Carbohydrates of Legume Flours Compared With Wheat Flour. II. Starch[1]

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ABSTRACT

Starch yield from various legume flours was considerably lower than values reported for wheat flour. In general, the legume starch granules were oblong and did not exhibit as wide a variation in size as wheat starch, which has a mixture of large, intermediate, and small spherical granules. The polarized-light photomicrographs indicated that the birefringence of the legume starch granules is similar among the various legumes but differs from that in wheat. The amylogram curves of the legume starches showed higher initial pasting temperature and higher viscosity than did wheat starch, which would indicate a higher resistance to swelling and rupture. No peak viscosity during the hold period at 95°C occurred with any of the legume starches, indicating that the pastes were relatively stable and that the granules did not rupture during stirring, which is not the case with wheat starch. Water-binding capacity values for the legume starches were similar to values reported for wheat starch except for mung bean starch, which had a lower value. The range in amylose contents obtained for the legume starches in this study was slightly lower than the range reported for wheat starch. Acetylated wheat starch amylose had a higher molecular weight than did the acetylated legume starch amyloses except for lentil. The intrinsic viscosity values for the different legume starch amyloses showed the same trend when compared with the molecular weight values except for that of lentil amylose.

Kawamura et al (1955) developed a method to isolate starch from various legumes by treatment with a 0.2% NaOH solution, washing with water, and dehydration with ethanol and ether. Schoch and Maywald (1968) found that the separation of pure starch was difficult with certain legumes because of the presence of a highly hydrated fine fiber fraction (presumably from the cell walls enclosing the starch granules) and also the high content of insoluble protein. Various workers (Hall and Sayre 1971, Kawamura et al 1955, Lineback and Ke 1975, McEwen et al 1974, Rockland and Jones 1974) have studied the size and characteristics of legume starch granules. They found good agreement among various legume starch granules of the same species. In general, legume starch granules were ellipsoid, kidney-shaped, or irregularly swollen, with an elongated hilum. Scanning electron photomicrographs of legume starch granules showed a relatively smooth surface or two or three lobes, with no evidence of fissures as seen with the light microscope.

The Brabender hot-paste viscosity pattern of various starches appears to be determined not only by the extent of swelling of the starch granules and the resistance of the swollen granules to dissolution by heat or fragmentation by shear (Lineback and Ke 1975), but also by the presence of soluble starch, which is leached from the granule structure (Allen et al 1977, Miller et al 1973) and the interaction or cohesiveness between the swollen granules (Leach 1965). Schoch and Maywald (1968) classified the viscosity patterns of "thick-boiling" starches into four types. They found navy bean and lentil starches gave Type C Brabender curves, which showed no pasting peak but rather a high viscosity that remained constant or increased during cooking. Mung bean starch showed a mixed viscosity pattern—Type C at low concentration and Type B at high concentration.

Kawamura and Tada (1957) reviewed the methods to fractionate amylose and amylopectin, with particular emphasis on the legume starches. Montgomery and Senti (1958) presented a method for separating amylose from amylopectin of starch by an extraction-sedimentation technique. Kawamura (1969) found that the estimation of amylose content in the legume starch is complicated by the method used and also that the starch content, amylose content, and molecular weight of starch increased with maturation of the legume seeds.

The objectives of this study were to isolate starch from various legume flours, examine the physicochemical properties, and investigate the amylose fraction of each legume starch. Comparison of information on legume starches and on wheat starch could provide useful knowledge about the use and effect of legume flours on dough and baking properties.

MATERIALS AND METHODS

The five legume flours used in this study were described previously (Naivikul and D'Appolonia 1978). The legumes used included navy beans, pinto beans, lentils, faba beans, and mung beans.

Isolation and Chemical Analysis

Legume starches were isolated according to the method of McEwen et al (1974). The legume flour (500 g) was extracted with 0.016*N* NaOH solution by mixing in a Waring Blendor for 2 min. Water-soluble material was removed by centrifugation (2,000 × *g* for 20 min). The crude starch was washed three times with water, twice with 70% ethanol, and two additional times with water. The supernatant and sludge were removed and discarded after each washing. The prime starch was air dried (three days) and passed through a 70-mesh sieve.

Characterization of Legume Starches

The isolated starches were analyzed for purity by measuring protein, fat, moisture, acid-detergent fiber, and ash content. The methods used were those previously described for legume flours (Naivikul and D'Appolonia 1978).

The size and shape of each legume starch was studied with a Nikon-Microscope Model L-Ke equipped with an automatic Nikon-Microflex Model AFM camera (Nippon Kogaku, K.K., Japan). Photomicrographs were taken at a magnification of 400 using both normal and polarized light. A Brabender VISCO/amylo/GRAPH® equipped with a 700-cmg cartridge was used to study the pasting properties of the various legume starches, according to the procedure described by Medcalf and Gilles (1965). The water-binding capacity of the legume starches was determined by the procedure of Yamazaki (1953) modified by Medcalf and Gilles (1965).

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Fractionation of Legume Starches

Amylose and amylopectin were obtained from the starch using the aqueous leaching procedure described by Montgomery and Senti (1958). The amylose content in the legume starches was determined by the blue-value method (Gilbert and Spragg 1964). The calculation for legume amylose content was obtained from the equation described by Kawamura (1969).

Potter and Hassid's method (1948) was used to determine the molecular weight of amylose obtained from the various legume starches. The molecular weight of the amylose triacetate was determined from osmotic pressure measurement using a recording Osmometer Model CSM-2 (Melabs Scientific Instrument, Stanford Industrial Park, Palo Alto, CA). The intrinsic viscosity of the amylose fractions from the various legume starches were determined by Leach's method (1963).

RESULTS AND DISCUSSION

Analytic and Physical Data of Isolated Legume Starches

The amount of starch recovered from each of the legume flours is shown in Table I. The range in starch yield varied from 33.8 to 41.9%, which is considerably lower than the 70–80% obtained for wheat flour (Pomeranz 1971). Schoch and Maywald (1968) reported 27, 38, and 37% starch yields from navy bean, lentil, and mung bean seeds, respectively. Lineback and Ke (1975) reported a 37% starch yield from horsebean flour. The results of previous studies were thus comparable to the present results (Table I), except those of navy bean. The differences in the navy bean starch yield could be attributed to the method used for isolation and perhaps the sample source itself. Table I shows the chemical analysis of the isolated legume starches. The nitrogen content of the starches was low (0.06–0.07%), with values similar to those reported for wheat starch (Pomeranz 1971). The fat content of the faba bean starch was the lowest (0.22%), and that of the navy bean starch was the highest (0.52%). In general, wheat starch contains 0.5–0.8% fat (Pomeranz 1971), which was comparable to the amount in the legume starches except in faba bean starch. The mung and faba bean starches contained the highest and lowest ash contents—0.27% and 0.05%, respectively. In wheat starch, only a trace amount of ash has been reported (Pomeranz 1971). The acid-detergent fiber content of the isolated legume starches ranged from 0.05 to 0.26%, with faba and mung bean having the lowest and highest values, respectively. Fiber contents were higher in the legume starches than in the wheat starch, which contains only a trace amount (Pomeranz 1971). The methods used for starch isolation and for fiber determination may account for the higher value. Previous studies have not reported acid-detergent fiber content in isolated legume starches.

The data on water-binding capacity of the various legume starches are shown in Table I. Mung bean starch had the lowest water-binding capacity value (78.2%), and lentil starch had the highest value (92.4%). The navy and pinto bean starches had similar values, as expected, because they belong to the same species of leguminous seeds. Medcalf and Gilles (1965) suspected that the bound water of wheat starch was absorbed by the granules and adsorbed on its surface. No evidence is presented for this mechanism in legume starch. Water-binding capacity values for wheat starches ranged from 83.0 to 91.0% (Medcalf and Gilles 1965), which were similar to the legume starches with the exception of the mung bean starch.

Granule Size and Microscopic Appearance

The range in granule size of the isolated legume starches is shown in Table II, with their shape and birefringence pattern illustrated in Fig. 1 and 2, respectively. The size, shape, and birefringence of starch granules are often characteristic of the species of the plant and its maturity (Manners 1974). This was true for the navy and pinto bean isolated starches, which were similar in size, shape, and birefringence pattern, since they belong to the same species—*Phaseolus vulgaris*. Results on the granular size and shape of the *P. vulgaris* starches (navy and pinto bean) were comparable to previous reports (Kawamura et al 1955). The granules were irregular and elliptical, although some were small and round. The diameter width ranged from 12 to 36 μm and 16 to 18 μm, while diameter length ranged from 12 to 40 μm and 16 to 48 μm for the navy and pinto bean starches, respectively. Faba bean starch granules ranged from 12 to 24 μm in width and from 20 to 48 μm in length. The shape of the granules varied from small spherical to large oval and contained many irregularly shaped granules, which agrees with reports by Kawamura et al (1955) and Lineback and Ke (1975). Lentil starch contained somewhat smaller granules than navy, pinto, and faba bean and also contained more of the round-type granules. Their size ranged in width from 16 to 28 μm and in length from 16 to 32 μm. No data on the size and shape nor photographs of lentil starch granules have been published. The size of the mung bean starch granule was the smallest of the isolated legume starches studied. The granules ranged in width from 8 to 16 μm and in length from 12 to 32 μm. The mung bean starch granules were oval and irregular in shape, with some being round. Similar information was reported by Kawamura et al (1955). Legume starch granules differ from wheat starch, which has a mixture of large, intermediate, and small spherical granules (Kulp 1973).

In the light micrographs (Fig. 1), all of the legume starch granules showed dark bands that appeared as cracks and stria. Hall and Sayre (1971) suggested that the cracking dark bands were internal cracks and fissures that probably resulted from air drying of the starch, because they were absent from freshly picked green bean starch granules. In scanning electron microscope (SEM) photographs obtained by Hall and Sayre (1971), McEwen et al (1974), and Schoch and Maywald (1968), however, the legume starch granules were round to oval with shallow to pronounced furrows or grooves, none of which appeared to circle the granule completely. Also by SEM, the surface of the granules appeared to be smooth, with no breaks, which would not account for the pronounced cracks and striae seen with the light microscope.

The polarized-light photomicrographs (Fig. 2) indicate that the birefringence of the legume starch granules was similar for all five types of legumes. Each starch granule exhibited a dark cross, with the resultant effect of dividing the material into four brilliant segments (MacMasters 1953).

TABLE I
Analytic and Physical Data of Isolated Legume Starches[a]

Flour Source	Starch Yield (%)	Nitrogen (%)	Fat (%)	Ash (%)	Acid-Detergent Fiber (%)	Water-Binding Capacity (%)
Navy bean	40.3	0.06	0.60	0.14	0.15	83.8
Pinto bean	38.3	0.06	0.51	0.09	0.30	83.0
Faba bean	39.9	0.07	0.39	0.05	0.06	87.0
Lentil	42.5	0.07	0.46	0.20	0.10	92.4
Mung bean	34.5	0.06	0.50	0.27	0.30	78.2

[a]Values are expressed on 14% mb.

TABLE II
Granule Size of Isolated Legume Starches

Starch Source	Range (diameter)[a]	
	Width (μm)	Length (μm)
Navy bean	12–36	12–40
Pinto bean	16–28	16–48
Faba bean	12–24	20–48
Lentil	16–28	16–36
Mung bean	8–16	12–32

[a]Values reported are average of at least six readings.

Pasting Properties

Table III shows the pasting properties of the legume starches obtained with the Brabender VISCO/amylo/GRAPH®. Medcalf and Gilles (1966) described the terminology used to express the amylogram results. The temperature of initial pasting for this work is defined as the temperature at which an initial increase of 10 Brabender units (BU) in viscosity is reached. Normally, peak height is the maximum viscosity obtained; however, since no definite peak was obtained in this study, the value of 95°C was reported as peak height. Height at 15 min is the viscosity of the sample after the 15-min holding period at 95°C. The 50°C height is the viscosity of the sample after it has cooled to 50°C.

TABLE III
Pasting Properties of Isolated Legume Starches

Starch Source	Initial Pasting Temperature (° C)	Peak Height (BU)	95° C Height (BU)	15-Min Height (BU)	50° C Height (BU)
Navy bean	77.0	...	530	585	940
Pinto bean	77.0	...	435	528	840
Faba bean	66.0	...	770	838	1,860
Lentil	68.0	...	960	1,068	2,470
Mung bean	71.0	...:	1,240	1,340	2,500

The starch isolated from navy and pinto bean flour had similar pasting properties. Of the five legume starches, they had the highest initial pasting temperature (77°C). Faba bean starch had the lowest initial temperature of pasting (66°C), and the lentil and mung bean starch had initial pasting temperatures of 68 and 71°C, respectively.

No values are reported for peak viscosity, because no distinct peak was obtained with the legume starches as is normally found with wheat starch (Fig. 3). The mung bean starch gave the highest 95°C height and 15-min and 50°C heights; these values progressively decreased with lentil, faba bean, navy bean, and pinto bean starches. Figure 3 also shows higher viscosity in legume starches than in wheat starch, which indicates that these starches have a higher resistance to swelling and rupture than does wheat starch (Lineback and Ke 1975). The shapes of the amylogram curve of the various legume starches during the hold period at 95°C was similar (no peak viscosity), indicating that the pastes were relatively stable and that the granules did not rupture during stirring, which is not the case with wheat starch (Lineback and Ke 1975).

Generally, amylograms of most starches fail to show the early stages of gelatinization, the steps in gelatinization, and the different gelatinization temperatures for different concentrations of starches (Sandstedt and Abbott 1964). This complicates a direct comparison between the present amylogram data of the various legume starches and previously reported data. The Brabender amylogram curves noted for the legume starches in this study, however, generally were similar to those reported by Kawamura (1969), Lineback and Ke (1975), and McEwen et al (1974). They indicated that navy bean,

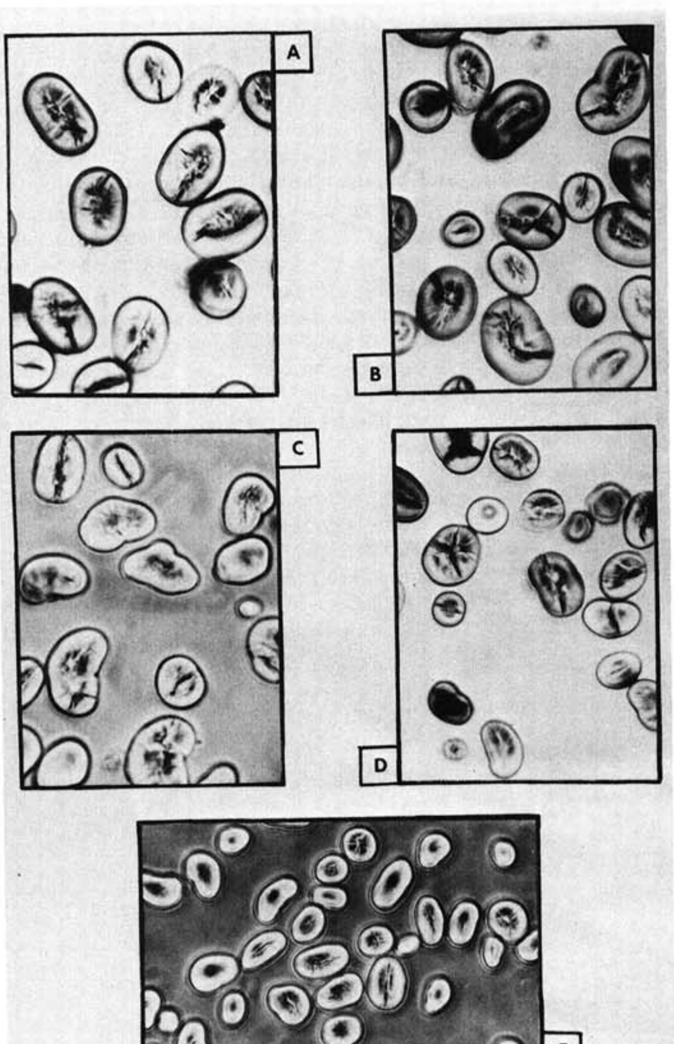

Fig. 1. Light micrographs of legume starch granules (×348). A, navy bean; B, pinto bean; C, faba bean; D, lentil; E, mung bean.

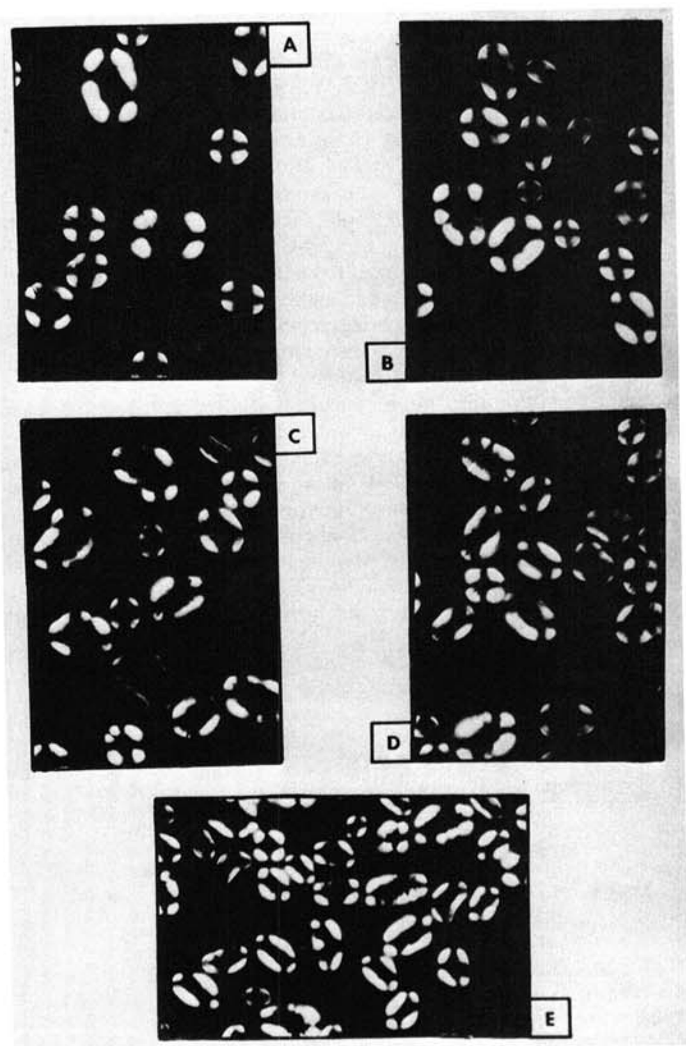

Fig. 2. Polarized-light micrographs of legume starch granules (×348). A, navy bean; B, pinto bean; C, faba bean; D, lentil; E, mung bean.

pinto bean, faba bean, and lentil starches gave Brabender pasting curves that were characteristic of a restricted type of swelling and contained no pasting peak, had a rather constant viscosity during heating at 95°C, and behaved similarly to cross-bonded starches. Thus, no evidence is still available to explain the observed results.

Mung bean starch differed from other legume starches in that it had a viscosity pattern that was highly concentration dependent. At a lower starch concentration its amylogram curves were similar to those of other legume starches, but at a higher concentration, the type of curve was similar to that found for wheat starch (Lineback and Ke 1975). In the present study, concentration was not varied because the amount of starch available was limited, so this phenomenon was not investigated.

An important factor to be considered in the use of legume flours in bread baking is the possible effect of the starch from these legumes on bread staling. Recently, Kim and D'Appolonia (1977) stated that the basic mechanism of bread staling involves changes analogous to crystallization of the starch fraction of the wheat bread crumb. They also showed amylograms of crumb slurries that had set back patterns different from starch, flour, or dough.

The differences noted in pasting properties of the legume starches warrant further studies of their retrogradation properties and influence in bread staling. The pasting curves of various legume starches in Fig. 3 suggest that faba bean, lentil, and mung bean might cause a different effect on staling than that of the other two legume starches because the increase in viscosity is greater during the cooling stage.

Fractionation of Legume Starches

The legume starches were fractionated into amylose and amylopectin by leaching of the granular starch with hot water and precipitating the amylose with butanol. The granule residue after extraction should consist only of amylopectin held in coherent form by bonds retained from the original granule structure.

The range in amylose contents obtained for the legume starches in this study (19.5–25.8%) (Table IV) was slightly lower than the range reported by Medcalf and Gilles (1965) for wheat starch (23.4–27.5%).

Kawamura (1969) found somewhat different amylose contents than those obtained in the present study. He reported amylose contents of 20.5 and 22.4% in broad bean starch (*Vicia faba*) and mung bean starch (*P. vulgaris*), respectively. Lineback and Ke (1975) reported that horse bean starch (*V. faba*) contained 30% amylose. Mukhtarova and Lovacheva (1973) found mung bean starches (three varieties) to contain amounts of amylose similar to those found in the present study.

The discrepancy in results on amylose content between previous reports and the present study could be explained by differences in the methods used to determine amylose content, measure iodine affinity (ie, colorimetric or potentiometric), and calculate the amylose content. Smirnova-Ikonnikova et al (1961) suggested that the large variation in amylose content of individual varieties of legume seeds depended on the area of growth but not the age of the seeds.

Variations in molecular weight values for amylose obtained from the different sources are evident from Table IV. The amylose isolated from the lentil starch had the highest molecular weight and that isolated from the pinto bean starch the lowest. The molecular weight values were determined on the triacetate derivative, which normally produces higher values than with deacetylated amyloses (Potter and Hassid 1948). The values in Table IV were considered to be an approximation of the molecular size of the amylose, because during the process of acetylation of starch with acetic anhydride at 60°C in the presence of pyridine, some degradation of the molecule could occur due to the elevated temperature as indicated by Potter and Hassid (1948). They reported that the molecular weight of deacetylated wheat starch amylose was 140,000 whereas the acetylated molecular weight product was 250,000.

Intrinsic viscosity is essentially a measure of the internal friction or resistance to displacement of high-polymeric molecules in solution. If properly used on a homologous series of a single molecular type, it provides an excellent criterion of relative molecular size (Leach 1963). Table IV shows the intrinsic viscosity values for the amylose fraction obtained from each legume starch. The mung bean amylose had the highest intrinsic viscosity value and pinto bean amylose the lowest. Amylose from wheat starches has been reported to have intrinsic viscosity values between 2.46 and 2.90 (Medcalf and Gilles 1965).

When compared with the molecular weight values for amylose, the intrinsic viscosity values for the different amyloses showed the same trend except for the values obtained for the molecular weight and intrinsic viscosity of lentil amylose. The reason for such results is difficult to explain.

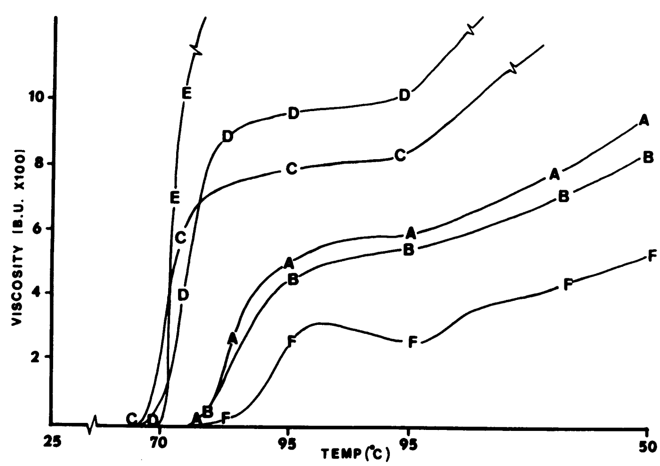

Fig. 3. Brabender amylograms of various legume starches compared with wheat starch. A, navy bean starch; B, pinto bean starch; C, faba bean starch; D, lentil starch; E, mung bean starch; F, wheat starch.

TABLE IV
Amylose Content, Molecular Weight, and Intrinsic Viscosity of Amylose Fraction From Various Legume Starches

Starch Source	Amylose[a] Content (%)	Molecular Weight	Intrinsic Viscosity (η)
Navy bean	22.1	165,000	0.74
Pinto bean	25.8	123,000	0.54
Faba bean	24.0	191,000	1.75
Lentil	20.7	312,000	1.85
Mung bean	19.5	245,000	2.42

[a]Values reported are average of two determinations.

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