

# Structural Characterization of Legume Starches.

## II. Studies on Acid-Treated Starches<sup>1</sup>

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### ABSTRACT

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Several purified starches (from smooth and wrinkled peas; adzuki, mung, and red kidney beans; green lentil; and corn) were hydrolyzed with 2.2*N* HCl at 35°C in a heterogeneous reaction mixture. Starch granules were eroded in two distinct stages, ie, a relatively fast hydrolysis of the amorphous or gel phase and a slower degradation of the starch crystallites. The hydrolysis rate for legume starches was slower than that for corn starch.

The mode of degradation of the chains of the amylopectin molecules was consistent with a cluster model. Debranching studies with pullulanase suggested that amylopectin constituted the main component of the starch crystallites in smooth pea, lentil, and adzuki bean starches, whereas short linear chains dominated the crystalline regions of wrinkled pea starch.

During treatment of starch granules with mineral acids, at temperatures below the gelatinization temperature, the amorphous regions are more rapidly hydrolyzed than are the crystalline areas (BeMiller 1965). Structural studies on acid-treated cereal and potato starches (Robin et al 1974, 1975) have provided useful information regarding the nature of the crystalline regions in the granule and have supported the cluster model (French 1972) for the molecular structure of amylopectin. Other studies (Evers and Juliano 1976, Maningat and Juliano 1979) have indicated that the susceptibility of rice starch to acid degradation is inversely correlated with both the amylose content and the gelatinization temperature. Our structural studies on the amylopectin fractions of several legume starches (Biliaderis et al 1981) have suggested that granule resistance to gelatinization may be influenced by the chain branching of the newly apposed branched starch molecules.

In view of these experimental findings and in anticipation that granule resistance to acid hydrolysis might correlate with other physical and chemical characteristics of starch, acid degradation studies were conducted with several legume starches. Structural

characterization of some of the acid-treated starches was also made. The results are discussed in the light of the current concepts of amylopectin structure and starch granule organization.

### MATERIALS AND METHODS

#### Starch

Corn starch was prepared according to Adkins and Greenwood (1966). The following legume starches were prepared and purified as previously described (Biliaderis et al 1979): smooth pea (*Pisum sativum* L. cv. Trapper), wrinkled pea (*Pisum sativum* L. cv. Venus), adzuki bean (*Phaseolus chrysanthos*), mung bean (*Vigna radiata*), red kidney bean (*Ph. vulgaris*) and green lentil (*Lens culinaris*). The proximate analysis and other physicochemical characteristics of these starches have been reported elsewhere (Biliaderis et al 1979, 1980).

Acid-modified starches were prepared by immersing granules (1.0 g/40 ml, in duplicate) in 2.2*N* HCl at 35°C. The starch slurries were shaken by hand daily to resuspend the starch granules. After various times, the insoluble residues were recovered on a sintered glass filter and then washed repeatedly with deionized water and ethanol. Each residue was finally dried to 10% moisture under vacuum at 20°C over P<sub>2</sub>O<sub>5</sub>. The extent of hydrolysis was determined by measuring the total carbohydrate in the filtrate (Dubois et al 1956), expressed as percent of initial starch. Kinetics of linterization are presented according to Robin et al (1974) by plotting log<sub>10</sub> (100/100-x) vs time (t), where x is the percent of hydrolyzed starch. Linear regression analysis showed a good fit of the experimental data for the first stage of the hydrolysis, with a first order type of process.

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Consequently, the apparent first order rate constants<sup>5</sup> were calculated using the equation:

$$\kappa = \frac{2.303}{t} \log_{10} \frac{100}{100-x}$$

in which  $\kappa$  is the initial hydrolysis rate constant.

### Chemical Analysis

Amylose contents, wavelength of maximum absorption ( $\lambda_{max}$ ) of the iodine-polysaccharide complexes, average gelatinization temperatures, and structural characteristics of the starches were determined as previously described (Biliaderis et al 1981). The branching pattern in legume amylopectins was determined by enzymatic debranching with pullulanase and was expressed as the ratio of short chains, those with a degree of polymerization (dp) of 14–18 glucose units, to long chains (dp of 45–55) as revealed by Biogel P-10 chromatography (Biliaderis et al 1981).

X-ray diffraction patterns of the starches were determined with a Philips x-ray diffractometer. Aqueous starch slurries were applied uniformly as thin layers on microscope slides and equilibrated at 90% rh for 24 hr. The operating conditions for the diffractometer were: copper  $K_{\alpha}$  radiation; high tension voltage, 35 KV; current, 16 mA; chart speed, 5mm/min; scanning angular velocity,  $1^{\circ}\theta/\text{min}$ ; time constant, 8 sec; and range scale, 400 cps.

Average starch granule sizes were determined by measuring 100 granules on photomicrographs ( $\times 300$  magnification) taken with a Carl Zeiss research microscope.

## RESULTS AND DISCUSSION

### Granular Susceptibility to Acid Hydrolysis

The solubilization profiles for some of the starches are presented in Fig. 1. The two-stage hydrolysis pattern was quite obvious in all cases. A relatively fast hydrolysis rate during the first eight days followed by a slower rate between 8 and 11 days has been reported for corn, waxy corn, wheat, potato, and rice starches (Maningat and Juliano 1979; Robin et al 1974, 1975). When the hydrolysis data were plotted as  $\log_{10} (100/100-x)$  (Robin et al 1974), the two-stage process was again evident (Fig. 2). The faster stage corresponds to the hydrolysis of the more amorphous parts of the starch granule. During the second stage, the crystalline material is slowly degraded (Kainuma and French 1971, Robin et al 1974). This is analogous to the phenomenon observed with cellulose and a number of semicrystalline synthetic polymers. Hydrolytic action in these materials occurs most rapidly in the disordered regions, whereas the crystalline areas are more resistant (Banks and Greenwood 1975).

To account for the slower hydrolysis rate of the crystalline parts of the starch granule, several hypotheses have been proposed (BeMiller 1965, Kainuma and French 1971). First, the dense packing of starch chains within the starch crystallites does not readily allow the penetration of  $H_3O^+$  into these regions. Second, acid hydrolysis of a glucosidic bond may require a change in conformation for the glucose unit, from chair to half-chair. Obviously, if the hydrolyzed bond exists within a crystallite, this change in conformation would require a high energy of activation and Sarko 1978a, 1978b), all glucosidic oxygens are buried in structures among the chains in starch crystallites (French 1972; Wu and Sarko 1978a, and 1978b), all glucosidic oxygens are buried in the interior of the double helix and are, therefore, far less accessible to acid attack.

The hydrolysis rate constants of the starches, calculated from plots  $\log_{10} (100/100-x)$  vs time, are presented in Table i. The  $\kappa$ , corresponding to the amorphous parts of the granule, showed considerable differences among the various starches. All the legume starches proved to be more resistant to acid hydrolysis

( $\kappa = 4.53-14.62 \times 10^{-2}$ , in days<sup>-1</sup>) than was corn starch, which was used as a control ( $\kappa = 19.22 \times 10^{-2}$ , in days<sup>-1</sup>). These results are in agreement with the kinetic data on starch solubilization (legume vs corn starch) with dimethyl sulfoxide and  $\alpha$ -amylase obtained by Rosenthal and Nakamura (1972). Differences in granular size

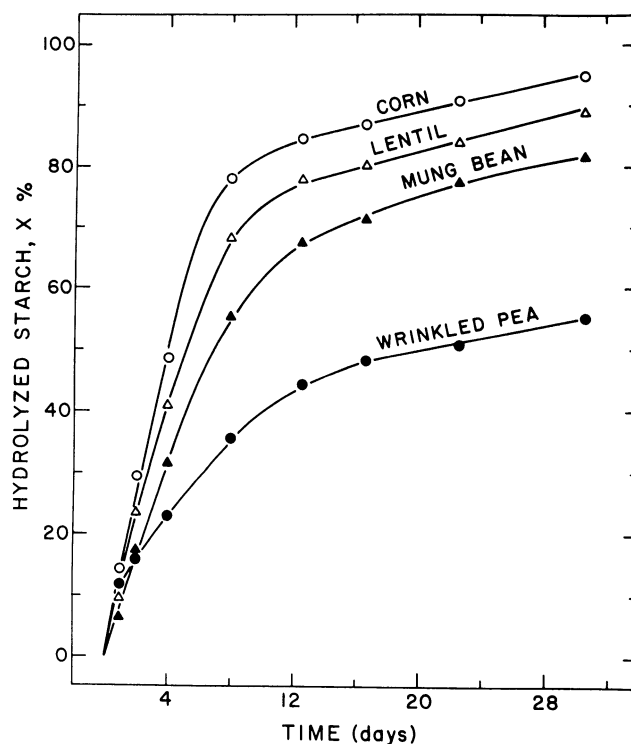


Fig. 1. Heterogeneous hydrolysis of starches in 2.2N HCl at 35°C.

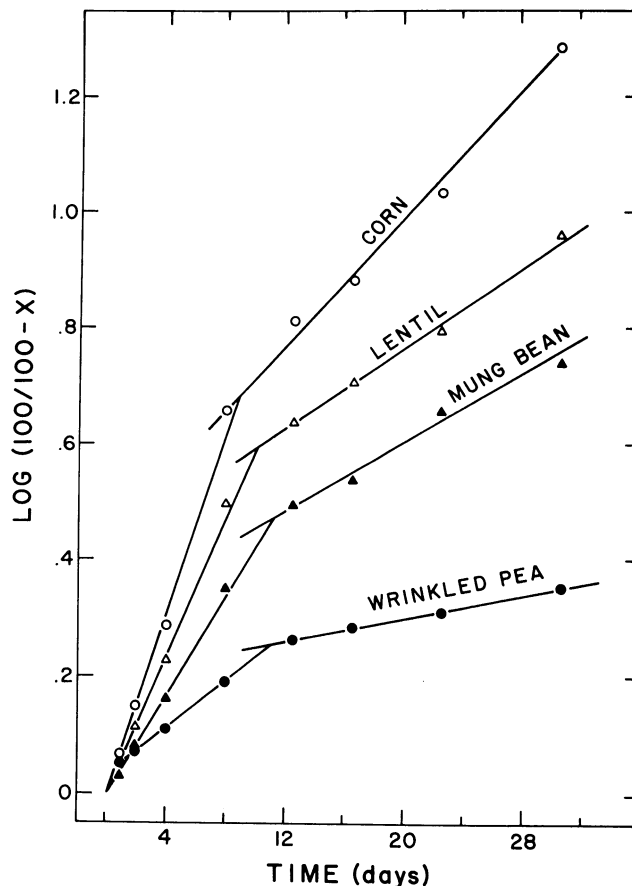


Fig. 2. Starch hydrolysis data plotted as  $\log_{10} (100/100-x)$  vs time.

<sup>5</sup>The reaction  $A (\text{starch}) \rightarrow B (\text{soluble products})$  is an oversimplified approach to the kinetics of the acid-mediated degradation of granular starch. This process would be better described by the equations  $A (\text{amorphous regions}) \xrightarrow{\kappa_1} C (\text{soluble products})$  and  $B (\text{starch crystallites}) \xrightarrow{\kappa_2} C (\text{soluble products})$ . However, no attempt has been made at a more rigorous treatment of the data.

TABLE I  
Hydrolysis Rate Constants and Granule Properties of Branched Starch Molecules of Legume Starches

| Starch                    | $\kappa \times 10^{-2}$ (days <sup>-1</sup> ) <sup>a</sup> | Average Gelatiniz. Temperature <sup>b</sup> (°C) | Granule <sup>c</sup> Size ( $\mu$ ) | Amylose Content (%) | Molar Ratio of Short Chains to Long Chains <sup>d</sup> |
|---------------------------|--|--|-------------------------------------|---------------------|---|
| Adzuki bean               | 7.34 (0.999) <sup>e</sup>                                  | 85   | 16 × 23                             | 34.9                | 6.3 ± 0.4   |
| Smooth pea                | 11.44 (0.999)  | 67   | 20 × 28                             | 33.1                | 7.7 ± 0.3   |
| Red kidney bean           | 12.41 (0.998)  | 66   | 21 × 28                             | 35.0                | 9.4   |
| Wrinkled pea              | 4.53 (0.997)   | 115  | 13 × 14                             | 64.0                | 4.2   |
| Lentil                    | 14.62 (0.999)  | 59   | 19 × 26                             | 45.5                | 9.6 ± 0.1   |
| Mung bean                 | 10.45 (0.999)  | 65   | 16 × 25                             | 34.9                | 7.2   |
| Corn, laboratory-prepared | 19.22 (0.998)  | 65   | 11 × 12                             | 22.4                | 10.5 ± 0.3  |

<sup>a</sup>  $\kappa$  is the apparent rate constant for the first stage of the hydrolysis curve,  $\log_{10} (100/100-x)$  vs time, calculated from the equation:  $\kappa = (2.303/t) \log_{10} (100/100-x)$ .

<sup>b</sup> Loss of birefringence of 50% of the granules.

<sup>c</sup> Average width times average length. Values reported are averages of 100 granules.

<sup>d</sup> Short chains ( $\bar{dp} = 14-18$ ), long chains ( $\bar{dp} = 45-55$ ) of the branched starch molecules (Biliaderis et al 1981).

<sup>e</sup> Numbers in parentheses give the correlation coefficient of the corresponding linear regression of the experimental data.

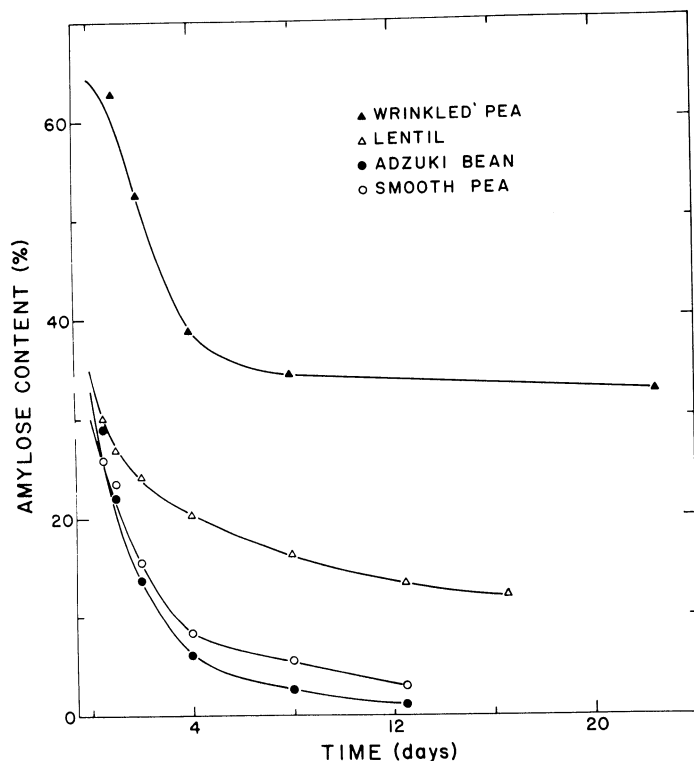


Fig. 3. Decrease in apparent amylose content of the starches as a function of the hydrolysis time.

between corn and legume starches may influence their erosion kinetics (the smaller the granule size, the larger the granule surface area available for acid penetration and, therefore, the faster the rate). However, observed differences in  $\kappa$  most likely reflect differences between these starches in the granular organization rather in granule size; that is, the legume starch granule appears to have a more rigid structure than that of corn starch.

Several granule properties (size, average gelatinization temperature, amylose content, and molar ratio of short to long chains) are also presented in Table I. Although  $\kappa$  is inversely correlated to some extent with average gelatinization temperature, no obvious relationship is apparent with granule size or amylose content. On the other hand, a significant correlation ( $r = 0.973$ ;  $P = 0.01$ ) was observed between  $\kappa$  and the molar ratio of short ( $\bar{dp}$  of 14-18) to long ( $\bar{dp}$  of 45-55) chains of the branched polysaccharide fraction of these starches. Because a higher hydrolysis rate is observed with starches having a greater molar ratio of short to long chains in their branched components, these data further support the previously proposed hypothesis for the detrimental effects of chain branching on granule rigidity (Biliaderis et al 1981).

However, more experimental evidence is required to further confirm this hypothesis.

The change in apparent amylose content for some of the starches, as a function of the hydrolysis time, is presented in Fig. 3. As hydrolysis time increased, the iodine-binding power of the starch decreased, confirming previous reports on potato starch (Robin et al 1974). This indicates a continuous decrease in the chain length of the linear material. However, these results do not mean that amylose is associated only with the amorphous regions of the granule. They only indicate that the length of the amylose chain segments participating in the starch crystallites is too short to allow normal binding of iodine. No differences were found in apparent amylose content changes among corn, mung bean, red kidney bean, smooth pea, and adzuki bean starches. However, lentil and particularly wrinkled pea starches showed a slower rate for the decrease in amylose content, presumably as a result of their high amylose content in the native state. We were interested to note that even after 65% hydrolysis of the wrinkled pea starch, the residual polysaccharide had an apparent amylose content of 26.0%. This indicates that a large portion of the iodine-binding material participates in the crystalline regions of this starch.

The x-ray diffraction patterns of three acid-treated legume starches and those of their native counterparts are shown in Fig. 4. The acid-treated samples retained the x-ray pattern of the native starches and even had sharper peaks at  $2\theta = 5.6^\circ$  (for all samples) and at both  $22$  and  $24^\circ$  (for wrinkled pea). Similar observations were also made with the rest of the starches. Sulfuric acid-treated starches (Nageli amyloextrins) and acid-treated rice and potato starches have also been reported to have sharper diffraction peaks than the native starches (Kainuma and French 1971, Maningat and Juliano 1979, Robin et al 1974). Kainuma and French (1971) have suggested that cleavage of just a few starch chains (in the amorphous regions) allows extensive "annealing," i.e., reordering of the chain segments, to give a more crystalline structure with a sharper x-ray pattern. Similarly, Wu and Sarko (1978a) have suggested that reordering of the crystalline structure might take place during acid hydrolysis by displacement of the water molecules in the crystallite cavities by double helices.

#### Structural Characterization of the Acid-Treated Legume Starches

Data depicting the structural characteristics of four acid-treated legume starches (smooth pea, adzuki bean, lentil, and wrinkled pea) are presented in Table II. With increasing hydrolysis, both the  $\lambda_{\max}$  of the iodine-polysaccharide complexes and the average chain length of the acid-treated starches decreased, indicating that progressive depolymerization of the starch molecules took place. This is consistent with the apparent amylose content changes shown in Fig. 3. Furthermore, the  $\beta$ -amylolysis limits increased with increase in hydrolysis, indicating that degradation of the branched polysaccharide molecules also occurred.

The elution profiles of the above starches, both before and after debranching with pullulanase, are shown in Figs. 5 and 6. With increasing hydrolysis, the elution patterns of the acid-treated

TABLE II  
Characteristics of Acid-Treated Legume Starches

| Starch       | Extent of Hydrolysis (%) | $\lambda_{\max}^a$ (nm) |            | $\beta$ -Amylolysis of Branched Starches (%) | Average Chain Length |            | $P_{\beta}^b$ (%) |
|--------------|--------------------------|-------------------------|------------|--|----------------------|------------|-------------------|
|              |                          | Branched                | Debranched |  | Branched             | Debranched |                   |
| Smooth pea   | 19                       | 560                     | 565        | 65.3 ± 1.3                                   | 77                   | 29         | 99.3              |
|              | 60                       | 525                     | 525        | 75.0 ± 1.5                                   | 37                   | 17         | 99.4              |
|              | 79                       | 515                     | 510        | 84.7 ± 0.7                                   | 20                   | 17         | 101.2             |
| Lentil       | 23                       | 565                     | 570        | 64.9 ± 1.3                                   | 66                   | 21         | 99.4              |
|              | 41                       | 545                     | 545        | 69.3 ± 1.0                                   | 29                   | 22         | 101.2             |
|              | 80                       | 545                     | 540        | 81.8 ± 1.7                                   | 27                   | 22         | 100.6             |
| Adzuki bean  | 25                       | 565                     | 565        | 66.1 ± 0.9                                   | 47                   | 21         | 98.1              |
|              | 45                       | 520                     | 520        | 74.6 ± 1.2                                   | 25                   | 15         | 98.4              |
|              | 80                       | 515                     | 515        | 82.3 ± 1.1                                   | 21                   | 16         | 99.8              |
| Wrinkled pea | 51                       | 565                     | 565        | 93.9 ± 1.7                                   | 31                   | 28         | 101.0             |
|              | 65                       | 555                     | 550        | 98.6 ± 1.4                                   | 25                   | 25         | 99.7              |

<sup>a</sup> Wavelength of maximum absorption.

<sup>b</sup> Percent  $\beta$ -amylolysis of debranched acid-treated starches.

smooth pea, lentil, and adzuki bean starches showed progressive decreases in both the molecular size and the range in the distribution of the eluted carbohydrate material. These results corroborate the decrease in average chain length (Table II) and are in agreement with those reported for acid-treated cereal and potato starches (Robin et al 1974, 1975).

Debranching and gel chromatography of the mildly acid-treated smooth pea and lentil starches (Fig. 5, a<sub>1</sub> and a<sub>2</sub>) revealed the typical bimodal chain distribution (chains with  $\overline{dp}$  of 45–55 and 15–17) of the debranched amylopectin fractions of these starches (Biliaderis et al 1981). The linearity of both chain populations was verified by the high  $\beta$ -amylolysis values (99%) of the debranched samples. A trace of gel-excluded linear material was detected for the lentil sample but no amylose type of material was detected for the acid-treated smooth pea starch. These results are consistent with the low  $\lambda_{\max}$  values of these starches (Table II) and the rapid decrease in the apparent amylose contents shown during the first stage of acid hydrolysis (Fig. 3). Therefore, amylose was degraded to shorter chain segments during the early stages of the acid treatment.

In contrast to lentil and smooth pea starches, the corresponding profile of the debranched mildly acid-treated adzuki bean starch (Fig. 6, a<sub>1</sub>) revealed, in addition to the amylopectinlike chain distribution, a small fraction of oligosaccharides with a  $\overline{dp}$  of 5–8. The fact that this fraction was absent in the debranched profile of the amylopectin of this starch (Biliaderis et al 1981) and the observation that the eluted long-chain population ( $\overline{dp}$  of 45–55) appeared as a shoulder rather than a discrete peak, suggests that the oligosaccharides with very short chains might represent segments of acid-degraded long chains. This hypothesis is further supported by the chromatogram of the debranched 45% acid-treated sample (Fig. 6, b<sub>1</sub>), in which the population of the long amylopectin chains has disappeared and the fraction with very short chains ( $\overline{dp}$  of 5–8) has increased pronouncedly. The presence of this fraction might reflect differences in the arrangement of the long chains in the amylopectin clusters of the adzuki bean compared to that of those in lentil and smooth pea starches.

The chromatograms of Figs. 5 and 6 show that as hydrolysis increased, the long linear chains ( $\overline{dp}$  of 45–55) disappeared and the debranched profiles of the acid-treated smooth pea, lentil, and adzuki bean starches were dominated by the population of the short ( $\overline{dp}$  of 15–22) chains. This implies that the long amylopectin chains were hydrolyzed more rapidly than the short ones, confirming the results of Robin et al (1974, 1975) for acid-treated cereal and potato starches. As acid hydrolysis proceeded, the profiles of the acid-treated starches (nondebranched) also gradually shifted toward the distribution consisting of short linear chains ( $\overline{dp}$  of 15–22) that characterized the debranched samples. Therefore, the crystalline areas of these legume starches also appear to be comprised of the short ( $\overline{dp}$  of 15–17) amylopectin chains and

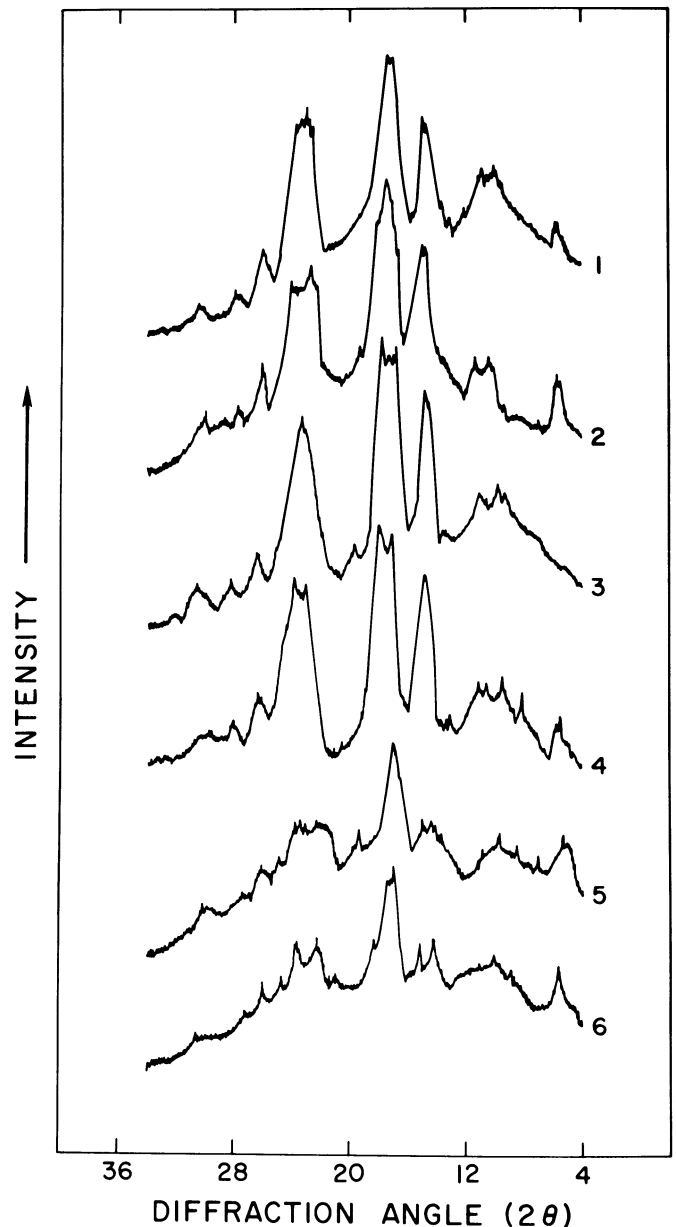


Fig. 4. X-ray diffraction patterns of native and acid-treated legume starches: 1, native smooth pea; 2, 82%-hydrolyzed smooth pea; 3, native adzuki bean; 4, 80%-hydrolyzed adzuki bean; 5, native wrinkled pea; 6, 65%-hydrolyzed wrinkled pea.

possibly short degraded amylose chains of similar length, as was previously suggested for the cereal and potato starches (Robin et al 1974, 1975). Thus the dimensions of the starch crystallite correspond to the length of the short ( $\overline{dp}$  of 15–17) amylopectin chains.

French et al (1971) and Watanabe and French (1980) reported that Sephadex G-50 chromatography of Nageli ( $H_2SO_4$ -treated) waxy corn amylopectin (40–50% of the original starch) showed three overlapping zones of the eluted carbohydrate material: 1) a multiply-branched high molecular weight fraction; 2) singly-branched material ( $\overline{dp}$  of 25); and 3) a linear fraction ( $\overline{dp}$  of 12). On the other hand, two peaks ( $\overline{dp}$  of 25 and 13–15) were obtained from 60–90% acid-hydrolyzed wheat, nonwaxy corn, waxy corn, and potato starches on Sephadex G-50 by Robin et al (1974, 1975). After debranching with pullulanase, most of the first ( $\overline{dp}$  of 25) fraction was converted into two chains with  $\overline{dp}$  of 12, indicating the branched nature of this population. In contrast to these studies, the Biogel P-10 profiles of the 80% acid-hydrolyzed smooth pea, lentil, and adzuki bean starches (Fig. 5,  $c_1$  and  $c_2$ ; Fig. 6,  $c_1$ ) did not show distinct separation of such structurally different eluted fractions. This difference in profiles may be the result of the higher amylose content of these starches; degraded amylose chains of various  $\overline{dp}$  may mask the elution of such branched fractions and therefore smooth the overall elution profile of the acid-treated legume

starches. The presence of the branched fractions, however, is readily recognized from the chromatography of the debranched starches, in which a pronounced increase appears in the short ( $\overline{dp}$  of 16 for adzuki beans and of 21 for lentil) linear chains. The fact that debranching gave rise to no other linear fractions of shorter  $\overline{dp}$  also suggests that both the main chain and branches of these fractions are of similar  $\overline{dp}$ .

In addition to the linear chains with  $\overline{dp}$  of 16–21, the unit chain profiles of the debranched smooth pea and lentil starches clearly showed shoulders at around  $\overline{dp}$  of 32 and 42, respectively. This material, which was linear (100.6–101.2%), was approximately twice the size of the corresponding short chains of these samples and, therefore, it may represent degraded amylose chains that were participating in the crystallites. A double helical (antiparallel helix or "hairpin" model; French 1972) conformation of these long chains in the crystallites would be compatible with the crystallite dimension and explain their resistance to acid hydrolysis.

The above interpretation of the experimental data leads to the conclusion that amylopectin is the main crystalline entity of the granule of smooth pea, lentil, and adzuki bean starches. The mode of chain degradation obtained during the acid treatment is compatible with the cluster model proposed for the amylopectin molecule by French (1972). This molecular structure (Fig. 7) is consistent with several experimental facts determined in this study.

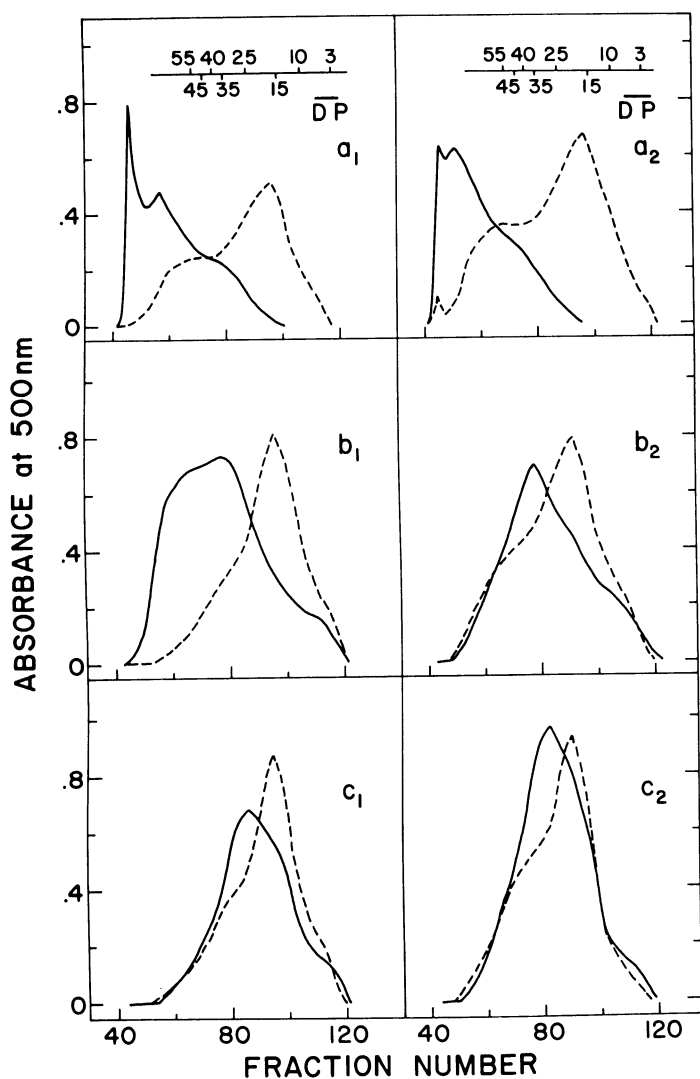


Fig. 5. Elution profiles of acid-treated legume starches before (—) and after (---) debranching with pullulanase on a Biogel P-10 column ( $2.6 \times 94$  cm) eluted with acetate buffer (0.1 M, pH 4.8), containing 0.02% sodium azide, at a flow rate of 16 ml/hr at 22°C.  $a_1$ ,  $b_1$ , and  $c_1$  = smooth pea starch, 19, 60, and 79%-hydrolyzed, respectively;  $a_2$ ,  $b_2$ , and  $c_2$  = lentil starch, 23, 41, and 80%-hydrolyzed, respectively. DP = degree of polymerization.

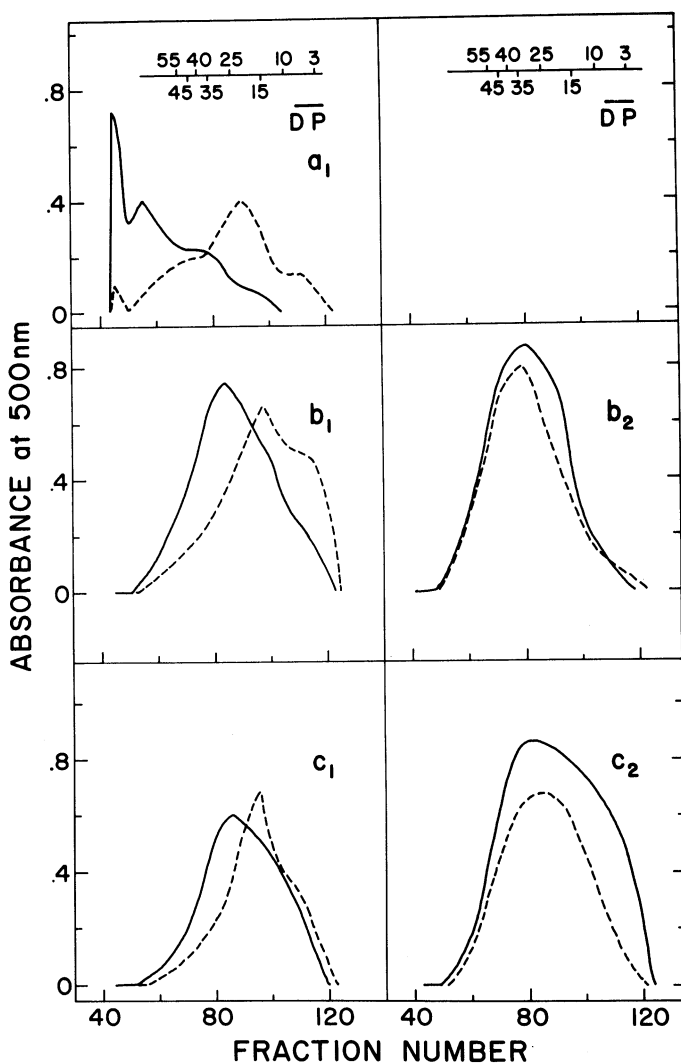
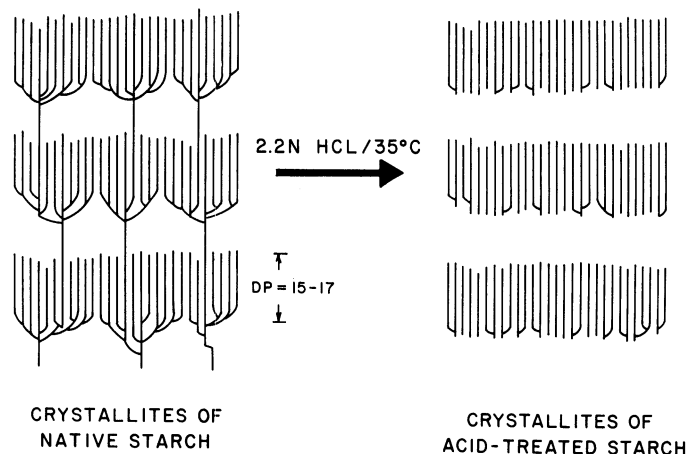


Fig. 6. Elution profiles of acid-treated legume starches before (—) and after (---) debranching with pullulanase on a Biogel P-10 column ( $2.6 \times 94$  cm) eluted with acetate buffer (0.1 M, pH 4.8), containing 0.02% sodium azide, at a flow rate of 16 ml/hr at 22°C.  $a_1$ ,  $b_1$ , and  $c_1$  = adzuki bean starch, 19, 60, and 79%-hydrolyzed, respectively;  $b_2$  and  $c_2$  = wrinkled pea starch, 51 and 65%-hydrolyzed, respectively. DP = degree of polymerization.



**Fig. 7.** The heterogeneous acid hydrolysis of the amylopectin molecules in granular starch. Amylopectin (**left**), in which the clusters of short chains constitute the starch crystallites, is preferentially hydrolyzed at the intercrystalline regions to give short segments of linear or lightly branched molecules (**right**) that remain crystalline and resistant to further acid hydrolysis. **DP** = degree of polymerization.

First, both native and acid-treated starches display a crystalline organization as revealed by their characteristic x-ray diffraction patterns. Second, native starch granules contain fewer organized regions that are selectively hydrolyzed during acid treatment. Accordingly, the extension of the long amylopectin chains over two successive chain clusters would be consistent with their rapid degradation at the early stages of the process (Fig. 7). Third, arrangement of the short chains in clusters can account adequately for the formation, size, and structure of the acid-resistant granular residues. Double helices formed among these short parallel chains and association with each other by extensive intermolecular hydrogen bonding would explain both the crystallinity and resistance to further acid degradation of the acid-treated starch samples. The heterogeneous acid hydrolysis of the amylopectin molecules in granular starch is summarized schematically in Fig. 7.

Although branched short-chain molecules were found in the residues of the acid-treated smooth pea, lentil, and adzuki bean starches, the experimental evidence shows quite unambiguously that short-chain linear material was the crystalline component of the wrinkled pea starch. Indeed, both the  $\beta$ -amylolysis (Table II) and the chromatographic profiles of the 64% hydrolyzed wrinkled pea starch before and after debranching (Fig. 6,  $c_2$ ) indicated that linear material was associated with the acid-resistant residues of this starch. Although chromatography was not attempted with samples less than 50% hydrolyzed, because of excessive retrogradation, Fig. 6,  $b_2$  and  $c_2$  show that the molecular size of the linear chains decreased very slowly over the entire range of the chain distribution as hydrolysis proceeded. Moreover, the distribution of the acid-resistant chains was wide and did not indicate any preference for accumulation of a particular  $\overline{dp}$  fraction. Thus, little ordering appeared in the dimensions of the starch crystallites of this starch. Retrograded amylose may form the crystalline structure of the high amylose starches (Banks and

Greenwood 1975, Kainuma and French 1971). A low degree of perfection and a three-dimensional order in the crystallites of such retrograded linear molecules would therefore be reasonable. Indeed, this concept is consistent with the described broad-chain distribution of the acid-resistant residues of wrinkled pea starch. Nevertheless, the overall crystalline structure in this starch would be very resistant to acid hydrolysis because of strong intermolecular hydrogen bonding among the aggregated amylose molecules.

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