Characterization of Pejibaye Starch[1]

J.-L. JANE,[2,3] L. SHEN,[2] and F. AGUILAR[4]

ABSTRACT

Starches were isolated from dried fruits of pejibaye palm (*Bactris gasipaes*) grown in the following regions of Costa Rica: Sarapiquí, Limón, Guápiles, Parrita, and Turrialba. Pasting, gelling, and thermal properties of the starches were investigated. Amylose and phosphorus contents, amylose molecular size, and amylopectin branch chain lengths were analyzed. Onset of gelatinization temperatures varied from 49 to 53°C. Enthalpy changes varied from 2.2 to 2.7 cal/g. Viscosity consistency of the starch pastes (8%, dry-starch basis) at 52°C varied from 520 to 1,100 BU (using a Brabender Viscoamylograph). Amylose contents varied from 8 to 19%, and phosphorus contents ranged from 0.049 to 0.054%. Branch chain lengths of amylopectin determined with the peak fractions had degrees of polymerization of 18 and 30 for short and long branches, respectively. Physical property variations were mainly attributed to differences in amylose contents and amylopectin structures.

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Geographical distribution of the pejibaye palm (*Bactris gasipaes* Kunth) extends from Honduras, Central America, to Bolivia, South America (Stone 1951, Antezana 1972, Mora-Urpi 1990). It was a tropical crop of great importance in several pre-Columbian civilizations. Fruits of pejibaye palm, 3–5 cm in size (Fig. 1), are rich in lipids (Hammond et al 1982) and carbohydrates (Salas and Blanco 1990). Because of its importance as a human and animal food source, commercial interest in pejibaye has been growing in recent years.

Great genetic variability has been reported for this crop between different growing regions (Mora-Urpi and Clement 1981, Mora-Urpi 1990). Therefore, an investigation of the functional properties and corresponding chemical structures of pejibaye starches isolated from different genetic variants may increase our understanding of structure-property correlations and utilization alternatives.

Starches were isolated from freeze-dried pejibaye fruits grown in the Costa Rican areas of Sarapiquí, Limón, Guápiles, Parrita, and Turrialba. Starch characterizations, such as micrographs of granules, pasting viscosity, gel strength, thermal properties, and retrogradation rates, were analyzed. Chemical structures, including amylose and phosphorus contents, molecular size distributions, and amylopectin branch chain lengths, were determined. The functional properties and chemical structures of these starches can be compared with those of the conventional cereal and tuber starches reviewed by Lineback (1984).

MATERIALS AND METHODS

Starch and Chemicals

Fruits of pejibaye palm grown in Sarapiquí, Limón, Guápiles, Parrita, and Turrialba were harvested in spring 1989, freeze-dried at the University of Costa Rica, and sent to Iowa State University for characterization. Starches were isolated from the dried fruits. Isoamylase (*Pseudomonas amyloderamosa* ATCC 21262) was purchased from Hayashibara Biochemical Lab., Inc. (Okayama, Japan). All other chemicals were reagent grade and used without further purification.

Starch Isolation

Dried pejibaye strips (0.5 × 0.5 × 2 cm, 200 g) were dipped in a mercuric chloride ($HgCl_2$) solution (0.01*M*, 1 L) for 1 hr to inhibit hydrolytic enzyme activity. The sample then was milled in a commercial blender (Hamilton Beach model 585-1) at full speed for 1 min. Starch was filtered through a cloth screen (200 mesh) with additional $HgCl_2$ solution. The milling procedure was repeated until only a small amount of starch was passing through the screen (Greenwood and Thomson 1959, de Willigen 1964). The starch was allowed to settle, then separated from the super-

[1]Journal paper J-14344 of the Iowa Agriculture and Home Economics Experiment Station, Ames; project 2863.
[2]Department of Food Science and Human Nutrition, Iowa State University, Ames 50011.
[3]Corresponding author.
[4]4, Food Technology Research Center, University of Costa Rica.

natant, resuspended in distilled water, and washed five or six times (until the supernatant was clear). The starch then was isolated and dried in a forced-air oven (40°C, 48 hr). Moisture content in the starch was about 10% (determined by oven drying at 120°C, 3 hr). Lipid in the starch was removed with aqueous methanol solution (85%) in a Soxhlet extractor (16 hr).

Microscopy

Scanning electron micrographs were taken with a JEOL JSM-35 scanning electron microscope (Tokyo, Japan). Starch samples were sprinkled on adhesive tapes, attached to specimen studs, and coated with gold-palladium.

X-Ray Diffraction Pattern

X-ray diffraction patterns of the starches were obtained with copper, nickel-foil filtered, K_α radiation (Zobel 1964). Operation was at 30 μA and 40 kV. Slits were 3°/0.15°, and scanning speed was 1°/min.

Pasting and Gelling Properties

Pasting curves of starches (8%, dry-starch basis [dsb]) were determined using a Brabender Viscoamylograph (model VA-V) (Hackensack, NJ), following the general procedures of Smith (1964). The starch suspension was equilibrated to 30°C, then heated at a rate of 1.5°C/min with constant stirring at 75 rpm. The paste was held at 97.5°C with continuous stirring for 30 min and then cooled to 52°C at the same rate of 1.5°C/min. The starch paste then was transferred to aluminum pans (5 cm i.d. × 2.5 cm) wrapped with aluminum foil around the wall for extra depth (Takahashi et al 1989). The samples were sealed in

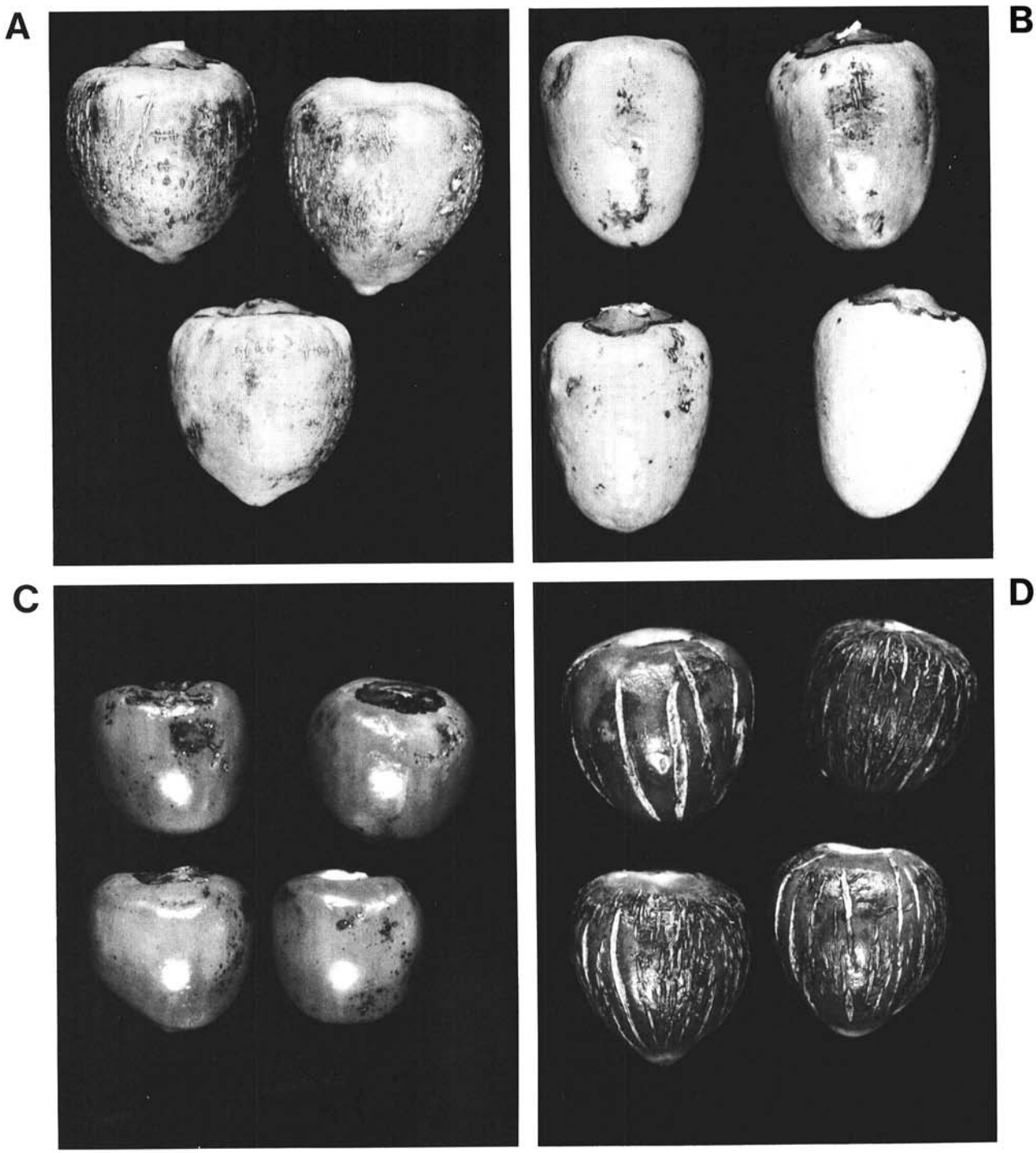

Fig. 1. Photographs of pejibaye fruits grown in the Costa Rican regions of **A,** Sarapiquí; **B,** Limón; **C,** Guápiles; **D,** Turrialba.

plastic bags and stored at two temperatures and time regimes (25°C for 4 hr and 4°C for 72 hr) for gel strength analysis.

Gel strengths of the starch pastes were measured with a Voland texture analyzer (model TA) (Scarsdale, NY). Immediately before each measurement, the foil wrap was removed, and the surface gel was removed with a cheese cutter. This exposed a fresh, smooth surface for texture analysis. A 2-mm-diameter probe and a 3-mm distance of penetration at the speed of 0.2 mm/sec were used through the analysis.

Differential Scanning Calorimetry (DSC)

Starch gelatinization and retrogradation were analyzed with a Perkin-Elmer differential scanning calorimeter (DSC-7, Norwalk, CT) equipped with an intracooling I system. Aluminum sample pans (Perkin-Elmer) were used for the analyses. Starch (about 2 mg, dsb) was weighed in the sample pan, mixed with distilled water (about 8 mg), and sealed. The heating rate was set at 10°C/min. Enthalpy changes, integrated by using DSC-7 standard software, were calibrated on the melting of indium metal. Each gelatinized sample was kept in individually marked glass vials and stored at 4°C for 28 days. The samples were analyzed for starch retrogradation (enthalpy changes) by DSC as previously described.

Amylose Content Analysis

Amylose contents were determined by both iodine potentiometric titration (Schoch 1964) and gel-permeation column chromatography (GPC) (Colonna and Mercier 1984). A Sepharose CL-2B column (2.6 cm i.d. × 75 cm) was used for the analysis. Fractions (5 ml) were collected and analyzed with a dual-channel Technicon AutoAnalyzer II (Bran & Lubbe, Elmsford, NY) for total carbohydrate content and blue value. Total carbohydrate content was analyzed by using the anthrone-sulfuric acid method (Wright and Gann 1966); blue value was analyzed by following the method of Juliano (1971). Molecular size distributions were analyzed by gel permeation chromatography using pullulan standards (Shodex P-82 Standard, Waters, Milford, MA). The average molecular size of the starch was not determined.

Determination of Phosphorus Derivatives

Defatted starch was used for phosphorus derivative analysis to eliminate possible interference from lipid phosphorus. Phosphorus contents were analyzed by dry-ashing the starches, followed by molybdenum blue spectrophotometric analysis (Smith and Caruso 1964).

Amylopectin Branch Chain Length

Starch (20 mg) was dissolved in distilled water (3.2 ml) by stirring the solution within a water bath at 96°C for 1 hr. An acetate buffer solution (0.1*M*, 0.4 ml) was added, and the pH was adjusted to 3.5. *Pseudomonas* isoamylase (900 units; Lee et al 1968, Yokobayashi et al 1970) was added, and the mixture incubated at 40°C for 48 hr. Change in reducing value was used to monitor the reaction, which was stopped at completion by heating in a water bath at 96°C for 10 min. Branch chain length distribution was analyzed by using a Bio-Gel P-6 gel-permeation column. The chain length of each peak was determined by measuring reducing value using the Somogi-Nelson method (Somogi 1945), and total carbohydrate content was determined by using phenol-sulfuric analysis (Dubois et al 1956) at the peak fractions.

RESULTS AND DISCUSSION

Starch yields (percentages, dsb) from dried fruits of pejibaye are presented in Table I. Dried pejibaye fruits yielded up to 37% starch. Scanning electron micrographs of the starches displayed round, oval, and irregular granular shapes (Fig. 2A–D). All of the starches displayed the A-type X-ray diffraction pattern (data not shown) (Zobel 1964). During isolation, Turrialba and Guápiles starches settled easily as individual granules. Limón and Sarapiquí starches tended to stick together and were more difficult to isolate.

Defatted pejibaye starches displayed slight differences in thermal properties (e.g., onset [T_o], peak [T_p], and complete [T_c] gelatinization temperatures and enthalpy changes [ΔH]), as indicated by DSC analysis (Table II). Limón starch had the lowest T_o and T_c (49 and 59°C, respectively). Sarapiquí starch, on the other hand, had slightly higher T_o and T_c (53 and 62°C, respectively). Gelatinization temperatures of pejibaye starches were generally lower than those of corn (62–72°C), wheat (57–64°C), and potato (59–68°C) starches (Lineback 1984). Enthalpy changes of the pejibaye starches varied from 2.2 to 2.7 cal/g, which also were lower than those of corn, wheat, and potato starches (2.9, 2.9, and 4.8 cal/g, respectively). Retrogradations of the starches measured by enthalpy changes (Table II) after storage (4°C for 28 days) also are lower than that of the corn starch counterpart (0.92 cal/g).

In contrast to the similarity in gelatinization temperatures and enthalpy changes, the starches displayed distinctive differences in their amylographs (Fig. 3). Sarapiquí starch had the lowest pasting temperature (60°C) and the highest viscosity consistency (1,100 BU) at 52°C. Starch pastes of Sarapiquí, Parrita, and Guápiles displayed little or no viscosity loss during the 30-min shearing at 97.5°C. Turrialba starch, having the highest pasting temperature (90°C) and the lowest viscosity, showed a continuous increase in viscosity when sheared at 97.5°C. Under similar conditions, Limón starch suffered a significant loss in viscosity, which we believed was due to low amylose content (about 8%) (Table I). Gel strength of the starch pastes after storage (25°C for 4 hr and 4°C for 72 hr) also exhibited differences (Table III). Limón starch with low amylose content displayed the least gel strength.

Amylose content varied (Table I). Turrialba starch had the greatest amylose content (19%) among the varieties tested. The starch displayed a slow increase in viscosity (Fig. 3). When viewed under a light microscope, Turrialba starch exhibited a limited swelling pattern during heating, whereas Sarapiquí starch displayed a prompt swelling, the largest among the varieties (data not shown). The other three were intermediate to these extremes.

Phosphorus content determined by molybdenum blue analysis (Smith and Caruso 1964) showed no significant differences among the varieties (Table I). The amount of phosphorus content in pejibaye starch, about 0.05%, is greater than that in normal corn starch (0.017%) but lower than that in potato starch (about 0.07%) analyzed by the same method.

Gel permeation column chromatograms of the starches also showed no significant difference in amylose molecular size (peak degree of polymerization [DP] of about 500, based on pullulan standards). Isoamylase debranching of the starches showed that the amylopectins have similar branch chain lengths (DP about 18 and 30 for short and long branch chains, respectively). The branch chain lengths are shorter than those of normal corn amylopectin (DP 20 and 39 for short and long branch chains, respectively). Distributions of the branch chain lengths (Table

TABLE I
Yield and Amylose and Phosphorus Contents of Pejibaye Starches

Source	Starch Yield (% dsb)[a]	Amylose Content IA[b] (%)	Amylose Content GPC[c] (%)	Phosphorus Content[d] (%)
Sarapiquí	23.8 ± 1.9	15.3	16.8 ± 1.4	0.050 ± 0.001
Limón	32.4 ± 3.2	7.9	8.7 ± 0.9	0.050 ± 0.001
Guápiles	34.7 ± 1.1	15.2	16.9 ± 1.1	0.049 ± 0.001
Parrita	20.4 ± 0.4	15.3	14.9 ± 0.7	0.054 ± 0.002
Turrialba	36.7 ± 1.7	18.8	19.1 ± 0.3	0.051 ± 0.002

[a]The data are averages of two replicates. dsb = Dry-starch basis.
[b]Iodine affinity (single analysis).
[c]Gel permeation column chromatography using Sepharose CL-2B gel. The data are averages of two replicates.
[d]The data are averages of three replicates.

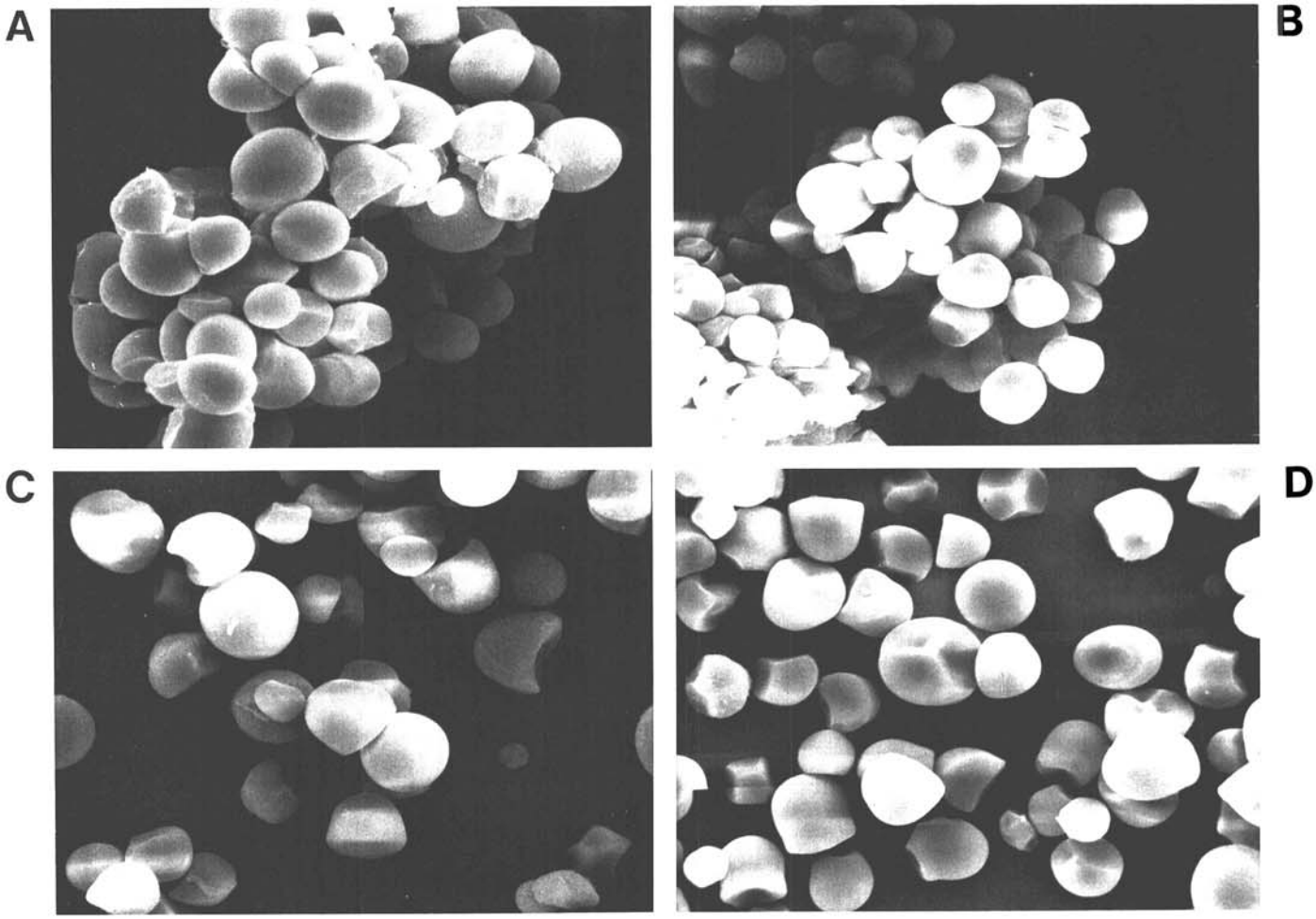

Fig. 2. Scanning electronic micrographs of starches isolated from pejibaye fruits grown in different regions in Costa Rica. **A,** Sarapiquí; **B,** Limón; **C,** Guápiles; **D,** Turrialba. Bar = 10 μm.

TABLE II
Pejibaye Starch Thermal Properties[a]

					Retrograded Starch[c]	
Source	T_o	T_p	T_c	ΔH[b] (cal/g)	T_o	ΔH (cal/g)
Sarapiquí	52.7	57.1	62.5	2.7 ± 0.1	ND[d]	ND
Limón	48.7	53.0	58.8	2.3 ± 0.1	36.5	0.33 ± 0.04
Guápiles	52.5	56.2	60.5	2.2 ± 0.2	40.5	0.57 ± 0.06
Parrita	51.2	55.4	60.5	2.2 ± 0.1	ND	ND
Turrialba	51.0	54.9	59.9	2.4 ± 0.0	37.3	0.56 ± 0.05

[a]Starch samples (~ 2 mg, dry-starch basis) and distilled water (~ 8 mg) were used for analyses. T_o, T_p, and T_c = onset, peak, and complete gelatinization temperatures, respectively.
[b]Enthalpy change. Averages of three replicates.
[c]After storage at 4°C for 28 days, averages of two replicates.
[d]Not determined.

IV) indicated that Sarapiquí starch had a low proportion of long branch chain (11.7%) in the amylopectin compared with other varieties (13.4–14.6%). It is plausible that fewer long branch chains, participating in two or more adjacent crystalline clusters in amylopectin, may provide more freedom (less restriction) to granule swelling, as evidenced by low pasting temperature (60°C).

These structural studies of pejibaye starch suggested that swelling, viscosity, and gelling properties of the starches are affected by their amylose contents and amylopectin structures. This suggests that starch with fewer long branch chains in the amylopectin has lower pasting temperature and greater swelling power, which is desirable in many uses.

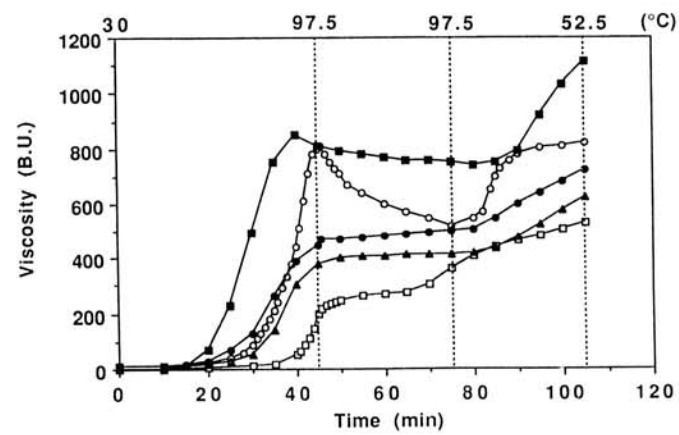

Fig. 3. Viscoamylograms of various starch pastes (8%, dry-starch basis). ■ = Sarapiquí; ○ = Limón; ● = Guápiles; ▲ = Parrita; □ = Turrialba. Sensitivity cartridge 700 cm/g; bowl capacity 500 ml. Sample analyses were done in duplicate.

TABLE III
Gel Strength of Pejibaye Starch Paste (8%, dsb)[a]

Source	4 hr at 25°C	72 hr at 4°C
Sarapiquí	4.0 ± 0.1	5.3 ± 0.1
Limón	3.0 ± 0.1	3.8 ± 0.1
Guápiles	5.3 ± 0.1	7.1 ± 0.1
Turrialba	3.8 ± 0.1	6.9 ± 0.1

[a]The data reported are averages of five replicates. dsb = Dry-starch basis.

TABLE IV
Branch Chain Length Distribution of Pejibaye Starch[a]

Source	Long-Branch Chain[b] (%)	Short-Branch Chain[c] (%)
Sarapiquí	11.7 ± 1.3	88.3 ± 1.3
Limón	14.6 ± 1.2	85.4 ± 1.2
Guápiles	13.8 ± 1.6	86.2 ± 1.6
Parrita	13.4 ± 1.1	86.6 ± 1.1
Turrialba	14.4 ± 0.6	85.6 ± 0.6

[a]The data reported are averages of three replicates.
[b]Peak branch chain length: DP 30.
[c]Peak branch chain length: DP 18.

CONCLUSIONS

Starch yields from freeze-dried fruits of pejibaye were between 20 and 37%. The starch displayed round, oval, and irregular shapes and an A-type X-ray diffraction pattern. DSC analysis showed that the starches had onset temperatures of gelatinization between 49 and 53°C and enthalpy changes between 2.2 and 2.7 cal/g. Sarapiquí starch had the lowest pasting temperature (60°C) and the highest viscosity consistency (1,100 BU) at 52°C, whereas Turrialba starch had the highest pasting temperature (90°C) and the lowest viscosity consistency (510 BU). Amylose contents in the starches varied from 8 to 19%, and phosphorus contents were around 0.05%. Gel permeation column chromatography showed no difference in amylose molecular size of the varieties. Branch chain lengths of the amylopectin were DP 18 and 30 for short and long branches, respectively.

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