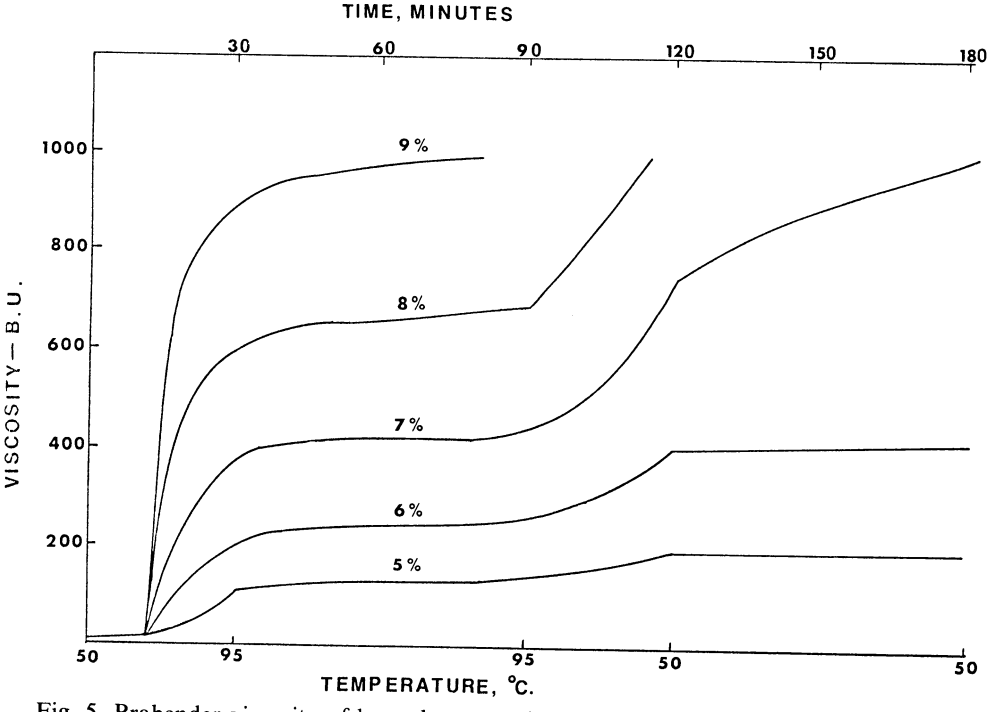

Fig. 5. Brabender viscosity of horse bean starch. Concentrations are given in per cent (w/v) of dry basis starch.

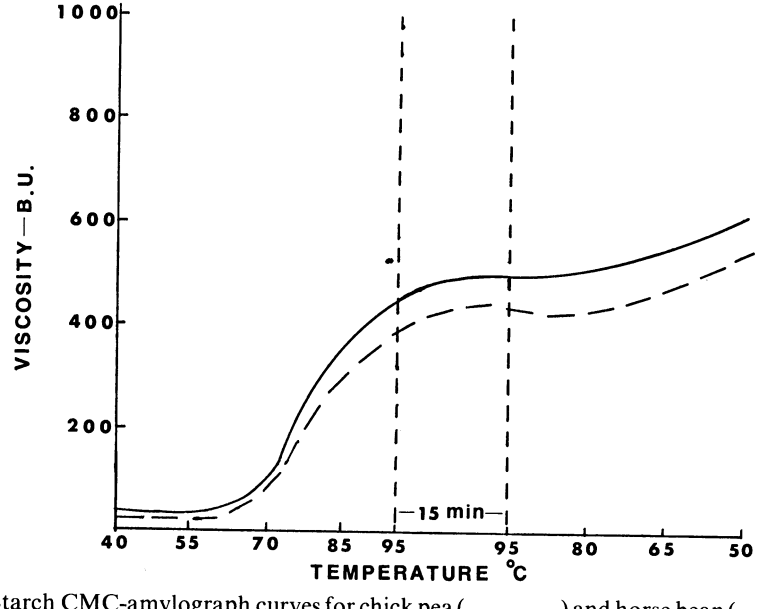

Fig. 6. Starch CMC-amylograph curves for chick pea (_____) and horse bean (- - - - -) starches (corrected for viscosity of CMC; 4.4% (w/v) starch on dry basis).

TABLE II
Iodine Affinity of Whole Starch, Amylose, and Amylopectin[a]

Source	Starch	Amylose	Amylopectin
Chick pea	6.08	19.2	3.08
Horse bean	6.28	17.1	2.21

[a]Values are per cent iodine affinity.

TABLE III
Amylose Content of Chick Pea and Horse Bean Starches[a]

	Method		
Starch	Colorimetric[b]	Calculated[c]	Fractionation[d]
Chick pea	20.0 ± 0.5	30.4	20.6 ± 1.5
Horse bean	30.0 ± 1.2	31.4	29.3 ± 0.6

[a]Values are per cent on dry basis plus the average deviation from the mean and are an average of three determinations.
[b]Method of McCready and Hassid (19).
[c]For calculation, "pure" amylose is assumed to absorb 200 mg iodine/g of amylose (16,27).
[d]Values from fractionation of starch.

TABLE IV
Carbohydrates in 70% Ethanol Extracts from Chick Pea and Horse Bean Flours[a]

	Chick Pea Flour		Horse Bean Flour	
	Distribution as % of		Distribution as % of	
Carbohydrate	Carbohydrate	Flour	Carbohydrate	Flour
Rhamnose	1.3 ± 0.2	0.11	1.3 ± 0.1	0.10
Xylose	0.1 ± 0.0	0.01	0.4 ± 0.1	0.03
Fructose	2.1 ± 0.1	0.18	0.4 ± 0.1	0.03
Glucose	4.0 ± 0.1	0.34	0.4 ± 0.1	0.03
Galactose	1.3 ± 0.0	0.11	1.1 ± 0.2	0.08
Sucrose	33.7 ± 0.4	2.82	40.8 ± 0.6	3.17
Maltose	2.1 ± 0.1	0.18	0.5 ± 0.1	0.04
Unknown I	0.6 ± 0.1	0.05	3.0 ± 0.1	0.23
Raffinose	7.7 ± 0.3	0.65	5.8 ± 0.5	0.45
Unknown II	20.9 ± 0.5	1.75	1.2 ± 0.1	0.09
Stachyose	27.3 ± 0.6	2.29	23.1 ± 0.4	1.80
Origin	2.7 ± 0.2	0.23	25.8 ± 0.4	2.01
Total sugars[b]	103.8	8.71	103.8	8.06

[a]Each value is an average of three determinations on dry basis plus the average deviation from the mean. A glucose standard curve was used to determine each carbohydrate.
[b]Total soluble carbohydrate in the original flour samples was 8.38% in chick pea and 7.78% in horse bean.

considerably higher than the 56° to 59°C reported (15) for a number of wheat starches. The higher pasting temperature for the two legume starches indicates that these starches have a higher resistance to swelling and rupture than does wheat starch. Maximum viscosities are nearly the same as those observed after 15 min at 95°C, indicating that the pastes are relatively stable during stirring at 95°C.

Fractionation of Starch

The starches were fractionated into their amylose and amylopectin constituents using an extraction procedure (16) prior to precipitating the amylose with butanol. Chick pea starch yielded 20.6% amylose and 73.2% amylopectin based on the dry weights of the fractions and the initial starch. Horse bean starch yielded 29.3% amylose and 66.8% amylopectin. Kawamura (5), using alkali gelatinization prior to precipitation of the amylose with butanol, obtained 22.0% amylose and 37.7% amylopectin from broad bean (horse bean) starch.

Iodine affinity values for whole starch, amylose, and amylopectin were determined by potentiometric titration (18) and are shown in Table II. The value of 6.08% for chick pea starch compares with previously reported values ranging from 5.65 to 6.70% (1,2,24,25). Values of 5.70% (5) and 4.5% (23) have been reported for broad bean (horse bean) starch; we observed 6.28%. Iodine affinities reported (5,26) for horse bean amylopectin (0.49 and 1.75%) and amylose (15.6 and 16.6%) are reasonably close to the values of 2.21 and 17.1%, respectively, obtained by us.

The amylose content of the two legume starches was determined (Table III) by fractionating the starches, by a colorimetric method (19), and by calculation from the iodine affinity of the whole starch, assuming that pure amylose absorbs 200 mg iodine/g of amylose (16,27). There was good agreement between the methods for horse bean starch, which yielded an amylose content of 30%. Kawamura (5) reported an amylose content of 20.5 and 26.8%, depending on the method used to measure iodine affinity (*i.e.*, colorimetric or potentiometric); Greenwood and Thomson (23) reported an amylose content of 24%, calculated from iodine affinity. For chick pea starch, the colorimetric method and fractionation data yielded an amylose content of 20%; the value calculated from the iodine affinity of the whole starch is much higher, 30%. Singh *et al.* (25), who reported amylose contents of 26.8 and 29.0% for starches from two varieties of chick pea, calculated them from iodine affinities using 21.2% as the value for pure amylose. Tolmasquim *et al.* (28) reported that iodine affinity values for legume starches generally range from 6.0 to 7.5%, indicating an amylose content of 30 to 37.5%, based on an assumption of an iodine affinity of 20.0% for pure amylose. Based on that assumption and using the iodine affinities previously cited (1,2,24,26), the amylose content of chick pea starch would be in the range 28.3 to 33.5%. The reasons for the discrepancy in results from the methods used in this study are not known.

Low-Molecular-Weight Carbohydrates in Chick Pea and Horse Bean Flours

Table IV lists carbohydrates identified in a 70% ethanol extract of chick pea and horse bean flour together with the amount of each carbohydrate expressed as a percentage of the total extractable carbohydrates and of the flour.

Carbohydrates were identified by cochromatography with known standards in two solvent systems. The 70% ethanol extracts from chick pea flour and horse bean flour contained rhamnose, xylose, fructose, glucose, galactose, sucrose, maltose, raffinose, stachyose, and two unidentified carbohydrates. Some differences were observed in the distribution of the carbohydrates in these two flour samples (Table IV). Chick pea flour has a slightly higher amount of monosaccharides than does horse bean flour. Sucrose (followed by stachyose and raffinose) is the predominant low-molecular-weight carbohydrate in both flour samples. Nigam and Giri (6) reported chick pea seed to contain 2.4% sucrose, 2.5% stachyose, 1.0% raffinose, and 4.2% stachyose. Horse bean seeds have been reported (7) to contain 2.5% stachyose, 0.65% raffinose, 2.3% verbascose, and a trace of ajugose. Pritchard *et al.* (8) observed sucrose, glucose, fructose, galactose, stachyose, raffinose, isomaltose, maltotriose, maltotetraose, and maltopentaose in an 80% ethanol extract of field beans (horse beans) with sucrose concentrations ranging from 0.2 to 5.2%. Cerning *et al.* (9) detected verbascose, stachyose, raffinose, sucrose, and, in some cases, traces of glucose and fructose in ethanol extracts from a series of 16 horse bean samples (from various geographical origins) grown in different areas of France. Sucrose comprised 20 to 25% of the total sugars. The two unidentified carbohydrates observed in our study with relative mobilities greater and less than raffinose, yielded fructose, glucose, and galactose upon acid hydrolysis. These two carbohydrates probably belong to the galactosylsucrose series, and one may be verbascose, detected in extracts from both chick peas and horse beans by previous investigators. The total soluble carbohydrate extracted from chick pea flour (8.38%) was higher than the 6.7% previously reported (29). The total soluble carbohydrate in horse bean flour (7.78%), also greater than the 7.54% reported by Tanusi *et al.* (7), was in the range (5.7 to 14.2%) observed by Pritchard *et al.* (8).

Conclusion

Chick pea and horse bean starches are similar to other legume starches possessing granules that resist swelling and rupture and yield a cooking viscosity pattern similar to cross-bonded starches. The two starches have a relatively high amylose content, about 30%, apparently characteristic of legume starches. The value for the amylose content of chick pea starch depended on the method of measurement. Chick pea and horse bean flours contain approximately 8% of low-molecular-weight carbohydrates, with sucrose predominant. This value is greater than that normally found in wheat flour; thus, one might anticipate increased browning in baked products containing these legume flours. The presence of stachyose and raffinose might also contribute to problems involving flatulence if these flours were consumed in large amounts. Wheat flour would probably not be supplemented with more than 20% of these legume flours, in which case flatulence and increased browning would be minimized.

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[Received January 21, 1974. Accepted September 16, 1974]