New Starch Resource: Study of Some Characteristics of the Starch by Enzyme Methods

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Babassu starch, a new starch resource, was isolated by alkaline treatment, and some characteristics were studied by enzyme methods. Babassu starch maintains an appreciable viscosity even after being kept at 95 °C for 20 min in the presence of bacterial α-amylase. This enzyme hydrolyzes granular tapioca starch slowly and granular babassu starch more slowly, as compared to corn starch. The relative solubilities of the granular starches in dimethyl sulfoxide are remarkably similar to the relative rates of hydrolysis of the starches by bacterial α-amylase. Microscopic findings are also confirmed with correlation between hydrolysis by α-amylase and solubilization by dimethyl sulfoxide. Enzyme susceptibility of the starch has no relationship to the patterns of swelling of starches. It was also found that there is some relationship between β-amylolysis and α-amylase susceptibility.

Babassu, *Orbygnia speciosa* in the Palmacea, is a native plant widely spread in northern and northeastern Brazil. It has been calculated that this plant occupies a total of 14 000 000 ha in these regions (Coco de Babacu, 1977).

As shown in Figure 1, babassu fruit consists of an epicarp (external fiber layer), a mesocarp (mixture of starch and fiber layer), an endocarp (hard wood layer), and nut. Conventionally, local people have obtained nuts by mechanical crushing in order to extract the oil. Consequently, there was no way to isolate starch from the mesocarp layer. Recently, the starch layer from the mesocarp has been isolated by a new mechanial process; the meal from the mesocarp contains approximately 65% starch (the mesocarp occupies about 23% babassu fruit)(Coco de Babacu, 1977). Because it is widely spread, this plant is considered to be a potential starch resource. The objective of this study was to examine some characteristics of the starch by enzyme methods.

MATERIALS AND METHODS

Preparation of Starches. Babassu meal obtained from the endocarp of Babassu fruit was mixed with 0.2% NaOH solutions with frequent agitation during daytime and then allowed to settle overnight at room temperature. The supernatant was decanted, and the precipitate was washed with water and centrifuged to separate insoluble materials. The precipitate was resuspended in water and screened through 150-mesh screen before final centrifugation. The wet starch was dried at room temperature by passing warm dry air over the surface. The dried starch was treated in the Soxhlet apparatus with ether for 5 h. The corn and tapioca starches were obtained from commercial laboratories.

Brabender Viscosity Curves. Brabender curves were determined using a Brabender amylograph as described by Mazurs et al. (1957).

Solubility of Granular Starches in Dimethyl Sulfoxide. The solubility in dimethyl sulfoxide was determined by the method described by Leach and Schoch (1962). Samples of granular starches weighing 0.5 g were suspended in 100 mL of anhydrous dimethyl sulfoxide in 250-mL Erlenmeyer flasks and agitated at 100 rpm at room temperature. Periodically the flask was removed and centrifuged. A 50-mL aliquot of supernatant was removed and added to 150 mL of methanol. The mixture was digested on the steam bath for 1 h and then allowed to stand overnight. The precipitated starch was then filtered and thoroughly washed with methanol and dried in the oven for 5 h at 110 °C.

Viscosity Reduction with Bacterial α-Amylase. The effect of bacterial α-amylase action was tested by the use of a Brabender amylograph as described by Goering and Brelsford (1965). Two hundred and sixty SKB units of bacterial α-amylase was added to 500 mL of starch suspension (8%) which had previously adjusted to pH 6.0. This mixture was introduced into the Brabender amylograph and heated at 1.5 °C/min to 95 °C and then held for 20 min at this temperature.

Hydrolysis of Granular Starches by Bacterial α-Amylase. The mixture of 1 g of granular starches and 60 000 SKB units of bacterial α-amylase in 100 mL of 0.05 M phosphate buffer pH 6.0 was covered with toluene and shaken on a rotary shaker (100 rpm) at room temperature for 60 h. Periodically, small amounts of samples were taken for determination of reducing substances by the method as described by Somogyi (1945).

Hydrolysis of Solubilized or Gelatinized Starches by β-Amylase and Pullulanase. β-Amylolysis was carried out by incubating a mixture of 100 mL of 0.5% solubilized starch solution (prepared by dissolving 0.5 g of granular starches in 20 mL of 1 N NaOH and diluted to 100 mL after adjusting to pH 6.0, with 1 N acetic acid) or gelatinized starch solution and 1000 units of purified soybean β-amylase at 40 °C. Samples were taken at 5, 10, 20, 40, and 60 min and assayed for total reducing sugars. After incubation for 60 min, 40 units of pullulanase (Hayashibara Biochemical Co.) was added to the digestion mixture and again incubated for 100 min.

Determination of Swelling Power. Granule swelling was determined by the procedure described by Leach and Schoch (1959). The mixture of granular starches (2 g in 50 mL of H_2O) and the suspension were incubated in a water bath maintained at a temperature ranging from 50 to 95 °C for 30 min with shaking (50 rpm). After incubation, the suspension was centrifuged to separate the supernatant from the sedimented starch paste. The paste and bottle were then weighed.

Determination of Protein and Ash. The protein content was determined by the Kjeldahl method as described in AOAC (1960). Ashing was carried out in a muffle furnace.

RESULTS AND DISCUSSION

Chemical Composition. Chemical analysis of all starches indicated that they contained the normal amounts of ash and protein.

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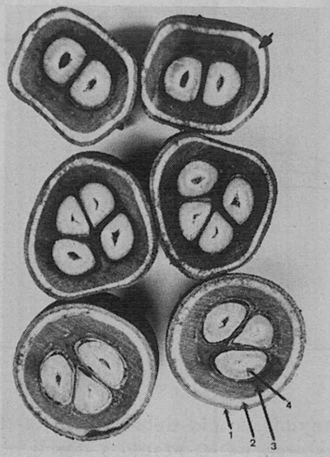

Figure 1. Cross section of Babassu fruit: (1) epicarp (external fiber layer), (2) mesocarp (mixture of starch and fiber layer), (3) endocarp (hard wood layer), and (4) nut.

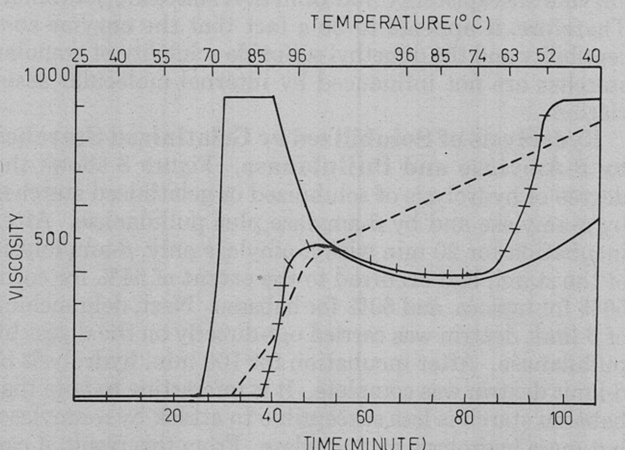

Figure 2. Brabender viscosity curve: (———) tapioca starch, (++++++++) corn starch, and (- - -) babassu starch.

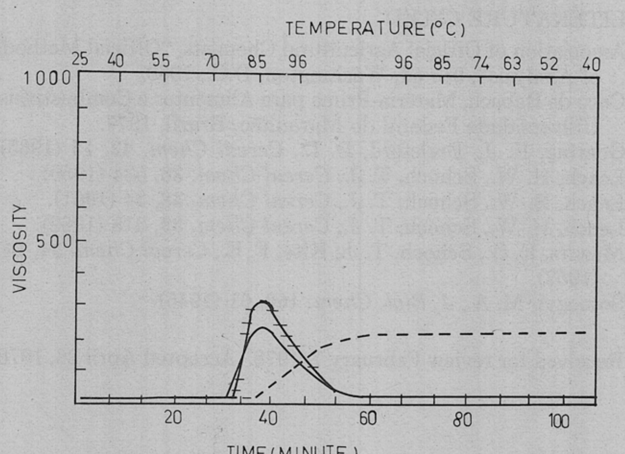

Figure 3. Brabender viscosity curve with bacterial α-amylase: (———) tapioca starch, (++++++++) corn starch, and (- - -) babassu starch.

Brabender Viscosity Curves. As shown in Figure 2, babassu starch has not exhibited reduction of initial pasting peak after cooking 20 min at 95 °C, which indicates high stability of the paste during cooking. This suggests that the babassu starch has different viscosity characteristics as compared to corn and tapioca starches. Figure 3 illustrates the effect of bacterial α-amylase action on three starches. Corn and tapioca starches exhibited substantial reduction of viscosity after cooking for 20 min at 95 °C, but babassu starch maintained appreciable

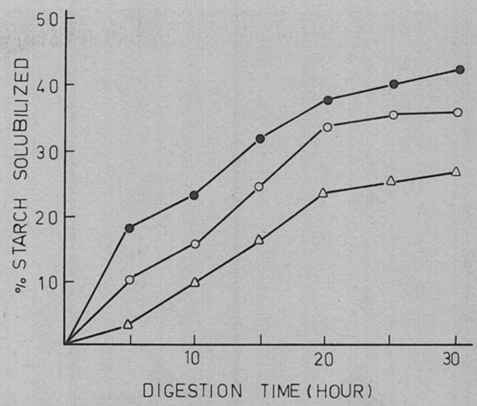

Figure 4. Solubility of granular starches in dimethyl sulfoxide: (-●-●-) corn starch, (-O-O-) tapioca starch, and (-Δ-Δ-) babassu starch.

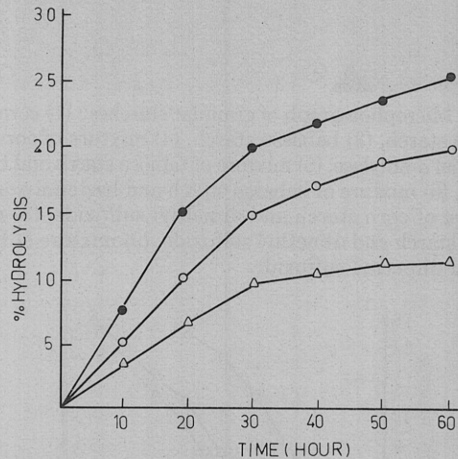

Figure 5. Effect of bacterial α-amylase on hydrolysis of granular starches: (-●-●-) corn starch, (-O-O-) tapioca starch, and (-Δ-Δ-) babassu starch.

viscosity even after being held at 95 °C for 20 min. Bacterial α-amylase effectively hydrolyzed corn starch. Tapioca starch was hydrolyzed less rapidly than corn starch, while babassu starch was slowly hydrolyzed.

Solubility of the Granular Starches in Dimethyl Sulfoxide. Solubilization of granular starches in dimethyl sulfoxide are shown in Figure 4. For this work, 0.5% solution of each starch was prepared by shaking the mixture for 24 h at room temperature. The relative solubilities of the granular starches in dimethyl sulfoxide are remarkably similar to the relative hydrolysis of these starches by bacterial α-amylase as shown in Figure 5. Leach and Schoch (1961, 1962) previously reported that the solubility of granular starches in anhydrous dimethyl sulfoxide can be used as a measure of susceptibility to α-amylase action.

Microscopic Observation. Figure 6 illustrates microscopic observation of starch granules after being acted upon by both dimethyl sulfoxide and α-amylase. Corn starch rapidly developed darkened hilums and demonstrated progressive fragmentation of the granules in dimethyl sulfoxide. In contrast, the other starches showed a much slower granule attack. Corn starch also showed severe granule erosion in bacterial α-amylase solution. Babassu and tapioca starches show slight enzyme attack.

Swelling Power. The results for the patterns of swelling power of the starches are shown in Figure 7. Tapioca starch undergoes granule swelling at low temperature, but the swelling thereafter proceeds at a slower rate. This indicates weak bonding forces and wider range of bond strengths in the tapioca starch granule. By

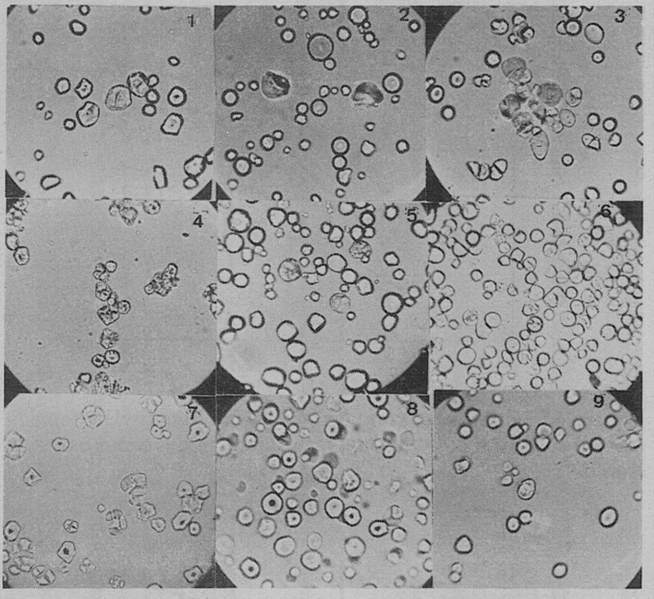

Figure 6. Microphotograph of granular starches: (1) corn starch, (2) tapioca starch, (3) babassu starch, (4) mixture of corn starch and bacterial α-amylase, (5) mixture of tapioca starch and bacterial α-amylase, (6) mixture of babassu starch and bacterial α-amylase. (7) mixture of corn starch and dimethyl sulfoxide, (8) mixture of tapioca starch and dimethyl sulfoxide, (9) mixture of Babassu starch and dimethyl sulfoxide.

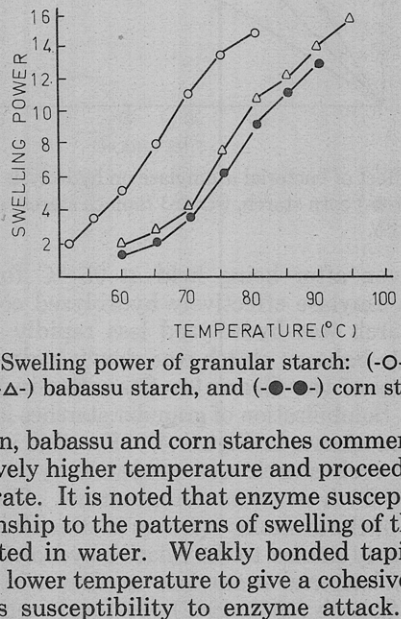

Figure 7. Swelling power of granular starch: (-O-O-) tapioca starch, (-Δ-Δ-) babassu starch, and (-●-●-) corn starch.

comparison, babassu and corn starches commence to swell at a relatively higher temperature and proceed thereafter at a slow rate. It is noted that enzyme susceptibility has no relationship to the patterns of swelling of the starches when heated in water. Weakly bonded tapioca starch swells at a lower temperature to give a cohesive paste, yet shows less susceptibility to enzyme attack. Strongly bonded corn and babassu starches show restricted swelling and give low paste, yet they differ considerably in both enzyme susceptibility and dimethyl sulfoxide solubility. Therefore, it appears to be a fact that the enzyme susceptibility and the dimethyl sulfoxide solubility of granular starches are not influenced by internal molecular association.

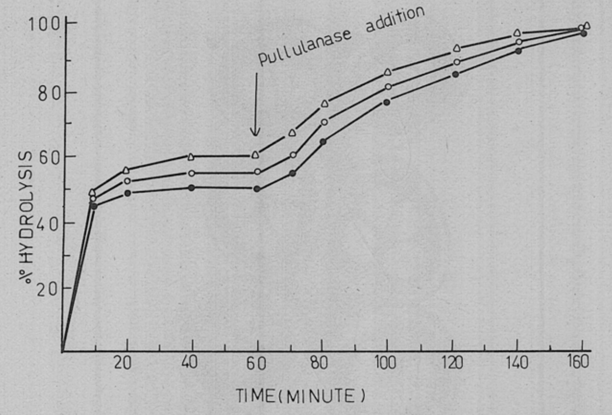

Figure 8. β-Amylolysis and debranching of β-limit dextrin: (-Δ-Δ-) babassu starch, (-O-O-) tapioca starch, and (-●-●-) corn starch.

Hydrolysis of Solubilized or Gelatinized Starches by β-Amylase and Pullulanase. Figure 8 shows the degree of hydrolysis of solubilized or gelatinized starches by β-amylase and by β-amylase plus pullulanase. After incubation for 20 min with β-amylase only, β-amylolysis of the starch had occurred to the extent of 54% for corn, 56% for tapioca, and 60% for babassu. Next, debranching of β-limit dextrin was carried out directly on the digest by pullulanase. After incubation for 100 min, hydrolysis of β-limit dextrin was complete. It is interesting to note that babassu starch is less susceptible to attack by α-amylase, but more hydrolysis by β-amylase. From this result, it can be inferred that babassu starch has a longer amylose chain than other starches.

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