Isolation and Characterization of Starch from Breadfruit[1]

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Cereal Chem. 58(4):282-286

ABSTRACT

The pulp of mature unripe breadfruit (*Artocarpus communis*) was freeze-dried. Starch was isolated from an aqueous slurry of the powder, and some of its physicochemical and structural properties were determined. Granular structure was evaluated by scanning electron, light, and polarized light microscopy. Molecular structure was studied by sequential hydrolysis with pullulanase and β-amylase and then fractionation of hydrolysis products by gel permeation chromatography. Starch granules were spherical and segmented (compound), 10–20 μm in diameter. Swelling and solubility exhibited a two-stage pattern and had values of 35-fold and 22%, respectively, at 95° C. A peak viscosity of 1,860 BU was observed at 95° C for an 8% starch suspension. Intrinsic viscosity of the starch solubilized in 1*N* KOH was 2.76 cS. Amylose content was 18.2%. β-Amylolysis of the starch was 58%. Debranched amylopectin contained chains with degrees of polymerization of 15 and 38–45 in a molar ratio of 4.4:1. About 15% of the debranched β-limit dextrin consisted of high molecular weight (degree of polymerization ≥600) linear polymers.

The breadfruit tree (*Artocarpus communis*) is native to tropical regions such as Malaysia, the South Pacific, and the Caribbean. The tree produces fruit primarily between May and August with some fruiting throughout the year. The fruit is round or ovoid, 3–8 in. in diameter, and weighs between 2 and 10 lb (Kennard and Winters 1960).

Breadfruit represents a valuable food resource. Its current usage, however, is limited by the poor storage properties of the fresh fruit (Thompson et al 1974). Conversion to flour would provide a more stable storage form as well as increasing its versatility. It has already been studied as a component of composite flour (Olatunji and Akinrele 1978).

Very little information has been published on the constituents of breadfruit. Knowledge of the starch, the primary component (68% of dry weight), is a prerequisite for exploring new uses of breadfruit. This study was undertaken to characterize the properties and structure of breadfruit starch.

MATERIALS AND METHODS

Very mature, unripe breadfruit from Puerto Rico was peeled, cored, cut into pieces, dried at 80° C, and ground into flour.

Isolation

Starch was extracted from the flour by forming a slurry with distilled deionized water (200 g of flour with 1,200 ml of water) in a large water-jacketed Waring Blendor, using low speed for 30 min. Water (20° C) was circulated through the jacket to avoid heat damage to the starch granules. After blending, the slurry was centrifuged at 4,080 × *g* for 15 min and the supernatant discarded. A yellow, gummy mucilage was scraped from the surface of the precipitate and also discarded. The precipitate was resuspended in distilled water and the separation process repeated. The resulting white starch precipitate was spread in a thin layer in aluminum trays and freeze-dried for 24 hr without shelf heat. The dry starch cake was pulverized into a fine powder using a spatula and a mortar and pestle. The purity of the starch preparation was determined by the procedure of Thivend et al (1972).

Chemical Analyses

Damaged starch was determined by its susceptibility to α-amylase, according to AACC method 76-30A (1969). A fungal amylase (*Aspergillus oryzae*, Fungamyl 1600, Novo Enzyme Corp., Mamaroneck, NY) was used. The alkaline ferricyanide method of Friedman et al (1962) was used to measure reducing sugars. The amount of maltose found in the sample after subtraction of the blank was multiplied by 0.95 to correct for the weight of water incorporated during starch hydrolysis. This was then divided by the total amount of starch corrected for moisture content. Because previous workers have found maximum starch hydrolysis by α-amylase to be 61% (Donelson and Yamazaki 1962), this result was divided by 0.61 to obtain percent damaged starch. The calculation is summarized as follows:

$$\frac{\text{maltose equivalent, } \mu g \times 0.95}{\text{starch, } \mu g, \text{ dry wt} \times 0.61} = \text{damaged starch, \%}$$

Moisture, ash, and protein determinations were made according to AOAC procedures (1970). Protein was calculated as nitrogen × 6.25. Fat was extracted with chloroform/methanol by the method of Folch et al (1957) and quantified gravimetrically.

Amylose content was determined according to the potentiometric titration procedure of Banks et al (1971).

Molecular structure was studied with sequential enzymatic hydrolysis and gel permeation chromatography according to methods outlined by Hood and Mercier (1978). In all cases, recoveries from the columns were ≥85%. Pullulanase (2,000 units per milliliter) was obtained from Hayashibara Biochemical Laboratories (Okayama, Japan). Starch dispersions (1% in distilled deionized water) were prepared by boiling for 4 min.

Microscopy

Suspensions of breadfruit starch or flour were dusted onto aluminum stubs, prepared with double-sided adhesive tape, and coated for 3 min with gold-palladium. Samples were viewed in an AMR-1000 scanning electron microscope at 10–20 kV.

Starch slurries were viewed with a Leitz Ortholux microscope equipped with both brightfield and polarized light optics.

Swelling and Solubility

Swelling and solubility patterns were studied according to the method of Leach et al (1959). Starch samples (3 g) were placed in 250-ml centrifuge bottles and heated in a 2-L glass water bath.

Viscosity and Pasting Characteristics

A Haake Rotovisco RV 1, equipped with a double gap beaker and rotor system NV, was used to determine the concentration range in which the viscosity of breadfruit starch is Newtonian (Hahn and Hood 1980). This was found to be ≤0.4% in 1*N* KOH.

Subsequently, a Cannon-Fenske capillary viscometer, size 100, was used to determine intrinsic viscosity on 0.1, 0.2, 0.3, and 0.4% starch in 1*N* KOH (Kerr 1950). The time required for the sample or 1*N* KOH menisci to pass between the two etched lines on the viscometer was measured with a stopwatch. Efflux time was calculated as:

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0009-0352/81/04028205/$03.00/0

$$\frac{\text{time for sample} - \text{time for KOH}}{\text{time for KOH}}$$

This value was multiplied by the instrument constant (0.015 cS per second) to yield specific viscosity, which was divided by the concentration of starch in the solution, as determined by the method of Thivend et al (1972), and plotted against the concentration of the starch solution. The resulting line was extrapolated back to zero concentration to determine intrinsic viscosity.

Starch slurries of 4, 5, 6, 7, and 8% in distilled deionized water were subjected to a heating-cooling cycle in a Brabender Visco/amylo/Graph (model VA-VE). Rotational speed was 75 rpm. Heating began at an initial temperature of 23°C and continued at 1.5°C/min to 95°C. After 60 min at 95°C, slurries were cooled at 1.5°C/min to 50°C, at which they were held for 60 min.

RESULTS AND DISCUSSION

The composition of breadfruit starch and of whole breadfruit are shown in Table I. Fresh breadfruit contained a high amount of starch but was quite low in protein. The unaccounted-for solids (23.3%) probably were other carbohydrates. The damaged starch value was 3.2%. Starch was 90% pure. This would suggest that the isolation procedure yielded starch of minimal damage and of relatively high purity. The amylose content was 18.2%.

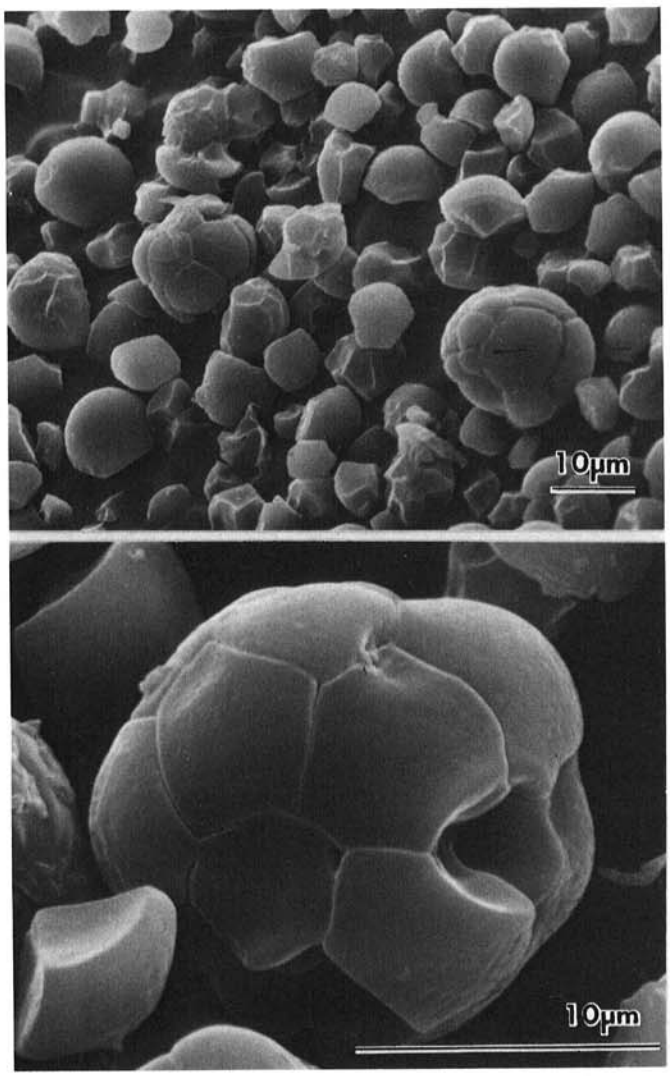

Fig. 1. Scanning electron micrographs of breadfruit starch. Multisided segments in the granules are evident.

The starch granules were spherical and segmented, ranging in size from 10–20 μm. Several multisided segments, predominately hexagonal, could be observed in scanning electron micrographs of the granules (Fig. 1). Some varieties of millet exhibit similar hexagonal shapes (Angold 1979). Most of the breadfruit granules were fractured into these smaller segments. They were not the result of oven drying at 80°C, milling, or starch extraction, because freeze-dried immature and mature unripe breadfruit also contained the segmented granules. Under polarized light, each segment had a maltese cross (Fig. 2). Whole granules showed a multiple cross pattern. Thus, like wrinkled-seeded pea (Banks et al 1974),

TABLE I
Composition of Fresh Breadfruit and Isolated Starch[a]

	Moisture	Ash	Lipid	Protein	Total Starch
Fresh breadfruit	64.3 ± 0.40	3.2 ± 0.04	3.6 ± 0.06	2.0 ± 0.04	67.9 ± 0.8
Starch	7.9 ± 0.01	0.3 ± 0.03	0.7 ± 0.01	0.4 ± 0.01	···

[a] Means ± SD, n = 3. Except for moisture, values are on a dry weight basis.

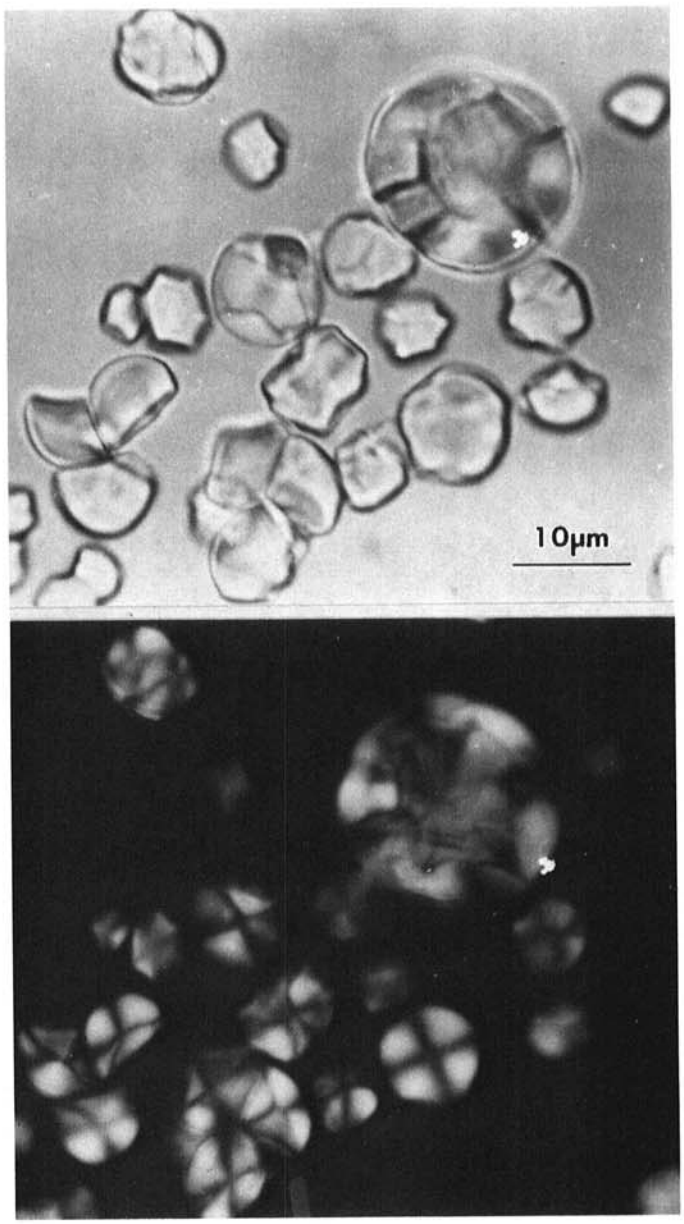

Fig. 2. Light **(top)** and polarized light **(bottom)** micrographs of breadfruit starch. Both micrographs were made of the same field. Note multiple cross pattern in intact granule and single maltese crosses in individual segments.

breadfruit starch granules appear to be true compound granules.

The swelling behavior is an indication of the water absorption characteristics of the granules during heating. Swelling increased rapidly around 60° C, leveled off, and increased again around 80° C (Fig. 3). This two-stage behavior has been observed for other starches (Leach et al 1959, Rasper 1969). It has been attributed to two sets of internal bonding forces that relax at different temperatures (Leach et al 1959).

The solubility pattern exhibited a two-stage behavior similar to the swelling pattern (Fig. 4). The two processes are closely related; however, percent solubility was significantly lower at each temperature than degree of swelling.

Viscosity

The intrinsic viscosity of breadfruit starch was found to be 2.76 cS. This is higher than reported values for wheat, cassava, and arrowroot starches but lower than those reported for yam (Ciacco and D'Appolonia 1978, Medcalf and Gilles 1965).

Brabender amylograms are shown in Fig. 5. At the lower starch concentrations (4 and 5%), considerable viscosity stability was evident throughout the heating-cooling cycle. This is evidence of restricted swelling and solubilization and of resistance to mechanical disintegration. The higher starch concentrations (7 and 8%) had higher peak viscosities (at 95° C) and considerable viscosity breakdown during prolonged heating and stirring. When the pasted starch was cooled, set-back (retrogradation) was evident. At the higher starch concentrations, the cooled gels exhibited a decrease in viscosity when held at 50° C. In general, the amylograms of breadfruit starch are similar to those of potato starch (Knight 1969).

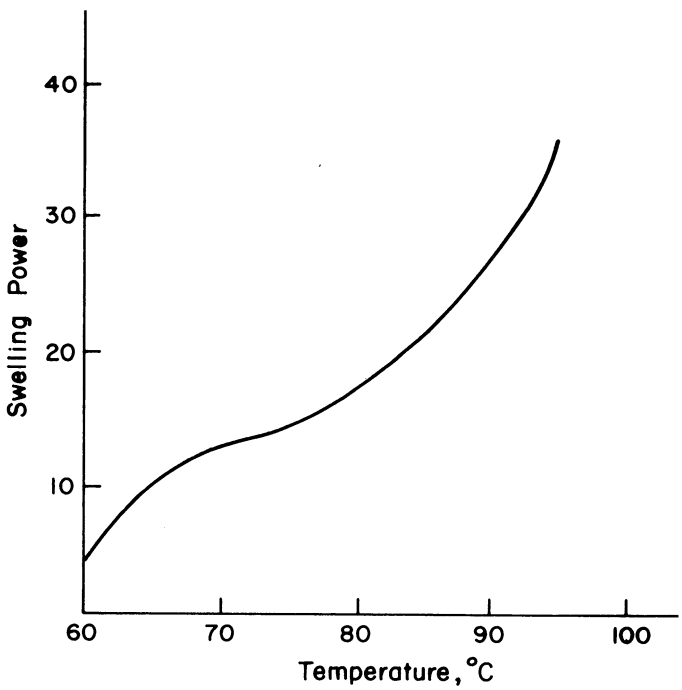

Fig. 3. Swelling pattern of breadfruit starch.

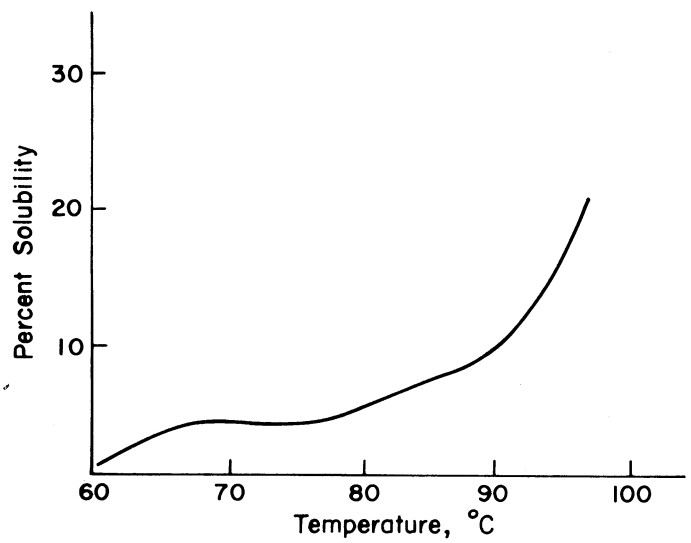

Fig. 4. Solubility pattern of breadfruit starch.

Molecular Structure

The Sephadex G-50 elution profile of pullulanase-debranched starch (P_1) revealed three major peaks: one at the void volume (V_0), a bimodal peak at degree of polymerization ($\overline{dp}$) 38–45, and a peak at $\overline{dp}$ 15 (Fig. 6). The amount of polysaccharide in each peak is summarized in Table II. Similar patterns have been observed for potato, maize, wheat, and tapioca starches (Hood and Mercier 1978; Lii and Lineback 1977; Mercier 1973; Robin et al 1974, 1975). The bimodal $\overline{dp}$ 38–45 peak is not common in debranched starch, although a similar split peak was noted by Kayisu and Hood (1980). Apparently two slightly different, yet distinct, populations of the heavier ($\overline{dp}$ 38–45) chains existed. The molar ratio of $\overline{dp}$ 38–45 to $\overline{dp}$ 15 chains was about 1:4.4. This ratio is lower than the ratio of $\overline{dp}$ 45 to $\overline{dp}$ 15 in other starches (Hood and Mercier 1978; Robin et al 1974, 1975). The peak at the V_0 represented 30% of the total polysaccharide and consisted primarily of amylose.

β-Amylase converted 94% of the debranched starch to maltose or maltotriose (Fig. 6). Thus, most of the polysaccharide at the V_0 in the P_1 digest was long ($\overline{dp}$ >60) linear polymers. A peak of 5% remained at the V_0 of the debranched starch hydrolyzed with β-amylase. This may represent branched amylose, as suggested by Mercier (1973), or possibly a pullulanase-resistant fraction of amylopectin. Pullulanase resistance might arise from α-(1,3) linkages—found in waxy maize starch (Wolfrom and Thompson 1956), from adjacent α-(1,6) linkages on the same chain (Whelan 1971), from the presence of single glucose stubs, and/or from a high molecular surface density that prevents penetration of the enzyme (Abdullah et al 1966). Kainuma and French (1969) demonstrated the presence of single glucose stubs in waxy maize starch but concluded that adjacent linkages were unlikely. Hood and Mercier (1978) also noted a small peak at the V_0 of debranched β-amylase

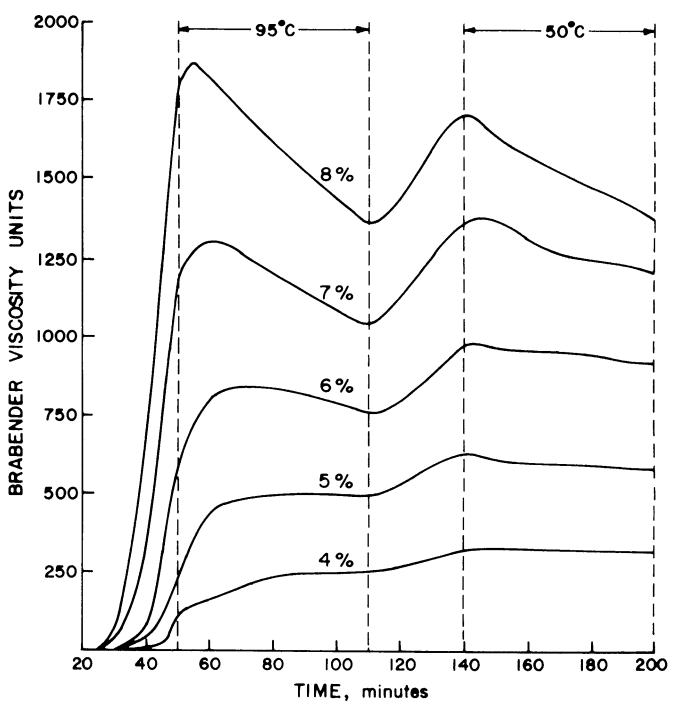

Fig. 5. Brabender amylograms of breadfruit starch at various concentrations.

TABLE II
Characteristics of Pullulanase-Debranched Breadfruit Starch and β-Limit Dextrin

Component	Percent[a]
Starch	
Pullulanase-debranched	
Chain length[b]	26
Degree of polymerization	
>60	30
38–45	27
15	43
Debranched and hydrolyzed with β-amylase	
β-Amylolysis[c]	94
Degree of polymerization >60	5
β-Amylolysis[c]	58
β-Limit Dextrin	
Debranched	
Chain length[b]	12
Degree of polymerization	
>60	8 (20)[d]
29–39	20 (47)
6–10	4 (10)
2–3	6 (15)
Debranched and hydrolyzed with β-amylase	
β-Amylolysis[c]	91
Degree of polymerization >60	2 (5)

[a] Unless otherwise noted, all values are expressed as percent of original starch.
[b] Polysaccharide in digest (glucose equivalents)/reducing capacity (glucose equivalents)
[c] Reducing capacity (maltose equivalents)/polysaccharide in digest (glucose equivalents) × 100
[d] Values in parenthesis are percent of total polysaccharide in the respective components.

hydrolyzed starch, which they tentatively identified as undebranched amylopectin.

The elution profile of the debranched β-limit dextrin (β_1P_1) revealed several peaks (Fig. 7). The peak at $\overline{dp}$ 29–39 was bimodal and probably represents the chains of $\overline{dp}$ 38–45 that were shortened in the preparation of the β-limit dextrin. The peak at $\overline{dp}$ 2–10 represents the maltosyl and maltotriosyl stubs remaining after the outer chains had been shortened by β-amylase and the inner chains of the amylopectin.

The large peak (20%) at the V_0 of the β_1P_1 is difficult to explain. When the β_1P_1 was fractionated on Sephadex G-100, the V_0 peak also represented 20% of the total polysaccharide recovered. Thus, this material had a $\overline{dp} \geq 600$. The chain length for the β_1P_1 was 12, compared to a value of about 10 for other starches (Hood and Mercier 1978, Mercier 1973), suggesting that debranching was incomplete. However, when the β_1P_1 was hydrolyzed with β-amylase, the peak at the V_0 was reduced to 5%. This indicates that 15% (ie, 20–5%) of the β-limit dextrin was comprised of high molecular weight ($\overline{dp} \geq 600$) linear polymers that could only be hydrolyzed with β-amylase after debranching. Considering the patterns in Figs. 6 and 7 and the data in Table II collectively, we are led to the tentative conclusion that this high molecular weight material is either branched amylose with branch points located near the nonreducing end of the molecule and/or amylopectin with some unusually long inner chains. These structures would be hydrolyzed to the branch points by β-amylase, leaving residues of long linear chains ($\overline{dp} \geq 600$) with maltosyl or maltotriosyl stubs. These long chains also would appear at the V_0 of the P_1 elution profile. No evidence has been reported that amylopectin contains long linear ($\overline{dp} \geq 600$) chains. Further molecular structure studies should focus on this apparently unique feature of breadfruit starch.

ACKNOWLEDGMENTS

We wish to express our appreciation to M. Cogan, R. Ferretti, and M. Liboff for their assistance in preparing the micrographs.

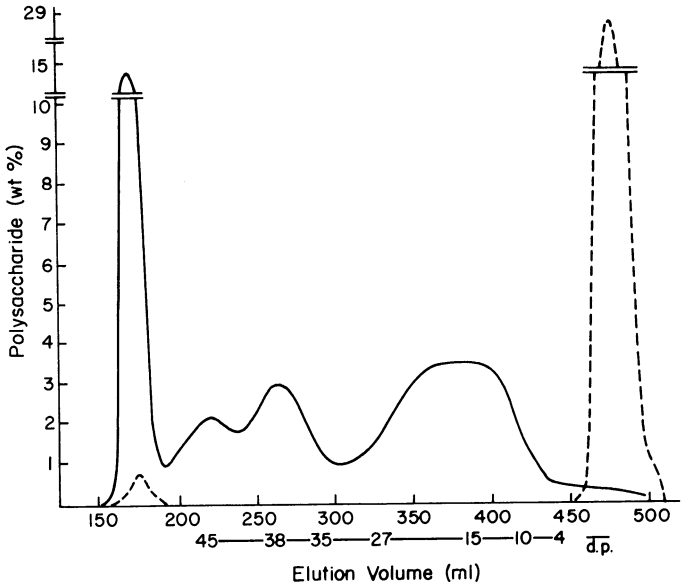

Fig. 6. Elution profiles from Sephadex G-50 of breadfruit starch. —— = debranched, --- = debranched and hydrolyzed with β-amylase.

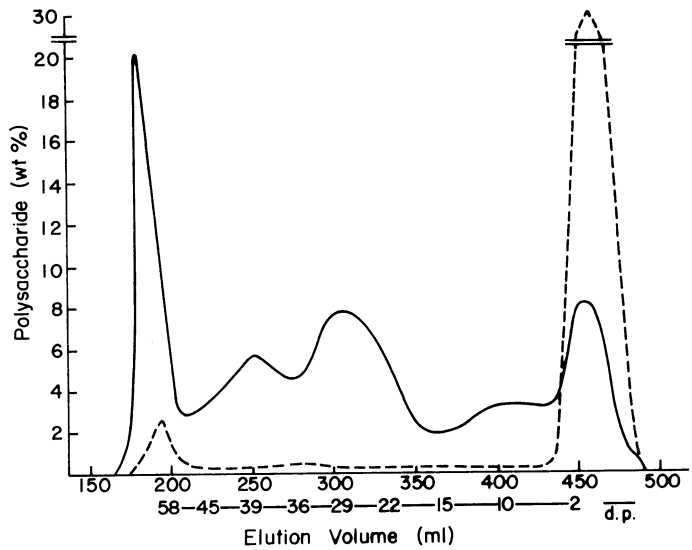

Fig. 7. Elution profiles from Sephadex G-50 of breadfruit β-limit dextrin. —— = debranched, --- = debranched and hydrolyzed with β-amylase.

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[Received December 20, 1979. Accepted November 24, 1980]