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Bioavailability of carbohydrate material stored in tropical fruit seeds

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Properties of starches derived from seeds obtained from tropical fruits used by the pulping industries were characterised and their response to enzyme attack ascertained. Despite similarity of the tissue from which starch was obtained, seeds starches varied depending upon the botanical source in all of the structural and physicochemical properties measured except in the pattern by which enzymes attack the native granule. Morphologically the granules were very different, possessing a variety of shapes and sizes; the composition and size of the macromolecular fractions were also distinct. The hydration properties and susceptibilities of the granules were found to be different, which was surprising considering the similarity in physiological function of the starches. Similarly, the susceptibility of intact granules to enzyme attack was different in extent, though all of the granules should undergo a similar pattern of attack, namely surface erosion. Correlation analysis revealed an apparent lack of correlation between the enthalpy of gelatinisation, assumed to represent degree of crystallinity, and enzyme susceptibility or swelling. It was concluded that, despite the existence of an underlying mechanism of hydrolysis, shaving of the granule surface and structural characteristics unique to a particular species may dominate. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The unique structural and physicochemical features of starch granules derived from different botanical sources have been principally attributed to genetic factors. Recent work suggests that environmental conditions may also be implicated in the expression of certain characteristics of the granule. Most comparative studies on starches derived from different botanical sources rely on material obtained from different plant tissues. Physiologically, starch serves as a source of storage carbohydrate, the precise duration of which is dependent upon the storage organ. Starch laid down in seeds acts as a reservoir for long-term storage and is deposited in large quantities in specialised plastids (amyloplasts). This starch is mobilised soon after germination, when it provides a source of carbon for the growing seedling. In contrast, starch may be temporarily accumulated in tissues, such as leaves. Starch synthesised during daylight will be degraded during periods of darkness to provide metabolic energy. Structural characteristics of the starch granule may well be related to the physiological storage pattern.

Seeds can be characterised on the basis of their main storage material, i.e. those containing lipids and those containing carbohydrates. After radicle protrusion, the event which defines the end of the germination process, carbohydrate-containing seeds experience composition changes characterised by loss of starch with a concomitant increase in free sugar. Changes in the storage material following early post-germination growth are the result of the activity of enzymes capable of degrading these polymers. Dry seeds contain mainly beta-amylase but, in germination, alpha-amylase is produced to the extent that up to 90% of the amylase activity in the seed can be attributed to this enzyme. Beta-amylase cannot attack the native starch granule. Starch polysaccharides are degraded by many enzymes *in vivo*, the pattern of breakdown reflecting the relative amounts of enzymes present. In plants it is probable that the initial attack on native granules is always initiated by alpha-amylases and complete degradation then results from the combined actions of other enzymes. The optimum pH for most of these enzymes is about 5.0–6.5 (Fogarty & Kelly, 1979).

Starch granules from different botanical sources, and often different plant tissues, possess a wide variety of characteristics, including their susceptibility to *in vitro* hydrolysis. This paper examines starch granules isolated from the same tissue but derived from a range of

botanical sources. Structural and functional characteristics of the granules are compared and correlated with the susceptibility of the granules to enzyme hydrolysis.

MATERIALS AND METHODS

The tropical fruits used for this study, rambutan, longan, durian, mango and jackfruit, were collected at two different times during the harvest period.

Starch was isolated from whole, cleaned seeds using the method of Schoch & Maywald (1968) with minor modifications: seeds were steeped in water overnight, washed and ground in a blender (Braun Multimix MX32) at low speed for 2 min. The slurry was filtered through a cloth bag (about 80 μm mesh) and the filtrate set aside to sediment the starch. Starch was reslurried in water and sedimented three times before final sedimentation in NaCl (10%) and toluene (0.02%). The collected starch was thoroughly washed with water and dried *in vacuo* at 50°C. Starch yield was expressed as the percentage recovery of the determined quantity of starch in the sample.

Size and shape of isolated starch granules were examined using a light microscope. Gelatinisation temperature was determined using a light microscope fitted with polarising filters and a hot stage. An aqueous suspension of granules (0.1%) was heated using a Kofler hot stage; the temperatures at which 10%, 50% and 90% of the granules were no longer birefringent were noted.

Dried starch samples were prepared for observation by scanning electron microscopy by sprinkling the starch onto double-backed adhesive tape attached to a circular specimen stub and coating with gold using a Baltzers SCD 004 sputter coater. The samples were viewed and photographed using a Phillips SEM 515 scanning electron microscope on AGFAPAN-APX 100 film.

Apparent amylose contents were determined colorimetrically with I_2/KI reagent (Morrison & Laignelet, 1983) and λ_{max} was recorded under assay conditions for total amylose. The major starch fractions were examined by high-performance size-exclusion chromatography (HPSEC) according to the method of Govindasamy *et al.* (1992).

Swelling and solubility determinations were carried out at 30°C and 90°C by the procedures of Leach *et al.* (1959).

Gelatinisation properties of the extracted starches were analysed using a Perkin-Elmer differential scanning calorimeter (DSC7; Norwalk, CT) equipped with an intercooler. Aluminium pans (Perkin-Elmer) were used for the analysis. Starch samples were slurried with water (20%, w/v) and mixed thoroughly. The starch slurry was dispensed, after mixing, into empty aluminium pans and hermetically sealed. Following equilibration at room temperature for 1 h, the samples were heated from 20°C to 110°C at a heating rate of 10°C per min. An empty pan was used as reference. Enthalpy changes, integrated using DSC 7 software, were calibrated on the basis of the melting enthalpy of indium metal. All pans were reweighed after cooling. To calculate enthalpy values accurately on a dry starch basis, each pan was carefully punctured, dried at 110°C for 2 h, and reweighed to determine moisture content of the scanned and rescanned samples.

Granule size distribution was determined by Coulter counter analysis. Starch granules (40–50 mg) were dispersed in 200 ml of electrolyte solution (ISOTON II diluant) and counted with a Coulter Counter (Industrial Model D; Coulter Electronics, UK). A 140 μm aperture was used.

Hydrolysis of native starch granules was completed using a reaction mixture containing 2.0% of starch granules. A 0.1 M acetic acid–sodium acetate buffer solution, pH 5.0 and 40 ppm Ca^{2+}, was incubated at 35°C with constant shaking. Hydrolysis was initiated following addition of a mixture containing 1% (volume of enzyme/weight of starch) of each of the enzymes glucoamylase (AMG, 300 units ml^{-1}; Novo Nordisk) and alpha-amylase (BAN, 480 units g^{-1}; Novo Nordisk). The reaction was stopped after 24 h by adding an equal volume of 0.4 mM $HgCl_2$ and incubating at 90°C for 20 min (Govindasamy *et al.*, 1992). The degree of hydrolysis was determined as outlined by Wang *et al.* (1995).

RESULTS AND DISCUSSION

These studies were intended to examine the starches as close to their native state as possible; thus we did not attempt to extract them from dried milled seed material, rather we extracted them from freshly harvested seeds.

The yields of pure starch recovered from the seeds varied from 6.7% (rambutan) to 18.4% (mango) (Table 1). The low amounts of starch extracted from

Table 1. Yields of pure starch and lipid content of starches extracted from tropical fruit seeds

Starch source	Yield pure starch (mg g^{-1} seed)		Lipid content (mg g^{-1} seed)	
	Sample 1	Sample 2	Sample 1	Sample 2
Jackfruit	15.4	12.7	0.70	0.70
Durian	4.2	1.8	0.24	0.07
Mango	18.4	4.0	0.00	0.00
Longan	17.9	12.1	2.96	2.44
Rambutan	7.7	6.7	0.77	0.75

Table 2. Size distribution (mean and range) of starches extracted from tropical fruit seeds

Starch source	Mean diameter (μm)	Size range (μm)	Mean diameter after hydrolysis (μm)	
			Population 1	Population 2
Jackfruit 1	11.0	4.2–47.0	11.0	5.4
Jackfruit 2	9.2	4.5–48.0	9.4	5.4
Durian 1	4.0	3.0–14.0	4.7	3.5
Durian 2	4.9	3.5–22.0	4.9	3.6
Mango 1	16.0	5.0–47.5	16.0	9.8
Mango 2	10.4	5.0–24.0		
Longan 1	11.2	2.5–45.0	11.2	5.4
Longan 2	10.5	2.5–46.0	10.5	5.2
Rambutan 1	10.8	2.0–46.0	7.6	
Rambutan 2	9.8	3.0–40.0	8.7	

some seeds is likely to be a result of low starch content rather than inefficient extraction procedures. Wastes from each of the washing stages were examined for starch by birefringence microscopy and little starch was found. Durian was difficult to check because of the small granules (average size >5 μm; Table 2), making granules that may have passed through the material used for straining difficult to detect. Extraction of starch from the fruits investigated was lower than the 20–40% that has been reported for other seed and legume starches (Hoover & Sosulski, 1985; Wankhede & Ramteke, 1981; Schoch & Maywald, 1968). Apparent higher yields of the other seeds are due to the lower moisture content of the starch sources; for example, cereals or legumes have seed moisture contents of less than 10%. Moisture contents of the seeds obtained from tropical fruits ranged from 40% to 60% (Powell & Oates, 1994).

Isolation of starch from jackfruit and longan was difficult because of the presence of insoluble flocculent material which settled with the starch to give a brownish deposit. Similar difficulties have been reported for a number of legume starches (Hoover & Sosulski, 1985). Debris attached to the granules is clearly evident in micrographs (Fig. 1(f)). After several acid washings, the granular surfaces appeared somewhat smoother, suggesting that the debris had been removed (Fig. 1(d)). It was not possible to remove the debris using solvents, and this suggests the non-lipid nature of this material. The limited commercial potential of some starches has been attributed to the combined effects of difficult extraction and the presence of insoluble flocculant protein and fine fibre associated with the isolated granules (Hoover & Sosulski, 1985). In addition, residual proteins may also affect the functional characteristics and digestibility of the starch granules. Residual protein associated with pea starch granules is thought to be derived from protein bodies, agglomerates, chloroplast membrane remnants and a fraction derived from the stroma. The purity of the starch was judged on the basis of composition and microscopic examinations which demonstrated the absence of any adhering protein.

Lipid content (Table 1) of some of the starches examined was higher than expected, particularly those from jackfruit, rambutan and longan. The lipid content of these starches was greater than 0.7% (Table 1), the highest being in longan (>2%). The higher lipid content of some of the starches is similar to the amounts reported for a number of oat starches (1.8–2.3%) (Morrison *et al.*, 1984). Functionally, the high lipid content of oat starches has been suggested to play a role in decreasing the rate and amount of degradation.

Starch granules were of different sizes and possessed a variety of structures and surface morphologies, dependent upon the botanical source (Fig. 1 and Table 2). Starch granules obtained from durian, rambutan and longan seeds were a mixture of rounded and angular multi-sided shapes, all with smooth surfaces. The angular nature of some of the surfaces may represent the consequence of close packing within the amyloplast or result from drying during the extraction process (Evers, 1979). Aggregates of starch granules could not be seen in any of the samples examined. This seems to indicate that the extraction procedure was effective in extracting individual granules. Granules of jackfruit seed starch were a variety of shapes, many appearing to be compound granules. In contrast, mango granules had a more elongated shape and were rough-surfaced. Despite morphological variations between the starches from different fruits, granules from the same source, but different batches, were similar in appearance.

Granules obtained from many of the seed varieties were round on one side and polygonal on the opposite side, suggesting that the granules grew in clusters, where they had acquired the shape of neighbouring starch granules (Hartunian Sowa & White, 1992). Some of the starch granules were more sensitive to the extraction procedure than others, as suggested by the large percentage of broken granules of durian, jackfruit and longan (Fig. 1(a)–(c)).

In comparison with starches derived from other botanical sources (e.g. corn, tapioca), granules examined were all comparatively small, mean sizes ranging from 5 μm (durian) to 16 μm (mango); all except durian showed wide size distributions (Table 2), although not to the extent seen in some of the cereal starches. The size of durian granules was spread over a narrow range, all

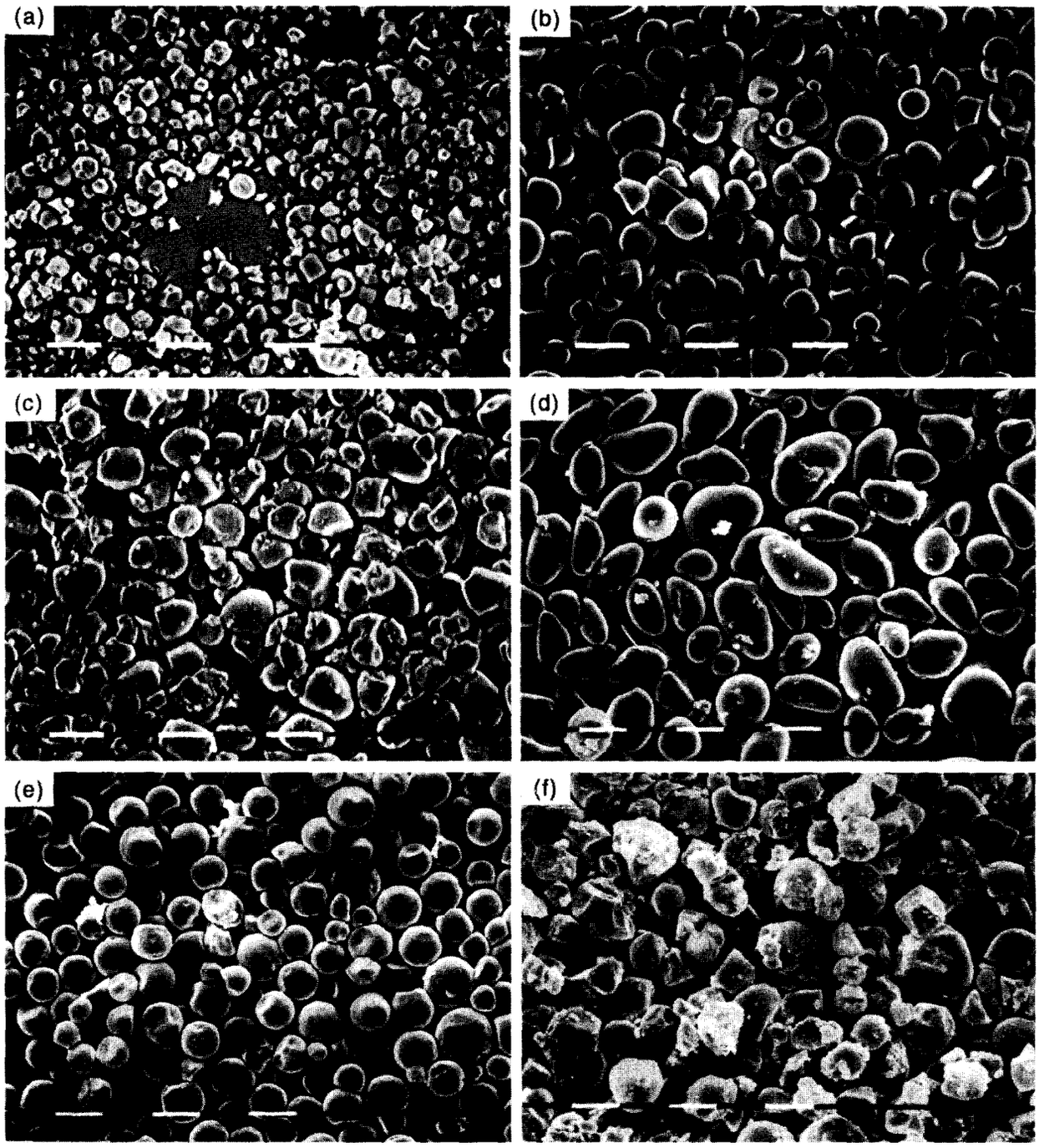

Fig. 1. Scanning electron micrographs of isolated seed starches: (a) durian; (b) jackfruit; (c) longan (first acid wash); (d) mango; (e) rambutan; (f) longan before acid washing. Bar denotes 0.1 nm.

granules falling within as 8 μm range. Wide size distribution is a common feature of most seed starches (Bewley & Black, 1985), and this may be a consequence of different environmental conditions during growth. If this is the case, then any commercial applications of this starch would have to take this into account. Little or no difference in mean size and size distribution between batches of each fruit type was evident, with the exception of mango which had a broader size range and larger mean size for one of the samples.

Differential scanning calorimetry (DSC) thermograms of the starches show characteristic endothermic transition enthalpies for native starches. All except jackfruit gave a single endothermic peak, characteristic of gelatinisation in the temperature range 30–120°C. Heating of both jackfruit starch batches was characterised by two endothermic peaks; the peak centred around 65°C representing gelatinisation, the second peak centred at about 80°C representing melting of the amylose–lipid complex. The high-temperature peak was present on re-heating, although diminished in size. Temperatures of gelatinisation, T_o, determined by DSC, ranged from 64.5°C to 78.3°C (durian 1 and mango 1, respectively) (Table 3). Little variation was found

Table 3. Thermoanalysis of starches extracted from tropical seeds (average of five replicates ± standard deviation)

Starch source	Thermoanalysis			Birefringence
	T_o (°C)	H_g (J g^{-1})	$T_{1/2}$ (°C)	98% loss (°C)
Jackfruit 1	64.7–0.14	2.87–0.18	7.5	70.6
Jackfruit 2	65.8–0.14	2.35–0.21	6.6	73.7
Durian 1	64.5–0.14	7.61–0.25	5.8	64.1
Durian 2	66.5–1.06	9.45–0.35	5.2	60.2
Mango 1	78.3–0.09	11.30–0.42	5.2	64.0
Mango 2	75.0–0.70	11.55–1.62	4.4	64.0
Longan 1	71.6–0.41	8.50–0.76	7.9	60.0
Longan 2	70.6–0.10	2.35–0.35	4.5	60.9
Rambutan 1	69.0–0.26	12.4–1.00	5.5	62.1
Rambutan 2	70.8–0.07	5.80–0.14	4.7	60.6

Table 4. Macromolecular composition of tropical seed starches

Starch source	Retention time (min)		Apparent MW of amylose	Amylose (%)	
	Amylopectin	Amylose		HPSEC	Iodine binding
Jackfruit 1	13.4	22.5	1.6×10^6	27.0	27.7
Jackfruit 2	13.1	23.3	3.2×10^5	28.1	28.5
Durian 1	13.5	23.4	2.5×10^5	28.3	29.0
Durian 2	13.6	22.5	1.6×10^6	23.1	25.2
Mango 1	13.8	23.6	1.6×10^5	21.1	20.3
Mango 2	13.6	23.2	4.0×10^5	29.0	26.9
Longan 1	13.7	22.6	1.2×10^6	27.7	26.0
Longan 2	13.4	23.4	2.5×10^5	26.5	27.7
Rambutan 1	13.1	23.6	1.6×10^5	22.8	26.4
Rambutan 2	13.4	23.1	5.0×10^5	21.5	27.0

between batches derived from the same botanical source. Temperatures of gelatinisation of the seed starches ranked in the order: mango > longan > rambutan > durian > jackfruit. Gelatinisation characteristics determined by monitoring loss of birefringence showed considerably less variability between botanical sources (Table 3). Enthalpy of gelatinisation was found to be dependent on botanical source and batch, ranging from 2.4 J g^{-1} to 12.4 J g^{-1} (Jackfruit 2 and Rambutan 1, respectively). At high water contents (>80%, w/w), enthalpy values will account for crystalline melting (Cooke & Gidley, 1992). The low enthalpy of gelatinisation for jackfruit may represent the occurrence of two thermally competing processes: endothermic melting of starch crystallites and exothermic formation of amylose–lipid complexes. Heating of longan seed starches in the presence of excess water did not appear to be associated with dissociation of the amylose–lipid complex in the temperature range examined.

Contrary to the reported similarities between DSC transition temperature and birefringence end-point by a number of authors (Biliaderis, 1990), birefringence end-point temperatures for the seed starches did not correlate with the DSC transition temperatures. Values ranged between 60.0°C and 73.7°C (Table 3). Both measures of gelatinisation monitor different events in the gelatinisation process.

With the exception of rambutan, the amylose contents determined by iodine–amylose complexing and HPSEC were in agreement. They ranged from 20.3% to 30.5% and from 21.1% to 30.6%, by iodine binding and HPSEC methods, respectively (Table 4). Amylose contents of the seed starches were similar to values reported for other seed starches (e.g. corn, 28–30%; Lineback, 1984). Despite little inter-variety variation in amylose contents, two of the starches, durian and mango, showed significant variation in amylose contents between the different batches. Retention time for the amylose fractions was 22.5–23.6, indicating the large

Table 5. Starch granule hydration properties and hydrolysis of tropical seeds starches

Starch source	Hydrolysis (%)	Solubility (%)	Swelling (%)
Jackfruit 1	41.9	18.0	30.4
Jackfruit 2	39.9	18.0	32.4
Durian 1	77.0	19.5	60.5
Durian 2	79.8	—	100.0
Mango 1	54.5	22.5	46.0
Mango 2	—	18.0	37.2
Longan 1	35.2	18.0	20.9
Longan 2	42.7	19.5	33.5
Rambutan 1	46.5	4.5	12.2
Rambutan 2	36.7	13.5	40.0

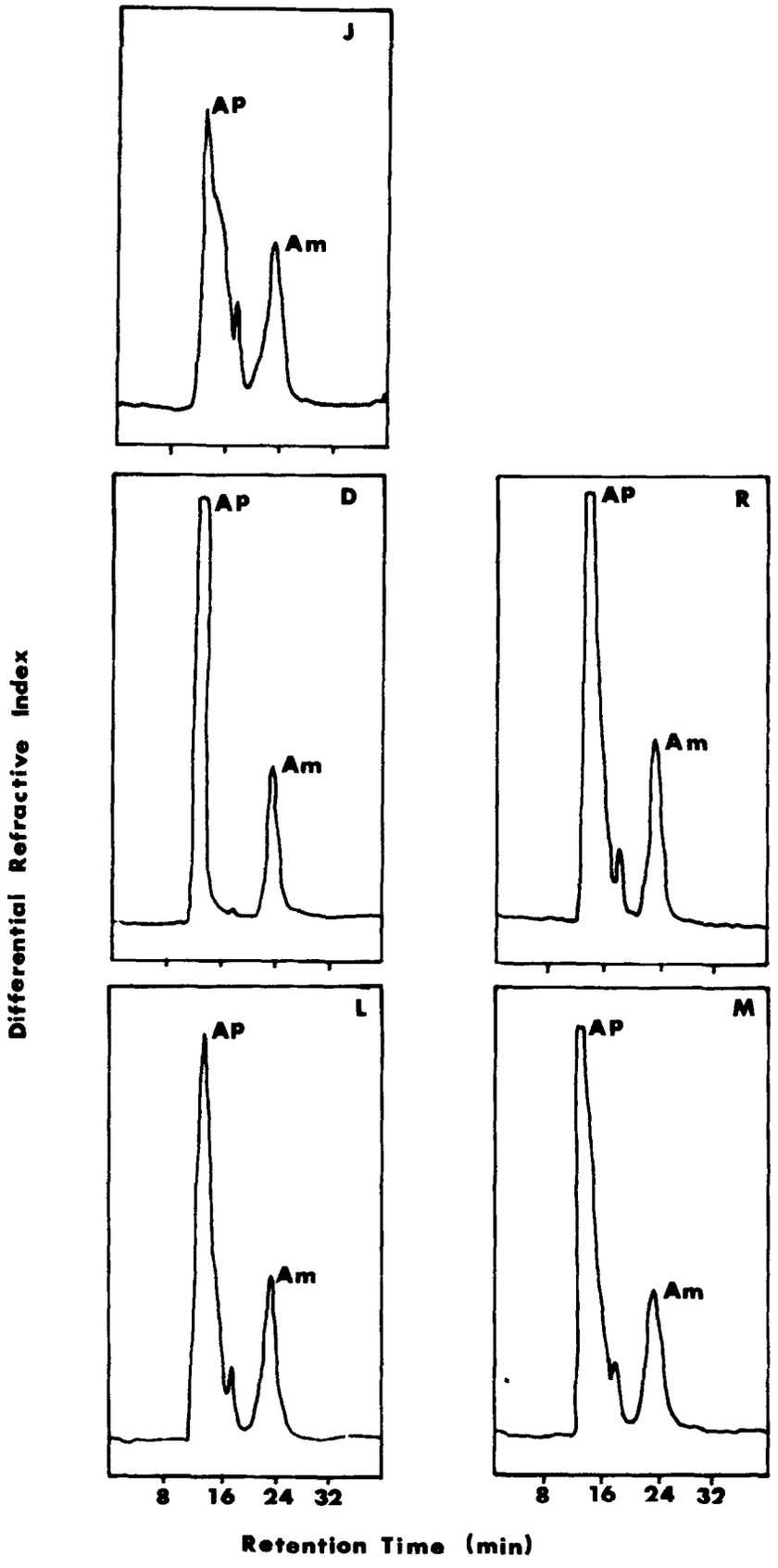

Fig. 2. HPSEC chromatograms of solubilised seed starches: (J) jackfruit, (D) durian, (R) rambutan, (L) longan, (M) mango. Ap, amylopectin; Am, amylose.

range of apparent molecular weights of 1.6×10^5 (mango and rambutan) to 1.6×10^6 (durian and jackfruit) (Fig. 2 and Table 4). These values were based on calibration using linear polymer standards. Since variations in hydrodynamic volumes were not taken into account, these values should be considered as approximations.

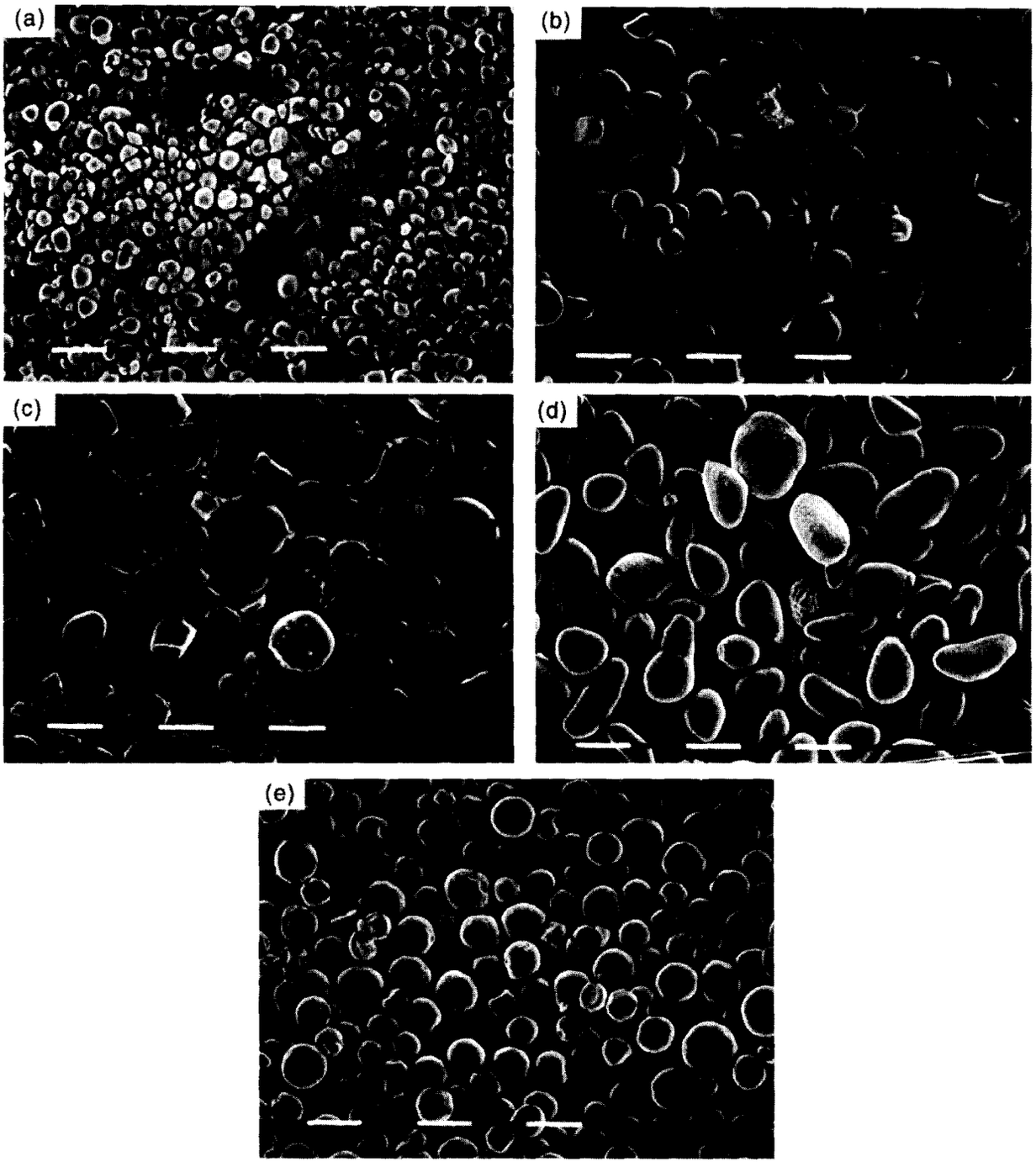

Fig. 3. Scanning electron micrographs of hydrolysed seed starch granules: (a) durian; (b) jackfruit; (c) longan; (d) mango; (e) rambutan. Bar denotes 0.1 nm.

The amylopectin of all the starches was associated with a fraction of slightly lower molecular weight, assumed to be intermediate material (Fig. 2), the proportion of which varied slightly between samples.

Solubility of the starches at 30°C was negligible and granular swelling minimal (below 2%). Solubility of the starches at 90°C increased to 18.0–22.5% (Table 5), highlighting similarities between the starches. The solubilities are in the range of values reported for other seed starches (Hizukuri *et al.*, 1988; El Faki *et al.*, 1983). Swelling behaviour, in contrast, was highly dependent on botanical source, between 12.2% and 100% (rambutan 1 and durian 1, respectively). The order of swelling was durian > mango > rambutan 2 > jackfruit = longan > rambutan 2 (Table 5). The various extents of swelling may be indicative of differing strengths of polymer interactions within the granule suggested by thermoanalysis. Tighter polymer interactions within the rambutan granule may give a more cohesive intra-granular architecture compared to durian. The size of the granule and hence available surface area can have a significant influence on the reactivity of the granule.

Hydrolysis of the starches under the standard conditions employed was highly variable (longan, 35.2%;

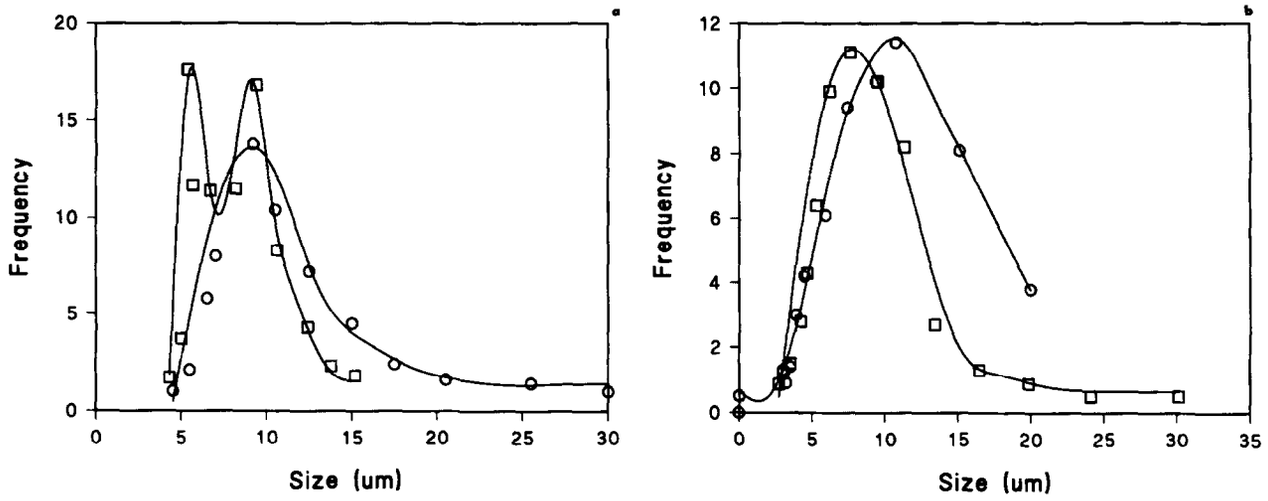

Fig. 4. Typical size distribution profiles for the two major classes of hydrolysed tropical seed starches: (a) jackfruit, bimodal population pattern; (b) rambutan, single population. □, native granules; ○, hydrolysed granules.

durian,79.8%; Table 5). Two of the starches showed differing extents of hydrolysis between samples: longan (35.2%, sample 1; 42.7%, sample 2) and rambutan (46.5%, sample 1; 36.7%, sample 2). This may reflect variation due to genetic or environmental factors which, if shown in other properties of the starches, would have to be addressed if these starches had a functional property suitable for a particular application. Hydrolysis of all other starches was within 2% for the same botanical source. The relative order of hydrolysis was durian > mango > rambutan > jackfruit > longan, similar to the order for granule swelling and suggesting comparable mechanisms for access to the granule interior.

In all of the samples some of the granules showed surface erosion (Fig. 3). The mean sizes of rambutan (samples 1 and 2) starch granules were reduced slightly when measured using a Coulter counter (Table 2 and Fig. 4(a)). All other seed starch granules appeared to have two separate populations with different susceptibilities to hydrolysis: one population had a mean size similar to that of the native granule; the second population had a lower mean size, and was presumably composed of hydrolysed starch granules (Table 2 and Fig. 4(b)). The pattern and extent of enzyme hydrolysis for corn and tapioca starches were comparable to those reported previously (results not presented).

Attempts to correlate the contributions of different granule properties with susceptibility to enzyme hydrolysis were made. Granule size, amylose/amylopectin ratio, degree of crystalline structure and degree of polymerisation of the granule components have been proposed to be critical factors in the hydrolysis of starch granules (Hoover & Sosulski, 1985). Many of these studies have used granules obtained from different storage tissues as well as botanical sources. Data presented in this study only partly support this proposal. Correlation analysis (Table 6) of the present data suggests an association between the ability of the granule to swell at 90°C and the rate of hydrolysis at 37°C ($R^2 = 0.86$, $P = 0.0007$). There was also a correlation between

Table 6. Correlation analysis[a] of granular characteristics

	Swelling	Solubility	T_O	H_g	Amylose content	Lipid content	Granule size	Hydrolysis
Hydrolysis	0.859	0.373	−0.351	0.415	−0.368	−0.406	−0.684	**1.000**
	0.007	0.258	0.290	0.209	0.266	0.216	0.02	**0.000**
Granule size	−0.645	0.061	0.703	−0.028	0.461	0.081	**1.00**	
	0.032	0.859	0.0160	0.934	0.153	0.814	**0.000**	
Lipid content	−0.352	0.006	−0.068	−0.330	−0.150	**1.00**		
	0.289	0.986	0.842	0.321	0.659	**0.000**		
Amylose content	−0.158	0.340	0.353	−0.458	**1.000**			
	0.642	0.307	0.287	0.156	**0.000**			
H_G	0.157	−0.218	0.412	**1.000**				
	0.644	0.520	0.208	**0.000**				
T_G	−0.315	0.055	**1.000**					
	0.346	0.872	**0.000**					
Solubility	0.541	**1.000**						
	0.086	**0.000**						
Swelling	**1.000**							
	0.000							

[a]Values given are R^2 (first row) and P (second row).

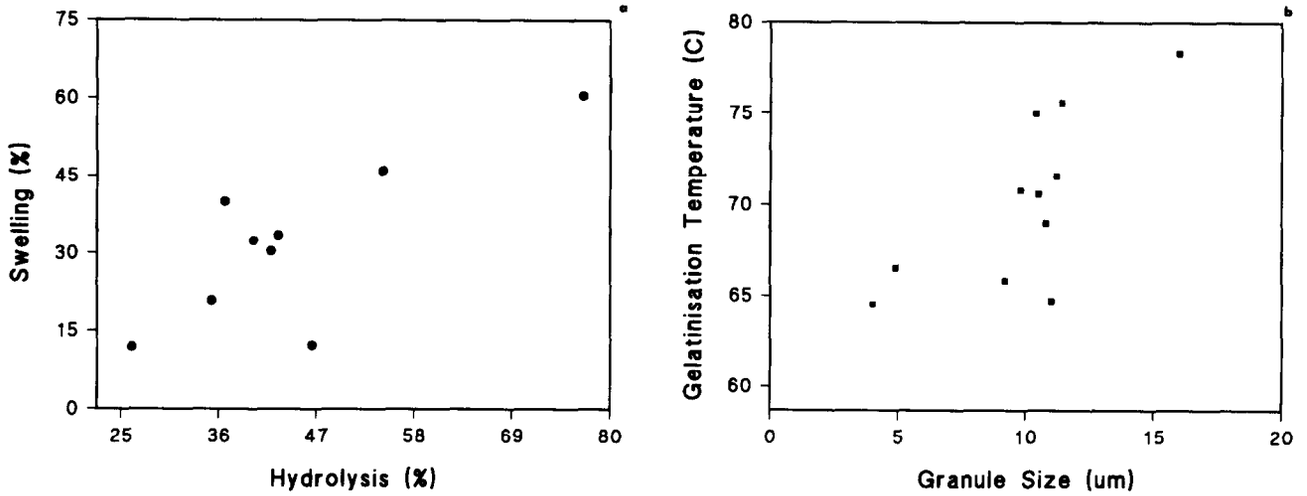

Fig. 5. Graphical representation of the main significant correlations: (a) granule swelling vs hydrolysis ($R^2 = 0.86$, $P < 0.01$); (b) gelatinisation temperature vs granule size ($R^2 = 0.7$, $P < 0.05$). Outliers are identified by reference to the seed source.

granule swelling and size of granule ($R^2 = -0.64$, $P = 0.03$). The size of the granule was correlated with gelatinisation temperature ($R^2 = 0.70$, $P = 0.015$). Amylose content seemed to have little effect on the extent of hydrolysis ($R^2 = 0.14$, $P = 0.27$).

Similarly, the enthalpy of gelatinisation was not a major factor in governing the extent of hydrolysis, swelling or solubility. The apparent lack of correlation between enzyme susceptibility or swelling and enthalpy of gelatinisation is somewhat surprising, if one assumes that the enthalpy of gelatinisation is an indication of extent of crystallisation. Factors other than crystallinity *per se* may govern granule swelling and hydrolysis. Accessible surface is important for both swelling and hydrolysis, although it will not account for all of the control in these processes. Lack of significant correlation between these properties and the enthalpy of gelatinisation may well explain the enzyme pattern, shown as surface erosion. Similarities in the comparative reactivity toward enzyme and swelling power suggest that the granules have regions accessible for hydration and enzyme activity. Access to the granular components would seem not to be a function of the extent of crystallinity but rather the spatial positioning of the crystalline regions within the granule.

Graphical representation of the significant correlations show that most starches are close to the regression line (Fig. 5(a) and (b)). Outlying points for all of the significant correlations were associated with the same seed type. These points, however, were from different seeds in the different graphs. This suggests that, despite the existence of an underlying mechanism of hydrolysis (shaving of the granular surface), structural characteristics unique to a particular species may dominate. Characteristics responsible for granular functionality are intricately interwoven in a unique fashion for each starch.

In conclusion, the seed starches were different in their properties and structural characteristics and the high lipid content of longan seed starches seemed to have little impact upon the functional properties of this starch. The seed starches share the same underlying mechanism of granular enzyme hydrolysis, namely shaving of the surface of the granule. Further work is required to determine whether this mechanism is the predominant mechanism for starches obtained from other plant tissues.

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