Structural Characterization of Legume Starches. I. Studies on Amylose, Amylopectin, and Beta-Limit Dextrins[1]

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

Cereal Chem. 58(6):496-502

ABSTRACT

Nine purified legume starches (from smooth and wrinkled peas; adzuki, garbanzo, mung, red kidney, navy, and faba beans; and green lentil) were fractionated into their amylose and amylopectin components. The fine structures of the isolated starch components and starch β-limit dextrins were investigated using hydrolytic enzymes (pullulanase, β-amylase, and glucoamylase) in conjunction with gel filtration chromatography (Biogel P-10). The unit-chain profiles of the debranched legume amylopectins were characterized by two overlapping distributions with average degrees of polymerization of 45-55 (II-chains) and 14-18 (III-chains). The average chain length for most of these amylopectins ranged from 20 to 26. Wrinkled pea starch contained branched polysaccharide molecules with a chain length greater than that of normal amylopectin. The limiting viscosity numbers of the legume amylose fractions ranged from 136 to 251 ml/g. The incomplete β-amylolysis of the legume amyloses (78.4-89.4%) was due to the presence of a relatively small number of α-(1→6) linkages in the molecule. A hypothesis was developed to explain starch gelatinization and granule rigidity based on the molecular characteristics of the branched polysaccharide fractions of these starches.

The application of enzymatic methods to the structural analysis of amylaceous polysaccharides has provided useful information regarding the fine structure of these α-D-glucans (Marshall 1974). Thus, debranching of amylopectin and glycogen, using purified pullulanase (E.C. 3.2.1.41) and isoamylase (E.C. 3.2.1.68) preparations, combined with gel filtration chromatography has led to the postulation of new models for the molecular structure of these polysaccharides (French 1972, Gunja-Smith et al 1970, Robin et al 1974). Although several cereal and tuber starches have been characterized by these techniques (Atwell et al 1980; French et al 1971; Gunja-Smith et al 1970; Hood and Mercier 1978; Ikawa et al 1978; Lii and Lineback 1977; Mercier 1973; Mercier and Whelan 1970; Robin et al 1974, 1975), no comparative studies have been made on legume starches.

This investigation was conducted to determine the structural characteristics of the components of nine purified legume starches. An attempt was also made to relate these characteristics to gelatinization properties of the starch granule.

MATERIALS AND METHODS

Starch Isolation

The following legume starches were prepared from their seeds by a wet-milling process (Biliaderis et al 1979): smooth pea (*Pisum sativum* L. cv. Trapper), wrinkled pea (*Pisum sativum* L. cv. Venus), adzuki bean (*Phaseolus chrysanthos*), garbanzo bean (*Cicer arietinum*), mung bean (*Vigna radiata*), green lentil (*Lens culinaris*), red kidney bean (*Ph. vulgaris*), navy bean (*Ph. vulgaris*), and faba bean (*Vicia faba* L. cv. Diana). The proximate analysis and other physicochemical characteristics of the purified starches were reported previously (Biliaderis et al 1979, 1980). Waxy corn and potato starch were obtained from a commercial source (A. E. Staley, Inc., Decatur, IL).

Iodine Affinity

Iodine affinities (IA) for the defatted (by hot extraction in 85% aqueous methanol for 48 hr) starches and the amylose and amylopectin fractions were determined by potentiometric titration at 30.0 ± 0.1°C (Schoch 1964). Amylose contents were calculated by assuming that pure amylose has an iodine affinity of 20.0%. The wavelength of maximum absorption (λ_{max}) of the iodine-polysaccharide complexes was determined as described previously (Biliaderis et al 1979).

Gelatinization Midpoint Temperatures

Gelatinization midpoint temperatures were determined according to Schoch and Maywald (1956). The recorded temperatures correspond to loss of birefringence by approximately 50% of the starch granules.

Limiting Viscosity Numbers of the Amyloses

Limiting viscosity numbers (η), of the amyloses dispersed in 1*N* KOH, were obtained with a modified Ubbelohde viscometer according to Greenwood's method (1964). An approximation of the average degree of polymerization ($\overline{dp}$) for each amylose was calculated from the relation $\overline{dp} = 7.4 \times \eta$ (Cowie and Greenwood 1957).

Fractionation of Starches

Two methods were used for starch fractionation. The first was by the dimethyl sulfoxide (DMSO)-thymol granule dispersion and selective precipitation of the amylose fraction (Banks and Greenwood 1967a) and the second by selective aqueous leaching of the glycerol-pretreated granular starches (Montgomery and Senti

[1]National Research Council of Canada Paper 19591. Taken in part from the Ph.D. thesis of C. G. Biliaderis, University of Saskatchewan, 1980.

[2]Biotechnology Section, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon.

[3]Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon.

[4]National Research Council of Canada, Ottawa, Ontario.

0009-0352/81/06049607/$03.00/0

1964). IA was adopted as the criterion by which to assess the purity of the amylose and amylopectin fractions so obtained. Yields of the amylose fractions were determined by expressing the weight of the recovered material as percent of the apparent amylose content of the original starch sample.

Total Carbohydrate by Enzymatic Hydrolysis

Total carbohydrate concentration was determined by enzymatic hydrolysis with glucoamylase (Diazyme L-100, Miles Lab., Inc., Elkhart, IN) at 50°C. The digests were made by mixing aliquots (1–2 ml) of polysaccharide solution (acetate buffer 0.1 *M*, pH 4.8), containing 30–250 μg of α-D-glucan, with 0.5 ml (0.5 IU) of glucoamylase. Glucose was subsequently quantitated by the D-glucose oxidase method (Banks et al 1970), using Statzyme Glucose 500 nm reagent (Worthington Biochemical Corp., Freehold, NJ).

β-Amylolysis

The β-amylolysis experiment was conducted by adding 0.1 volume of 0.1%, w/v, barley β-amylase (Fluka, A.D., Buchs, S.G., Switzerland) in water (8 IU/ml) to 1.0 volume of the buffered (0.05 or 0.1 *M* acetate, pH 4.7) polysaccharide solution (1–2 mg/ml). When the reducing power became constant, the solutions were boiled to inactivate the enzyme and the percent of β-amylolysis was calculated as:

$$\% \beta\text{-amylolysis} = \frac{\text{Reducing capacity (as maltose)}}{\text{Total carbohydrate (as maltose)}} \times 100$$

Reducing sugars were determined according to Dygert et al (1965), and total carbohydrate was measured by enzymatic hydrolysis as described.

The β-limit dextrins of amylopectins and starches were prepared by treating higher concentrations (5–6 mg/ml) of buffered (0.1 *M* acetate or 20%, v/v, dimethyl sulfoxide in 0.1 *M* acetate, pH 4.7) polysaccharide solutions with β-amylase for 48 hr as described. The β-amylolysis of wrinkled pea starch was done with 40%, v/v, DMSO in acetate buffer. The digestion was repeated twice, and the hydrolysate was dialyzed (Spectrapor membrane; molecular weight cutoff: 6,000–8,000; Spectrometrics Co., Los Angeles, CA) against water at 2°C and finally freeze-dried.

Debranching with Pullulanase

Branched polysaccharides, 40 mg, were debranched with 32 IU (0.08 ml) of crystalline pullulanase (Hayashibara Biochemical Lab. Inc., Okayama, Japan) in 5 ml of solution (20% DMSO in 0.1 *M* acetate buffer, pH 5.5) at 37°C for 24 hr (Harada et al 1972, Mercier and Kainuma 1975, Mercier and Whelan 1970). The digest vials were subsequently heated in boiling water for 20 min to inactivate the enzyme. Insoluble material (3–7%), presumably retrograded linear fraction, was removed by centrifugation (10,000 × *g* for 20 min), and the supernatants were analyzed according to Mercier and Whelan (1970) as follows: 1) total carbohydrate concentration by enzymatic hydrolysis, 2) total reducing power by the Nelson's reducing sugars method (1944), 3) percent β-amylolysis, and 4) gel chromatography on Biogel P-10.

The average chain length ($\overline{CL}$) of the branched starch molecules was determined by the following equation (Marshall 1974):

$$\overline{CL} = \frac{\text{Total carbohydrate (as glucose)}}{\text{Reducing capacity after debranching (as glucose)}}$$

Gel Filtration Chromatography

Biogel P-10 (Bio-Rad Laboratory, Richmond, CA) columns (2.6 × 94 cm) were slowly packed using a gel applicator (Pharmacia Ltd., Montreal, Quebec). Debranched polysaccharide solutions (5–7 ml) were applied to the columns and eluted at 22°C with acetate buffer (0.1 *M*, pH 4.8), containing 0.02% sodium azide, by the ascending method at constant flow rate (16 ml/hr). Fractions of 4 ml were collected and assayed enzymatically for total carbohydrate content. The columns were calibrated by fractionating debranched waxy corn amylopectin (80 mg) according to Mercier and Whelan (1970). The $\overline{dp}$ of each eluted fraction was determined by dividing the total carbohydrate concentration by its reducing capacity. The debranched chains of the amylopectins fell into two molecular weight categories, designated chain fractions II and III. The molar ratio of these two chain populations was calculated by the following equation, assuming a symmetrical weight distribution of chains around the peak fractions:

$$\text{Chain molar ratio} = \frac{\text{(Total carbohydrate of III-chains)}}{\text{(Total carbohydrate of II-chains)}} \times \frac{(\overline{dp} \text{ of II-chains})}{(\overline{dp} \text{ of III-chains})}$$

RESULTS AND DISCUSSION

Fractionation of Starches

The DMSO-thymol fractionation scheme (Banks and Greenwood 1967a) provided relatively pure amylopectin fractions for all legume starches except wrinkled pea starch. The IA of these fractions (1.00–1.82) were comparable to the values of 1.00–1.60 reported earlier for several smooth pea amylopectins (Greenwood and Thomson 1962) but significantly higher than those (0.4–0.6) of cereal amylopectins isolated by the same method (Banks and Greenwood 1967a). Although we achieved an excellent fractionation for potato starch (IA of 18.98 and 0.32) for amylose and amylopectin, respectively), the IA values for the thymol-amyloses recovered from the legume species indicated that, even after two recrystallizations with butan-1-ol, these fractions were contaminated with material resembling amylopectin (IA of 9.5–18.0). Similar trends in the purity of both the amylose and amylopectin fractions were also obtained by employing the dispersion conditions of Schoch (1957). Therefore, in order to prepare amylose fractions of greater purity, we used the glycerol pretreatment and aqueous leaching procedure developed by Montgomery and Senti (1964). Amylose fractions (IA of 18.48–20.00) with yields ranging from 55 to 65% of the apparent amylose contents of the corresponding starches were obtained by this method. However, the granule residues (amylopectin fractions) were less pure than the amylopectins from the dispersion experiments. Therefore, thymol-amylopectins and leached amyloses were used for subsequent studies on structural characterization of the legume starch components.

In contrast to the other legume starch fractions, the thymol-amylopectin fraction of wrinkled pea showed a very high capacity to bind iodine (IA 5.26). We suggest that this fraction was extensively contaminated with amylose and/or branched glucan molecules of longer chain length than those of a typical amylopectin. Greenwood and Thomson (1962) also experienced difficulties in obtaining amylopectins of low IA from this starch; their minimum reported value was 2.7. Their studies further indicated that this abnormality was due to the presence of a linear short-chain material, which, because of its low molecular weight, could not complex with thymol and therefore contaminated the amylopectin fraction.

Characterization of Legume Starch Components

Preliminary experiments using the whole starch samples indicated that extensive retrogradation took place during debranching, presumably because of the high amylose content. We therefore restricted the structural studies to isolated starch fractions. We have assumed that such fractions would be representative of the total amylopectin, but we also recognize that the validity of this assumption is open to question because these fractions were recovered in less than 100% yield. The elution profiles of the debranched amylopectins on a Biogel P-10 column are shown in Figs. 1–3. The $\overline{CL}$, IA, percent β-amylolysis after debranching, and percent distribution among the major peaks are given in Table I.

The unit-chain distribution of each debranched sample revealed two major populations of differing chain length. The longer chains (II-chains) consisted of linear material with a $\overline{dp}$ of 45–55, whereas

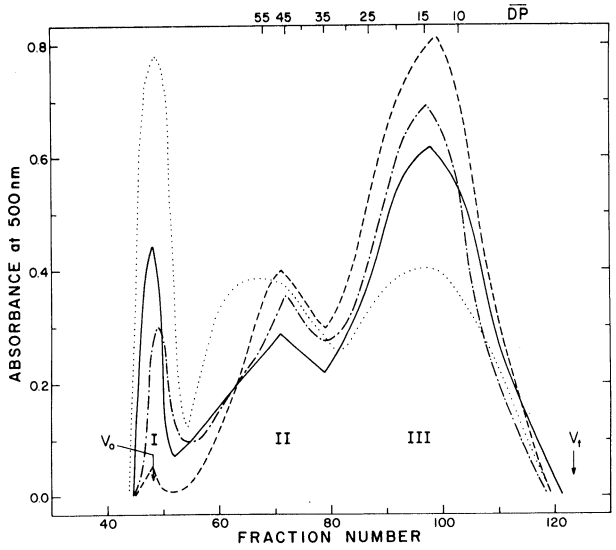

Fig. 1. Elution profiles of pullulanase-debranched amylopectins from waxy corn (----), red kidney bean (—), garbanzo bean (-·-·), and wrinkled pea (····). $\overline{DP}$ = average degree of polymerization, V_o = void volume, V_t = total volume, I–III = chain fractions.

the shorter chains (III-chains) had a $\overline{dp}$ of 14–18. These results are in agreement with previous studies on waxy corn (Gunja-Smith et al 1970, Mercier and Whelan 1970), tapioca starch (Hood and Mercier 1978), potato starch (Robin et al 1974), corn and wheat starches (Robin et al 1975), and wheat amylopectins (Lii and Lineback 1977). The presence of the II-chain and III-chain populations leads to the conclusion that the amylopectin structure in these legume starches can be described by the revised Meyer model proposed by Gunja-Smith et al (1970) as well as by the cluster models of French (1972) and Robin et al (1974). However, Marshall (1974) has pointed out the lack of experimental evidence to establish whether both chain populations arise from the same polysaccharide molecule. He suggested that the bimodal distribution of chains might arise from the debranching of two distinct populations of polysaccharide. Atwell et al (1980) have recently investigated the above hypothesis by studying the β-limit dextrin of wheat amylopectin. They showed that the structural characteristics of the overall amylopectin population were uniform and concluded that the bimodal distribution of chains came from two populations that existed within the same macromolecule.

A third minor peak (fraction I) was also observed in all chromatograms at the void volume. The amount of this fraction was considerably greater for some of the debranched legume amylopectins (3.1–20.2%) than for the waxy corn (0.5–1.8%). The

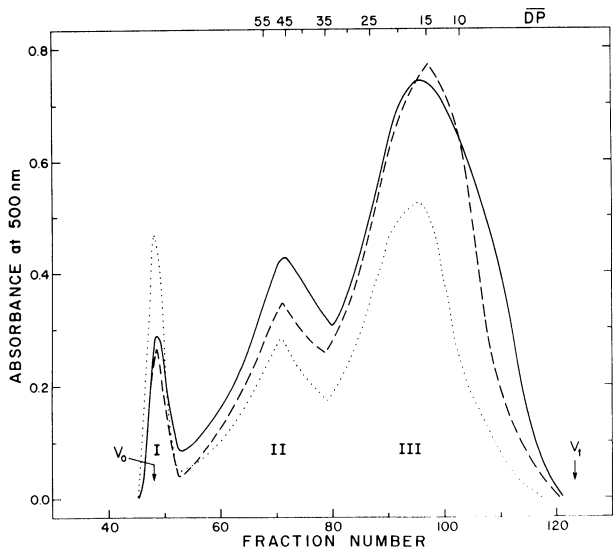

Fig. 2. Elution profiles of pullulanase-debranched amylopectins from mung bean (—), lentil (----), and smooth pea (····). $\overline{DP}$ = average degree of polymerization, V_o = void volume, V_t = total volume, I–III = chain fractions.

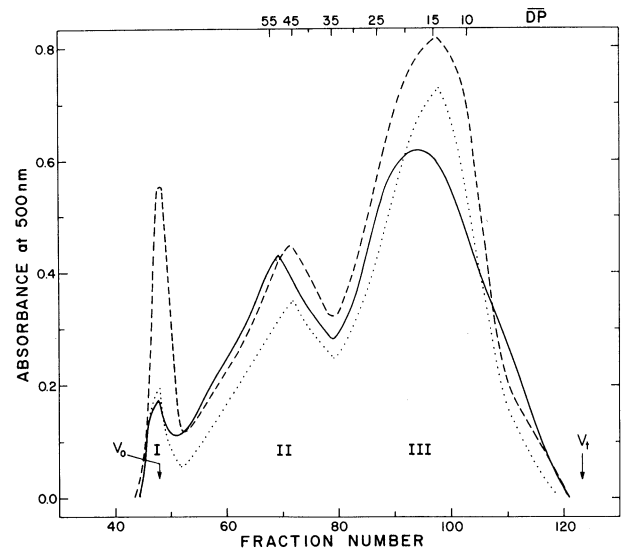

Fig. 3. Elution profiles of pullulanase-debranched amylopectins from navy bean (----), adzuki bean (—), and faba bean (····). $\overline{DP}$ = average degree of polymerization, V_o = void volume, V_t = total volume, I–III = chain fractions.

TABLE I
Characteristics of Isolated[a] Legume Amylopectins

Sample	Iodine Affinity	Average Chain Length	P_β[b] (%)	Distribution of Chains (%)			Molar Ratio of III-chains to II-chains
				I	II	III	
Adzuki bean	1.00	26	96	4.1	31.4	64.5	6.3 ± 0.4
Adzuki bean[c]	···	25	97	5.4	30.0	64.6	···
Garbanzo bean	1.51	22	96	7.0	25.0	67.9	8.0
Smooth pea	1.28	22	97	8.6	25.0	66.4	7.7 ± 0.3
Smooth pea[c]	···	22	96	7.6	24.3	68.1	···
Red kidney bean	1.82	20	96	9.2	23.1	67.7	9.4
Wrinkled pea	5.26	34	98	20.2	35.2	44.6	4.2
Lentil	1.17	20	97	5.1	23.7	71.2	9.6 ± 0.1
Lentil[c]	···	20	96	5.4	23.4	71.2	···
Navy bean	1.30	22	97	8.0	27.5	64.5	7.5
Faba bean	1.00	21	98	3.1	26.5	70.4	8.9
Mung bean	1.15	23	96	4.8	26.8	68.4	7.2
Waxy corn	0.00	19	99	0.5	23.9	75.6	10.7 ± 0.2
Waxy corn[c]	···	19	97	1.8	23.5	74.7	···

[a] Method of Banks and Greenwood (1967a).
[b] Percent β-amylolysis of pullulanase-debranched amylopectins.
[c] Debranching experiment repeated.

most plausible explanation for the presence of this fraction is that it represents incompletely debranched polysaccharide and/or an amylose type of material (Abdullah et al 1966, Gunja-Smith et al 1970, Robin et al 1974). However, the high percent of β-amylolysis achieved for all the debranched digests (96–99%, Table I) strongly suggests that fraction I consisted mainly of an amylose type of material. This linear fraction may be a contaminant of the isolated amylopectins or it may be an integral part of the branched molecules that was liberated during debranching.

The $\overline{CL}$ for these amylopectins ranged from 20 (lentil, red kidney bean) to 26 (adzuki bean) and was greater than that (19) of waxy corn (Table I). These values are comparable to or slightly higher than those reported for waxy corn (20), waxy sorghum (20), wheat (17–20), triticale (15), rye (26), and potato (23) amylopectins (Lee and Whelan 1966, Lii and Lineback 1977). The chain molar ratios ranged from 4.2 to 9.6 for all legume amylopectins and were lower than that (10.7) for waxy corn.

The elution profiles of the thymol-amylopectin fractions showed that wrinkled peas (Fig. 1) had a higher carbohydrate content in fraction II than did the other legumes. Consequently, wrinkled pea amylopectin had the highest $\overline{CL}$ (34) and the lowest chain molar ratio (4.2). Early structural studies by Potter et al (1953) and Greenwood and Thomson (1962) also indicated that the amylopectin component from wrinkled peas had a much higher $\overline{CL}$ (36) than that of the amylopectin from smooth peas (26). However, on ultracentrifugation (Greenwood and Thomson 1962), the wrinkled pea amylopectin was found to contain a short linear material (for which β-amylolysis was 100%), which accounted for the apparently longer $\overline{CL}$ of the amylopectin fraction. The same observation was also made for the amylopectin fractions isolated from high-amylose corn starches (Banks et al 1974). In view of these results, we thought that some of the chains in fraction II might be present as contaminants in the sample before debranching. However, chromatography of the undebranched sample revealed only one peak at the void volume of the column. Therefore, we concluded that fractions II and III both arose only during debranching. Ikawa et al (1978) have reported similar chromatographic profiles for debranched high-amylose corn starches, in which the carbohydrate content of fraction II was equal to or even higher than that of fraction III.

The properties of the isolated amylose components of the legume starches are presented in Table II. The η ranged from 136 (wrinkled pea) to 251 (mung bean). The corresponding $\overline{dp}$ values were 1,000–1,900 glucose units. In contrast to the legume amyloses, potato amylose had a much higher η (430) and a $\overline{dp}$ of 3,200. The range in η for legume amyloses is lower than the values reported for cereal (wheat, 330; rye, 335; barley, 355; and oat, 435) and potato (410) amyloses isolated by granule dispersion experiments (Banks and Greenwood 1967b). One might argue that this difference in molecular size between legume and cereal amyloses is due solely to the fact that the former have been isolated by aqueous leaching experiments at yields of 55–65% (Table II) and, therefore, might not be representative samples of the linear starch fractions. However, wrinkled and smooth pea amyloses isolated by granule dispersion experiments have been reported (Greenwood and Thomson 1962) to have η of 140 and 180, respectively, which are comparable to the results of the present study. Therefore, legume amyloses appear to have a lower molecular weight than do those of cereal and potato.

The β-amylolysis limits of the legume amyloses ranged from 78.4 (mung bean) to 89.4% (lentil) and are higher than the values reported for cereal (72–78%) and potato (76%) amyloses (Banks and Greenwood 1967b). However, the β-amylolysis limits of wrinkled pea (82%) and smooth pea (81%) amyloses as found by Greenwood and Thomson (1962) are comparable to those obtained in the present study. The incomplete β-amylolysis of amylose has

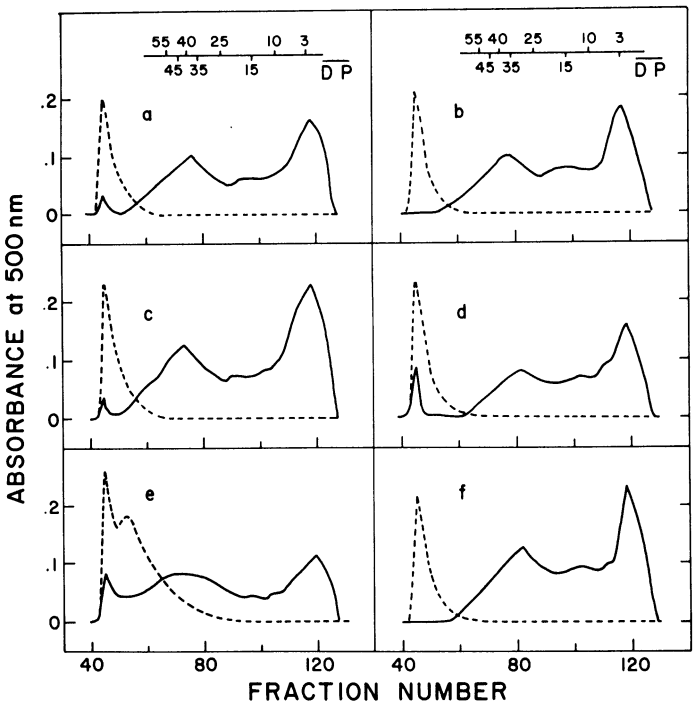

Fig. 4. Elution profiles of β-limit dextrins (····) and their products after pullulanase-debranching (—). **a,** smooth pea starch; **b,** smooth pea amylopectin; **c,** adzuki bean starch; **d,** lentil starch; **e,** wrinkled pea starch; **f,** waxy corn β-limit dextrins. $\overline{DP}$ = degree of polymerization.

TABLE II
Characteristics of Isolated[a] Legume Amyloses

Sample	Iodine Affinity	Yield[b] (%)	η[c] (ml/g)	Average Degree of Polymerization (glucose units)	β-Amylolysis (%)	β + P[d] (%)
Adzuki bean	19.49	64.2	220	1,600	86.8 ± 1.8	102.8 ± 2.1
Garbanzo bean	18.88	60.3	174	1,300	85.9 ± 1.2	···
Smooth pea	18.84	62.8	194	1,400	85.0 ± 1.9	99.6 ± 1.9
Red kidney bean	20.00	···	180	1,300	88.8 ± 1.2	···
Wrinkled pea	19.82	55.4	136	1,000	86.5 ± 0.4	100.2 ± 1.5
Lentil	19.62	65.1	188	1,400	89.4 ± 1.1	101.3 ± 2.2
Navy bean	18.48	···	174	1,300	86.2 ± 1.1	···
Mung bean	19.43	···	251	1,900	78.4 ± 1.2	···
Faba bean	19.61	61.4	188	1,400	85.6 ± 1.5	···
Potato[e]	18.98	···	430	3,200	78.0 ± 1.3	···
Wrinkled pea[e]	17.99	···	144	1,100	84.7 ± 1.1	···

[a] Method of Montgomery and and Senti (1964).
[b] Expressed as percent of the apparent amylose content of starch.
[c] Limiting viscosity number.
[d] Concurrent action of β-amylase and pullulanase (P).
[e] Method of Banks and Greenwood (1967a).

been attributed to the presence of α-(1→6) linkages (Banks and Greenwood 1967b, Kjolberg and Manners 1963). Concurrent treatment of several legume amyloses with β-amylase and pullulanase (Table II) resulted in complete conversion of these polysaccharides into maltose. This provided additional evidence that α-(1→6) linkages constitute the only barrier to the action of β-amylase on amylose.

Characterization of Starch β-Limit Dextrins

The amylose retrogradation problem encountered during debranching and gel chromatography of the whole starch can be overcome by working with the corresponding β-limit dextrins. Therefore, an alternative approach to the elucidation of the structural characteristics of the legume starches was attempted by debranching their β-limit dextrins. The action of β-amylase on dispersed starch completely converts linear amylose and the outer chains of amylopectin into maltose and maltotriose, leading to a β-limit dextrin (Marshall 1974, Mercier 1973). Consequently, differences in elution profiles of debranched dextrins will indicate differences in the internal structure of the branched molecules. This study was restricted to the β-limit dextrins of waxy corn, smooth pea, wrinkled pea, adzuki bean, and lentil starches. The β-limit dextrin of smooth pea amylopectin was also used for comparison. The elution profiles of the β-limit dextrins and their debranched products are shown in Fig. 4. Their structural characteristics are presented in Table III.

TABLE III
Characteristics of β-Limit Dextrins

Sample	Average Chain Length	λ_{max}[a] (nm)	P_β[b] (%)
Starch			
Adzuki bean	12.1	530	105.4
Smooth pea	11.2	530	103.6
Wrinkled pea	21.3	555	98.9
Lentil	10.5	528	108.2
Waxy corn	9.7	525	110.1
Amylopectin			
Smooth pea	10.2	525	104.9

[a] Wavelength of maximum absorption.
[b] Percent β-amylolysis of pullulanase-debranched β-limit dextrin.

All β-limit dextrins except that of wrinkled pea starch were excluded from the gel. The latter exhibited an additional peak next to the gel-excluded material (Fig. 4e). Thus, the β-limit dextrin of this starch appears to contain an appreciable amount of low molecular weight branched material. This is consistent with the presence in wrinkled pea starch of a polysaccharide having molecular weight intermediate to those of typical amylose and amylopectin fractions (Biliaderis et al 1979).

The elution patterns of the debranched β-limit dextrins were different from those of the corresponding amylopectins shown in Figs. 1–3. The excluded material (Fig. 4a and c–e), which was verified as linear by a further β-amylolysis, must be derived from the slightly branched amylose fraction of these starches. The presence of limited branching in a fraction of the amylose has been shown for several isolated tuber and cereal amyloses (Banks and Greenwood 1967b). A peak with a $\overline{dp}$ of 30–40 replaced the peak with a $\overline{dp}$ of 45–55 observed in the isolated, debranched amylopectins. For some of the debranched samples, minor peaks also appeared at $\overline{dp}$ of 12–20 and 7. These peaks represent internal chains of the amylopectin fraction of starch that were only partially susceptible to β-amylase treatment. The last peak, with a $\overline{dp}$ of 2.5, contains maltose and maltotriose derived from the external stub branched of the β-limit dextrins.

The profiles of the debranched β-limit dextrins from smooth pea starch and smooth pea amylopectin (Fig. 4a and b) showed very little difference. The only obvious distinction between the two patterns was the presence of the gel-excluded material in that of the debranched starch β-limit dextrin. This presumably corresponds to the branched amylose fraction of the starch. Both samples had almost identical $\overline{CL}$ and λ_{max} of the iodine-polysaccharide complexes. These results suggest that the isolated smooth pea amylopectin was a representative sample of the entire branched polysaccharide fraction of this starch in terms of its internal fine structure.

Wrinkled pea starch β-limit dextrin showed a much higher

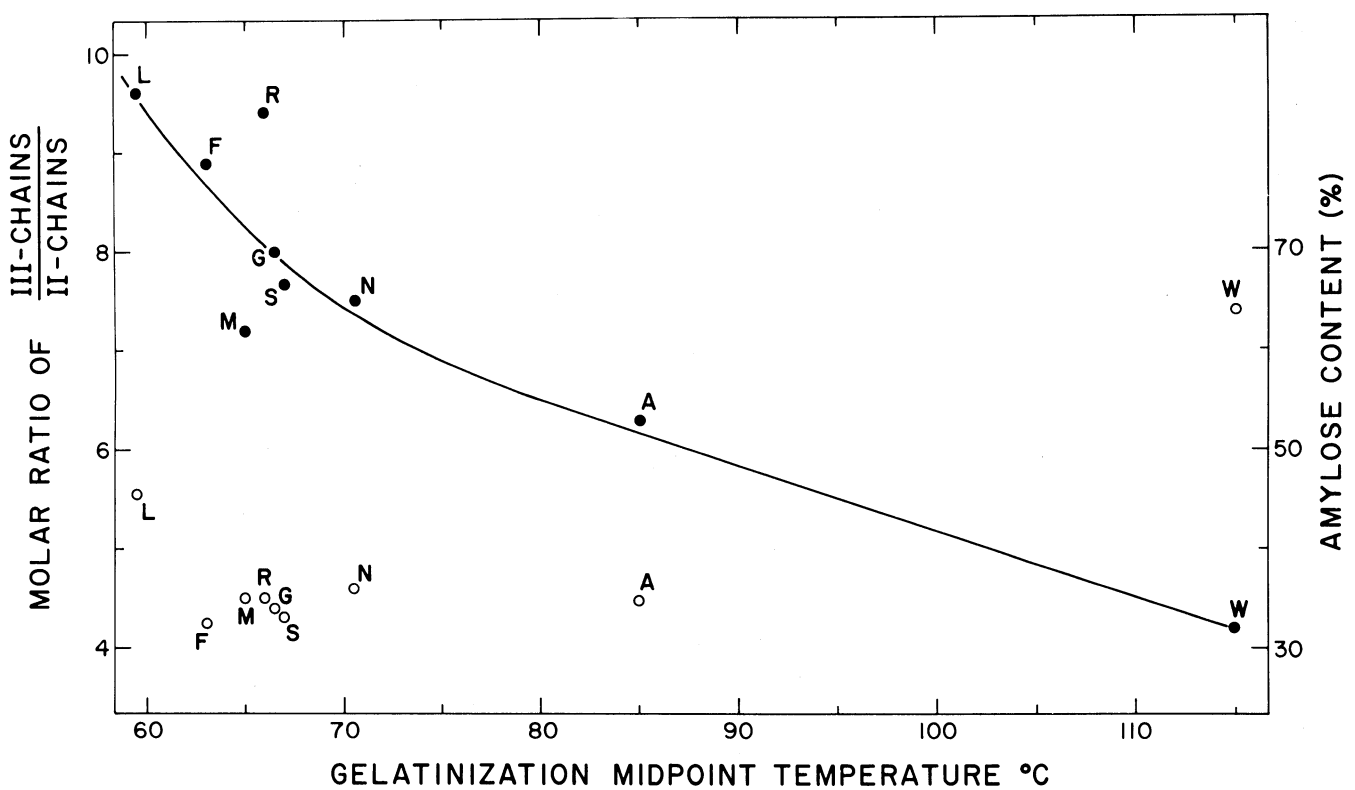

Fig. 5. Correlation of gelatinization midpoint temperature with the molar ratio of III-chains to II-chains (•) and amylose content (o). **L** = lentil, **F** = faba bean, **M** = mung bean, **R** = red kidney bean, **G** = garbanzo bean, **S** = smooth pea, **N** = navy bean, **A** = adzuki bean, **W** = wrinkled pea starches.

proportion of longer chains ($\overline{dp}$ greater than 25) than did those of the other starches (Fig. 4). Consequently, it had a much higher CL (21.3) and exhibited a λ_{max} at longer wavelengths (555 nm) than did the other starch dextrins. Mercier (1973) has reported a similar chromatographic profile (on Sephadex G-50) for the debranched β-limit dextrin of high amylose corn starch, in which most of the liberated chains had a $\overline{dp}$ greater than 30. These results, combined with the debranching data of the wrinkled pea amylopectin fraction (Fig. 1, Table I), strongly suggest that the branched polysaccharide fraction of wrinkled pea starch has its own unique structure, with longer chains than those found in other legume amylopectins. Greenwood and Thomson (1962) have attributed the long $\overline{CL}$ and high IA of this fraction to a linear short chain contaminant. However, that conclusion is not supported by the present experimental results. If the Greenwood and Thomson hypothesis were valid, the chromatographic profiles of debranched wrinkled pea amylopectin following preliminary treatment with β-amylase would be similar to those of the other starches. The chromatograms of Fig. 4 clearly indicate that this was not the case. More work is necessary to establish whether wrinkled pea amylopectin is homogeneous or whether it is a mixture of long $\overline{CL}$ molecules and normal branched molecules.

Relationship Between Gelatinization Temperature and Molecular Characteristics of Legume Starches

Granular starch is a semicrystalline polymer. Several reseachers (French 1972; Robin et al 1974, 1975; Watanabe and French 1980) have proposed that, for those starches containing appreciable quantities of amylopectin, the amylopectin chain clusters constitute the crystalline entities. In synthetic semicrystalline polymers, the extent of crystallinity and molecular organization depend upon various factors operative during the crystallization process (Cowie 1973). For example, an increase in chain branching is considered to lower the percentage of crystallinity, with a resulting decrease in melting temperature. The legume starches that we have studied are analogous to synthetic polymers in that a relationship exists between gelatinization temperature and the degree of branching of the branched chain components. Figure 5 is a plot of gelatinization mid-point temperatures vs the chain molar ratio. This ratio may be used as an index of the extent of chain branching (the higher the ratio, the higher the degree of branching) because, within this group of samples, the peak fractions of II-chains and III-chains were of similar $\overline{dp}$. The amylose contents are also plotted in Fig. 5 because resistance to gelatinization of starches with high amylose content is generally attributed to their high levels of amylose (Leach 1965).

The gelatinization temperature obviously correlates much more closely with the chain molar ratio than it does with the amylose content. Therefore, we suggest that chain branching may influence the organization of the starch granule. Although the presence of α-(1$\rightarrow$6) bonds perhaps induces the crystallization process by providing the initiation sites for ready formation of stable double helices (Borovsky et al 1979, Yamaguchi et al 1979), extensive branching may be detrimental to the overall crystallinity and rigidity of the granule in two ways. First, by analogy with synthetic polymers, chain branching will affect the organization of the newly apposed branched starch molecules. Second, chain branching may impose constraints on the molecular conformation of amylose and therefore limit the intramolecular and intermolecular hydrogen bonding between the apposed amylose molecules. However, the latter process would also be affected by the amount and the molecular size of the linear fraction, both of which influence its aggregation state (Banks et al 1974). More experimental evidence is obviously required to test this postulate. The gelatinization phenomenon is extremely complex; in addition to the structure of the granule and its components, other factors such as granule size and phosphorus and lipid contents are also important (Banks and Greenwood 1975).

LITERATURE CITED

ABDULLAH, M., CATLEY, B. J., LEE, E. Y. C., ROBYT, K., WALLENFELS, K., and WHELAN, W. J. 1966. The mechanism of carbohydrase action. XI. Pullulanase, an enzyme specific for the hydrolysis of α-(1$\rightarrow$6) bonds in amylaceous oligo- and polysaccharides. Cereal Chem. 43:111.

ATWELL, W. A., HOSENEY, R. C., and LINEBACK, D. R. 1980. Debranching of wheat amylopectin. Cereal Chem. 57:12.

BANKS, W., and GREENWOOD, C. T. 1967a. The fractionation of laboratory-isolated cereal starches using dimethyl sulfoxide. Staerke 19:394.

BANKS, W., and GREENWOOD, C. T. 1967b. Physicochemical studies on starches. The incomplete β-amylolysis of amylose. Staerke 19:197.

BANKS, W., and GREENWOOD, C. T. 1975. Starch and Its Components. Halsted Press: New York.

BANKS, W., GREENWOOD, C. T., and MUIR, D. D. 1970. The characterization of starch and its components. II. The semi-micro estimation of the starch contents of cereal grains and related materials. Staerke 22:105.

BANKS, W., GREENWOOD, C. T., and MUIR, D. D. 1974. Studies on starches of high amylose content. Staerke 26:289.

BILIADERIS, C. G., GRANT, D. R., and VOSE, J. R. 1979. Molecular weight distributions of legume starches by gel chromatography. Cereal Chem. 56:475.

BILIADERIS, C. G., MAURICE, T. J., and VOSE, J. R. 1980. Starch gelatinization phenomena studied by differential scanning calorimetry. J. Food Sci. 45:1669.

BOROVSKY, D., SMITH, E. E., WHELAN, W. J., FRENCH, D., and KIKUMOTO, S. 1979. The mechanism of Q-enzyme action and its influence on the structure of amylopectin. Arch. Biochem. Biophys. 198:627.

COWIE, J. M. G. 1973. Polymers: Chemistry and Physics of Modern Materials. International Textbook Ltd.: Aylesbury. Bucks, England.

COWIE, J. M. G., and GREENWOOD, C. T. 1957. Physicochemical studies on starches. VI. Aqueous leaching and fractionation of potato starch. J. Chem. Soc. 2862.

DYGERT, S., LI, L. H., FLORIDA, D., and THOMA, J. A. 1965. Determination of reducing sugar with improved precision. Anal. Biochem. 13:367.

FRENCH, D. 1972. Fine structure of starch and its relationship to the organization of the granules. J. Jpn. Soc. Starch Sci. 19:8.

FRENCH, D., KAINUMA, K., and WATANABE, T. 1971. Nageli amylodextrin: Hydrolysis of starch granules at room temperature by 15% sulfuric acid. Cereal Sci. Today 56:299.

GREENWOOD, C. T. 1964. Viscosity-molecular weight relations. Page 179 in: Whistler, R. L., ed. Methods in Carbohydrate Chemistry, Vol. 4. Academic Press: New York.

GREENWOOD, C. T., and THOMSON, J. 1962. Studies on the biosynthesis of starch granules. Biochem. J. 82:156.

GUNJA-SMITH, Z., MARSHALL, J. J., MERCIER, C., SMITH, E. E., and WHELAN, W. J. 1970. A revision of the Meyer-Bernfeld model of amylopectin and glycogen. FEBS Lett. 12:101.

HARADA, T., MISAKI, A., HIROSHI, A., YOKOBAYASHI, K., and SUGIMOTO, K. 1972. Characterization of *Pseudomonas* isoamylase by its action on amylopectin and glycogen: Comparison with *Aerobacter* pullulanase. Biochem. Acta 268:497.

HOOD, L. F., and MERCIER, C. 1978. Molecular structure of unmodified and chemically modified manioc starches. Carbohydr. Res. 61:53.

IKAWA, Y., GLOVER, D. V., SUGIMOTO, Y., and FUWA, H. 1978. Amylose percentage and distribution of unit chain-length of maize starches having a specific genetic background. Carbohydr. Res. 61:211.

KJOLBERG, O., and MANNERS, D. J. 1963. The action of isoamylase on amylose. Biochem. J. 86:258.

LEACH, H. W. 1965. Gelatinization of starch. Page 289 in: Whistler, R. L., and Paschall, E. F., eds. Starch Chemistry and Technology, Vol. 1. Academic Press: New York.

LEE, E. Y. C., and WHELAN, W. J. 1966. Enzymic methods for the micro-determination of glycogen and amylopectin, and their unit-chain lengths. Arch. Biochem. Biophys. 116:162.

LII, C. Y., and LINEBACK, D. R. 1977. Characterization and comparison of cereal starches. Cereal Chem. 54:138.

MARSHALL, J. J. 1974. Application of enzymic methods to the structural analysis of polysaccharides. Adv. Carbohydr. Chem. Biochem. 30:257.

MERCIER, C. 1973. The fine structure of corn starches of various amylose percentage: Waxy, normal and amylomaize. Staerke 25:78.

MERCIER, C., and KAINUMA, K. 1975. Enzymatic debranching of starches from maize of various genotypes in high concentration of dimethylsulfoxide. Staerke 27:289.

MERCIER, C., and WHELAN, W. J. 1970. The fine structure of glycogen from Type IV glycogen-storage disease. Eur. J. Biochem. 16:579.

MONTGOMERY, E. M., and SENTI, F. R. 1964. Separation of amylose from amylopectin of starch by an extraction-sedimentation procedure. J. Polymer Sci. 28:1.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375.
POTTER, A. L., SILVEIRA, V., McCREADY, R. M., and OWENS, H. S. 1953. Fractionation of starches from smooth and wrinkled seeded peas. Molecular weights, end-group assays and iodine affinities of the fractions. J. Am. Chem. Soc. 75:1335.
ROBIN, J. P., MERCIER, C., CHARBONNIERE, R., and GUILBOT, A. 1974. Lintnerized starches. Gel filtration and enzymatic studies of insoluble residue from prolonged acid treatment of potato starch. Cereal Chem. 51:389.
ROBIN, J. P., MERCIER, C., DUPRAT, F., CHARBONNIERE, R., and GUILBOT, A. 1975. Amidons lintnerises. Etudes chromatographique et enzymatique des chlorhydrique d'amidons de cereals, en particulier de mais cireaux. Staerke 27:36.
SCHOCH, T. J. 1957. Preparation of starch and starch fractions. Page 5 in: Colowick, S. P., and Kaplan, N. O., eds. Methods in Enzymology, Vol. 3. Academic Press: New York.
SCHOCH, T. J. 1964. Iodometric determination of amylose. Page 157 in: Whistler, R. L., ed. Methods in Carbohydrate Chemistry, Vol. 4. Academic Press: New York.
SCHOCH, T. J., and MAYWALD, E. C. 1956. Microscopic examination of modified starches. Anal. Chem. 28:382.
YAMAGUCHI, M., KAINUMA, K., and FRENCH, D. 1979. Electron microscopic observations of waxy maize starch. J. Ultrastruct. Res. 69:249.
WATANABE, T., and FRENCH, D. 1980. Structural features of Naegeli amylodextrin as indicated by enzymic degradation. Carbohydr. Res. 84:115.

[Received August 4, 1980. Accepted March 23, 1981]