Molecular Weight Distributions of Legume Starches By Gel Chromatography[1]

C. G. BILIADERIS,[2] D. R. GRANT,[3] and J. R. VOSE,[4] National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada S7N 0W9

ABSTRACT

The distribution of the molecular weight of the components of nine purified legume starches (smooth and wrinkled field peas; adzuki, garbanzo, mung, red kidney, navy, and faba beans; and green lentil) was investigated by gel chromatography. The Sepharose 2B columns were calibrated with dextrans of known molecular weight. Evidence was provided (% β-amylolysis and λmax of iodine-polysaccharide complexes) that the amylopectin fraction was excluded from the gel in all the starches. It was concluded therefore that the average molecular weight of this component was greater than 20×10^6. The distribution of components differed at different molecular weight ranges. The elution patterns of their components fractionated within the range of 0.2–0.9 Kav (primarily amyloses) were quite similar for all the legume starches except those of smooth and wrinkled seeded peas. The amount of polysaccharide material of both smooth and wrinkled pea starches was greater in the intermediate molecular weight range, $2 \times 10^6 <$ mol wt $< 20 \times 10^6$, as compared with those of the other legume starches. Correlation analysis between set-back viscosities (obtained from viscoamylograms) and percent components fractionated between 0.2 and 0.9 Kav showed significant correlation ($P = 0.05$).

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Recent research on the use of field peas as a protein crop in western Canada has resulted in the development of efficient processing techniques for separating protein and starch components from legume seeds (Vose et al 1976, Youngs 1975).

Although some work has been done in the area of basic chemistry of legume starches (Banks et al 1974, Greenwood and Thomson 1962, Kawamura 1969, Naivikul and D'Appolonia 1979, Rosenthal et al 1974), no rigorous research has been conducted to investigate their fine structure and the molecular weight distribution of their components. These molecular properties are important in determining the functionality of these starches.

Our objective was to determine by gel chromatography the molecular weight distribution of the components of nine different purified legume starches.

MATERIALS AND METHODS

The following dried legume seeds were obtained from a local supplier: smooth-seeded field peas (*Pisum sativum* L. cv. Trapper), wrinkled-seeded field peas (*P. sativum* L. cv. Venus), adzuki bean (*Phaseolus chryasnthos*), garbanzo bean (*Cicer arietinum*), mung bean (*Vigna radiata*), green lentil (*Lens culinans*), red kidney bean (*Ph. vulgaris*), navy bean (*Ph. vulgaris*), and faba bean (*Vicia faba equina* L. cv. Diana). Semipurified starches were prepared from the

[1]NRCC No. 17571.

[2]Graduate student, Interdisciplinary Food Science Program, University of Saskatchewan, Saskatoon.
[3]Dept. Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon.
[4]National Research Council of Canada, Prairie Regional Laboratory, Saskatoon.

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seeds by a wet-milling process. The seeds were ground with water in a single disc mill (Bauer Bros. Co., Springfield, OH) to give a coarse slurry. The slurry was defibered by means of a horizontal 75 μm vibratory screen (Sweco Separator, Southwestern Engineering Co., Los Angeles, CA). The starch was then removed and partially purified by centrifugation (2,400 rpm) in a basket centrifuge (Fletcher Works, Inc., Philadelphia, PA). Further purification was obtained by passing the starch slurry through a counter-current washing unit with 10 banks, each containing four nylon 1 × 10 cm cyclones (Dorr-Oliver Inc., Stanford, CT). The starches were then dried in a spray drier (Proctor-Schwartz Corp., Philadelphia, PA) and stored for future use. Final purification of these starches was done in the laboratory by repeated washing with 95% ethanol and screening (44 μm). The purified starches were then dried in a vacuum oven (50°C for 48 hr). Corn, waxy maize, and potato starch (A. E. Staley Inc., Decatur, IL) were used for comparison.

Standard AACC methods were used for proximate analysis. Protein was estimated as N × 6.25. Starch was assayed by the dual-enzyme semimicro method of Banks et al (1970), using α-amylase (Tenase, Miles Lab. Inc., Elkhart, IN), glucoamylase (Diazyme L-100, Miles Lab.), and Glucostat reagent (Worthington, Biochem. Corp., Freehold, NJ). Iodine affinities of the defatted (hot extraction with 85% methanol for 48 hr) starches were determined by potentiometric titration (Schoch 1964). Gelatinization temperature range was determined as described by Schoch and Maywald (1956). Amylograms were prepared on a Brabender Visco-Amylograph with a 700 cm/g sensitivity cartridge at 75 rpm using 8% w/w slurries.

Gel chromatography of the defatted starches was done according to a modification of Yamada and Taki's method (1976), using Sepharose 2B (Pharmacia Ltd., Montreal, Quebec) as the gel filtration medium. In this procedure 15 ml of a 0.4% perchloric acid solution containing 1.44 mg of NaOH per milliliter was added to the column before addition of the sample to diminish any tendency for the amylose to retrograde. This is particularly important in the first part of the column where the polysaccharide concentration is highest. Starch samples (90–100 mg) were prepared by dispersion at 2°C in 0.5 ml of 40% perchloric acid, diluted first with 5 ml of NaOH (1.44% w/v) and then to 50 ml with distilled water. Aliquots (5 ml) were applied to the column and eluted with water (4°C) by the ascending method at constant flow rate. Fractions (3 ml) were collected and analyzed (alternate samples) for total carbohydrates and λmax of absorption of the iodine-polysaccharide complexes. Total polysaccharides in the fractions were assayed by the phenol-sulfuric method (Dubois et al 1956), using a standard curve prepared with D-glucose. The iodine-polysaccharide complexes were prepared by mixing the fractions with 0.5 ml of iodine solution containing 0.0002 mg of I_2 and 0.002 mg of KI per milliliter. The parameters V_o and V_t for the systems were obtained by chromatography of Blue Dextran (Pharmacia Ltd., Montreal, Quebec) and KCl, respectively. The peak fraction detected with Blue Dextran (absorption at 280 nm) was taken as the value of V_o. The peak fraction that titrated (Cl^-) with $AgNO_3$ was used as V_t. For calibration of the columns, a series of linear dextrans (Pharmacia) was used: Dextran T-40 (mol wt 41,000), Dextran T-150 (mol wt 143,000), Dextran T-500 (mol wt 466,000), and Blue Dextran (mol wt 2,000,000). The chromatograms of the dextrans with one of the columns are shown in Fig. 1.

The chromatographic parameters such as gel-bed and flow rates are given in Figs. 5 (for column B) and 6 (for column A). The elution volume for each dextran (peak fraction) remained constant within $V_e \pm 1.5$ ml in repeated chromatographic measurements. Rechromatography of samples taken from different parts of the elution pattern (both dextrans and samples) did not significantly change the elution volume. Recoveries of the eluted polysaccharides were within 94–101% for all the samples applied to the column. The relationship between Kav and log mol wt for the dextrans is shown in Fig. 2. Kav is defined as $(V_e - V_o)/(V_t - V_o)$, where V_e is the elution volume, V_o is the exlusion volume, and V_t is the total volume.

Amylopectins were isolated by using Banks and Greenwood's method (1967). The smooth and wrinkled pea amyloses were prepared according to the method of Montgomery and Senti (1964). In this procedure, starch was pretreated with 85% glycerol for 1 1/2 hr at 88°C, the amylose was leached twice with water (98°C for 15 min), and the extracts were combined. The amylose was then precipitated as an amylose-butanol complex. The β-amylolysis was effected by adding 0.1 volume of 0.1% barley β-amylase (Fluka, A. D., Buchs, S. G., Switzerland) in water to 1.0 volume of the buffered (0.05*M* acetate, pH 4.7) polysaccharide solution. Digests were incubated at 37°C and the reaction was monitored by measuring the reducing power of the hydrolyzates. When the reducing capacity of the digests became constant (12–18 hr), the percent of β-amylolysis was calculated as:

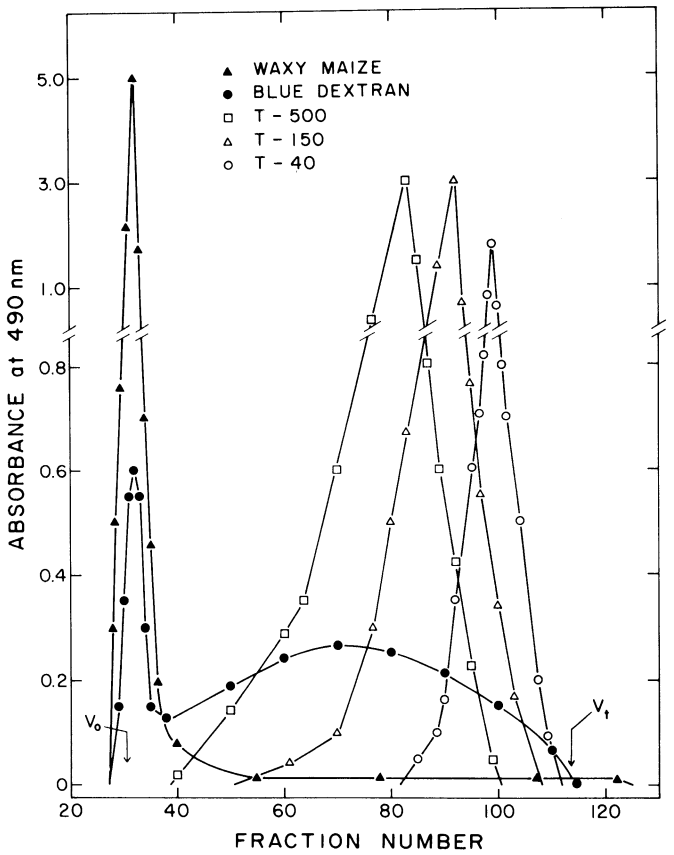

Fig. 1. Gel chromatography of dextrans and waxy maize starch. Column A: Sepharose 2B, gel-bed 2.6 × 64 cm, flow rate 18 ml/hr.

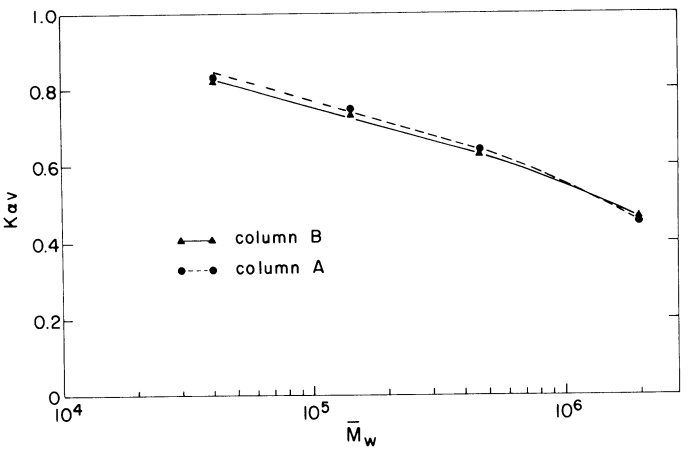

Fig. 2. Calibration curve based on the molecular weight values of the dextran standards.

$$\frac{\text{reducing capacity (as maltose)}}{\text{total carbohydrate (as glucose)}} \times 100$$

Reducing sugars were determined according to Dygert et al (1965), and total carbohydrates in the digests were assayed according to the previously mentioned dual-enzyme method (Banks et al 1970).

Limiting viscosity numbers of the amyloses (η) dispersed in 1N KOH, were obtained with a modified Ubbelohde viscometer according to Greenwood's method (1964).

RESULTS AND DISCUSSION

The proximate analysis and some physicochemical characteristics of the legume starches are given in Table I. The gelatinization temperature range for most of the starches is between 58 and 69° C, which agrees with reported vaues (Rosenthal et al 1974) for a series of legume starches. However, the adzuki bean has a considerably higher gelatinization temperature even though it had an amylose content similar to that of garbanzo bean, smooth pea, mung bean,

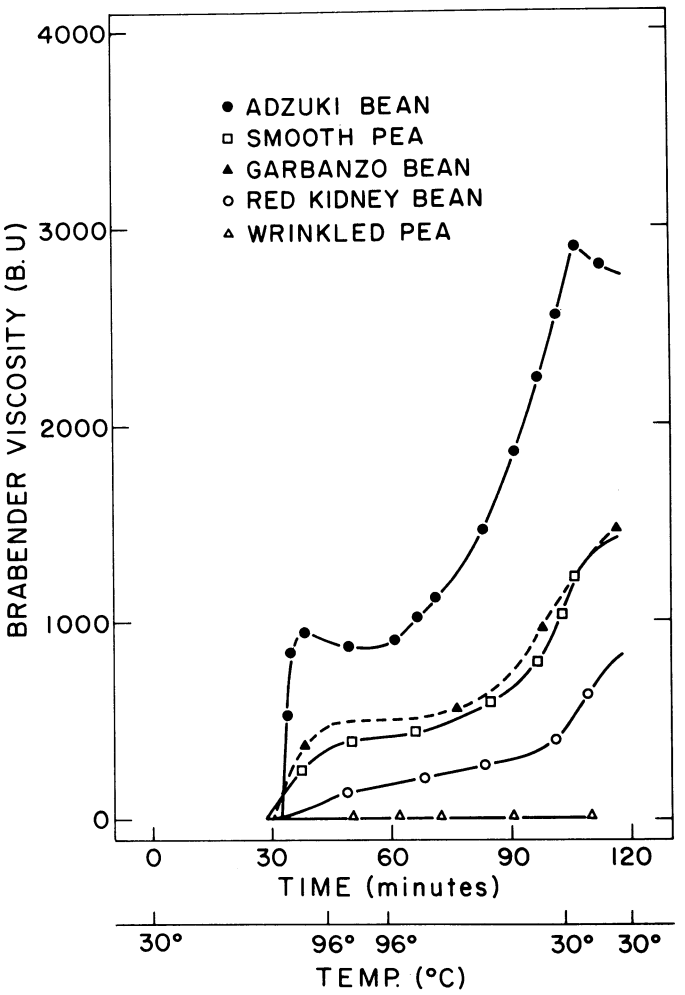

Fig. 3. Brabender pasting curves of adzuki bean, smooth pea, garbanzo bean, red kidney bean, and wrinkled pea starches.

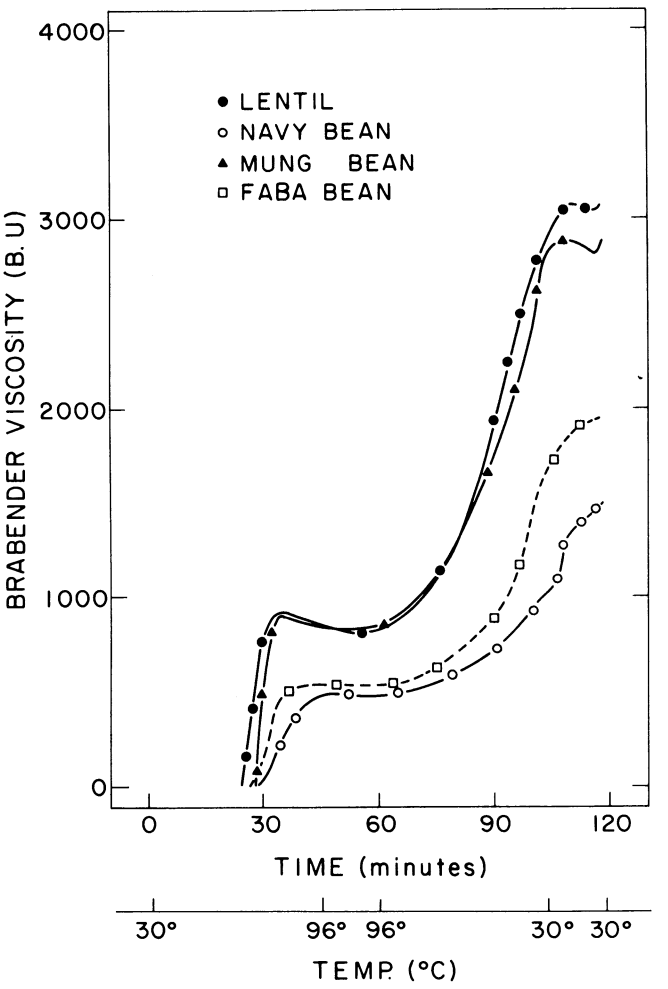

Fig. 4. Brabender pasting curves of lentil, navy bean, mung bean, and faba bean starches.

TABLE I
Proximate Analysis and Other Physicochemical Characteristics of Legume Starches

Starch	Starch (%)	Protein (%)	Lipid (%)	Crude Fiber (%)	Ash (%)	Gelatinization Temperature[a] (°C)	Iodine Affinity	Apparent[b] Amylose Content (%)
Adzuki bean	93.9	0.27	0.03	0.15	0.07	83–(85)–89	6.98	34.9
Garbanzo bean	95.1	0.36	0.14	0.09	0.05	65–(68)–71	6.81	34.1
Smooth pea	97.2	0.52	0.01	0.01	0.08	65–(67)–69	6.62	33.1
Red kidney bean	94.0	0.30	0.18	0.22	0.12	64–(66)–68	7.01	35.0
Wrinkled pea	94.8	0.46	0.01	0.18	0.08	> 99	12.80	64.0
Lentil	96.2	0.17	0.23	0.20	0.13	58–(59)–61	9.09	45.5
Navy bean	96.8	0.13	0.09	0.10	0.06	68–(71)–74	7.20	36.0
Mung bean	94.9	0.28	0.20	0.51	0.18	63–(65)–69	6.98	34.9
Faba bean	97.8	0.49	0.00	0.00	0.06	61–(63)–66	6.51	32.5
Corn	95.9	0.38	0.09	0.08	0.07	63–(65)–68	4.53	22.6

[a]Recorded temperatures correspond to loss of birefringence by 5, 50, and 95% of the starch granules.
[b]Assuming that "pure" amylose has an iodine-binding capacity of 20.0%.

red kidney bean, and faba bean. This might reflect differences in the internal structure of the granule of this starch. As expected, gelatinization of the wrinkled seeded pea starch occurred at temperatures greater than 99° C, presumably as a result of its high amylose content. The range of iodine affinities (6.51–12.80) and the corresponding apparent amylose contents (32.5–64.0%) are comparable with results reported by Banks et al (1974), Greenwood and Thomson (1962), Rosenthal et al (1974), Schoch and Maywald (1968), and Shahen et al (1978) for some of these starches. However, Kawamura (1969) and Naivikul and D'Appolonia (1979) obtained significantly lower amylose contents with a colorimetric method. These differences may arise from the different method for measuring the amylose content and also from the fact that variety (Rosenthal et al 1974, Shahen et al 1978) and physiological stage of the seeds (Banks et al 1974) could pronouncedly affect both the amylose/amylopectin ratio and the nature of the linear fraction. Our results and previous results (Rosenthal et al 1974) emphasize that the amylose content of most of these legume starches extends over a broad range (30–45%) compared with the relatively constant amylose content (20–22%) of mature cereal and tuber starches. Consequently, this group of starches could exhibit a wide spectrum of rheological properties.

Brabender pasting curves of the starches are given in Fig. 3 and 4. The highest pasting temperature, as defined by Medcalf and Gilles (1966), was that of the adzuki bean (78° C), and the lentil had the lowest (66° C). These values agree with the gelatinization temperatures of these starches (Table I). The values for the rest of the starches were 69–75° C. The viscosity patterns of the legume starches resemble those of chemically cross-linked starches, possibly as a result of extensive intermolecular hydrogen bonding (Rosenthal et al 1971). The wrinkled pea starch (64% amylose) behaved like the amylomaize starches, possibly because of the rigidity of its granule, which is imparted by the high content of the linear molecules. Only the adzuki bean starch produced a cohesive smooth gel after pasting; all others yielded opaque friable gels with a firmer texture than comparable corn gels.

The gel chromatographic patterns of the starches are shown in Figs. 5 (for Column B) and 6 (for Column A). All chromatograms had a similar pattern, a very high and narrow peak (macromolecules excluded by the gel) and a low curving section that occurred within the fractionation range of the Sepharose 2B. The general trend of the chromatograms is similar to those of other starches reported for the same (Yamada and Taki 1976) or different (Ebermann and Praznik 1975) conditions. Comparison of the carbohydrate material of the exclusion peak and some of the chemically isolated amylopectins (% β-amylolysis and λ max) provides strong evidence that the peak consists mainly of amylopectin. The data (Table II) indicate that both the β-amylolysis and λmax values of the peak polysaccharides were slightly lower than those of the isolated amylopectins. However, the fact that both λmax and β-amylolysis values were significantly higher than that of typical waxy maize amylopectin may be due to either 1) structural differences between

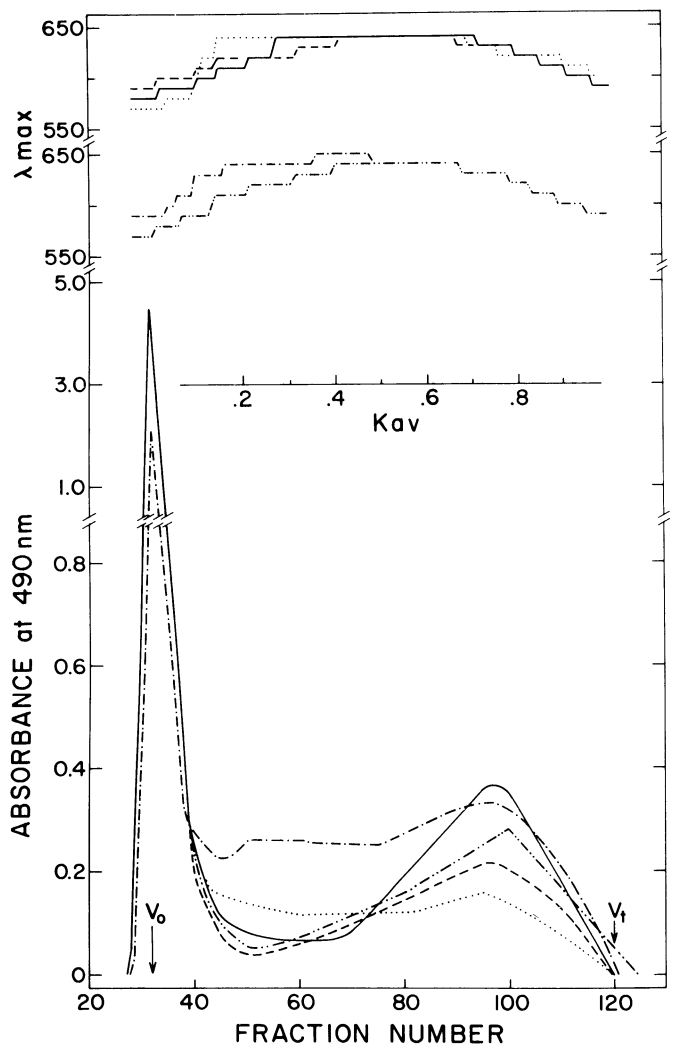

Fig. 5. Gel chromatography of starches. Column B: Sepharose 2B, gel-bed 2.6 × 67 cm, flow rate 17 ml/hr. Adzuki bean (——), smooth pea (·····), garbanzo bean (- - -), red kidney bean (-·-), and wrinkled pea (-··-) starches.

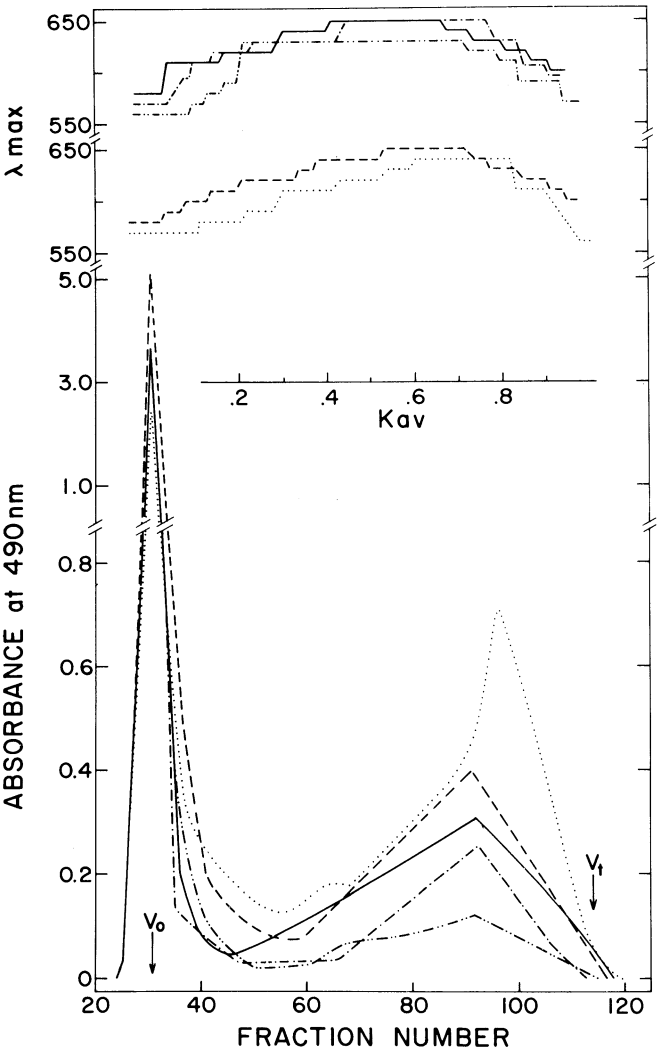

Fig. 6. Gel chromatography of starches. Column A: Sepharose 2B, gel-bed 2.6 × 64 cm, flow rate 18 ml/hr. Lentil (·····), navy bean (——), mung bean (---), faba bean (-·-), and potato (-··-) starches.

TABLE II
Comparison of Gel-Excluded Polysaccharides and Chemically Isolated Amylopectins of Legume Starches

Starch	Exlusion Peak Polysaccharides			Chemically Isolated Amylopectins		
	Fraction Number	λmax Range (nm)	β-Amylolysis (%)	Apparent Amylose[a] Content (%)	λmax (nm)	β-Amylolysis (%)
Smooth pea	27–40[b]	570–610	63.4	7.50	610	66.5
Navy bean	24–37[c]	580–610	61.9	8.30	610	65.4
Adzuki bean	27–40[b]	580–600	63.2	5.45	610	63.9
Faba bean	24–37[c]	570–590	62.6	5.40	610	66.2
Waxy corn (control)	27–40	540	55.9	0.00	570	57.2

[a]Based on the iodine affinities of the amylopectins and the assumption that "pure" amylose has a 20% iodine-binding capacity.
[b]Column B.
[c]Column A.

TABLE III
Percent Distribution of Molecular Weight of the Starches

Starch	mol wt $< 4 \times 10^4$	4×10^4 < mol wt $< 1.5 \times 10^5$	1.5×10^5 < mol wt $< 5 \times 10^5$	5×10^5 < mol wt $< 2 \times 10^6$	mol wt $> 2 \times 10^6$
Adzuki bean	7.5	7.7	9.3	8.8	66.7
Garbanzo bean	6.5	5.8	7.2	6.5	74.0
Smooth pea[a]	4.7	4.8	6.9	7.2	76.4
Red kidney bean	7.6	7.8	8.2	6.7	69.7
Wrinkled pea[a]	8.7	7.7	10.7	13.2	59.7
Lentil	9.9	12.5	12.0	6.3	59.3
Mung bean[a]	3.3	8.2	10.5	9.1	68.9
Navy bean	4.8	6.6	10.0	6.9	71.7
Faba bean[a]	1.3	6.2	10.2	8.1	74.2
Potato	0.9	3.2	5.8	4.5	85.6

[a]Data presented are means of duplicate chromatographic runs. Duplicate values had an average variation of 5.46% (standard error of mean = 1.26).

the amylopectins, 2) presence of an intermediate material with molecular properties between those of amylose and amylopectin, or 3) physical or chemical (via hydrogen bonding) entrapment of amylose in the amylopectin molecules. This difficulty in efficiently fractionating legume starches chemically was also observed by Greenwood and Thomson (1962). This certainly appears to be an inherent characteristic of the overall granule organization and could account for some of the hypotheses. Although no explanation can be definite now, contamination of the peak, expressed as percent amylose, must be low, according to the data (Table II). Because the exclusion limit of Sepharose 2B is 20×10^6 for polysaccharides, we concluded that all the amylopectins exceeded that molecular weight.

The retarded peaks in the chromatograms are comprised of the amyloses, which fractionate according to their molecular weight. This is strongly supported by these fractions' λmax values, which range between 620 and 650 nm. The percentage distributions in different molecular weight groups were calculated from the chromatograms (Table III).

The λmax profiles of the amyloses cannot be explained solely by the degree of polymerization. Differences in the bathochromic and hypsochromic shifts of the λmax at certain parts of the chromatograms for the different legume starches may reflect differences in the structure of their amyloses. Among the starches studied, both smooth and wrinkled seeded pea starches had chromatograms significantly different from those of the rest of the starches in that their low molecular weight fractions were distributed more uniformly within the range of 0.2–0.9 Kav. In the case of smooth pea starch, rechromatography did not significantly change the elution volume of fractions 55–60.

Gel chromatography of the leached amyloses of these two starches (smooth pea amylose: η = 192, iodine affinity = 18.84; wrinkled pea amylose: η = 136, iodine affinity = 19.82) showed elution patterns very similar to those of the retarded amylose components of the other legume starches. The yield of these isolated amyloses was only about 55–60% of the apparent amylose content, however, and consequently cannot be taken as representative of the entire linear fraction of these starches. Therefore, no conclusions can be made as to whether the fractions in the 0.2–0.5 Kav region present true molecular features of the linear components of these pea starches. One of the following possibilities could also be responsible for the results: 1) presence of a low molecular weight amylopectin (between 20×10^6 and 2×10^6), as a result of the physiological stage or genetic characters of these seeds; 2) presence of an intermediate material with molecular weight and properties between those of amylose and amylopectin; or 3) partial intermolecular association (covalent or hydrogen bonding) between the linear chains to give oligomeric structures of apparent higher molecular weight. Differences in the distribution were obvious between the different legume starches (Table III).

We attempted to explain the pasting properties of the starches (Fig. 3 and 4) on the basis of their molecular characteristics. The starches that exhibited the highest hot and cold gel viscosities (lentil, mung, and adzuki bean) had the highest values for percent components with mol wt $< 2 \times 10^6$. Significant correlation (r = 0.737, $P = 0.05$) was shown between set-back viscosities (from the viscoamylograms) and percent components fractionated within 0.2–0.9 Kav. In addition to percentage of content, however, other factors such as molecular organization of the granule and fine structure of the amylopectin must also be involved in the gelling properties of these starches.

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