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Characterization of Starch from Tubers of Yam Bean (*Pachyrhizus ahipa*)

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Detailed studies of the starch present in tubers of six accessions of *Pachyrhizus ahipa* (ahipa) have been carried out using starches from tubers of *P. erosus* (Mexican yam bean) and seeds of ahipa and wheat for comparison. Starch accounted for 56−58% of the tuber dry weight with granules occurring in a range of geometric forms and in sizes from below 5 μm to about 35 μm (mean about 10 μm in all accessions except two). The amylose content ranged from 11.6 to 16.8% compared with 16.9% in *P. erosus* tubers and over 23% in the seed starches. X- ray diffraction analysis showed A-type or C_A-type diffraction patterns. The chain-length distribution of the amylopectin after enzyme debranching showed a peak at DP11 similar to that of wheat starch, but had a less marked shoulder at DP 21−22 and contained a higher proportion of longer chains. Differential scanning calorimitry showed an endothermic peak corresponding to gelatinization with T_{max} ranging from 59 to 63 °C, which was similar to the T_{max} of wheat (about 64 °C). The composition of the ahipa starch may mean that it is suitable for food applications that require low amylose content and low retrogradation after processing.

KEYWORDS: Storage tubers; yam bean; *Pachyrhizus*; food processing

INTRODUCTION

Leguminous plants are widely grown for their protein-rich seeds, forming the second most important group of crop plants after the cereals. However, some leguminous plants also have tuberous roots, notably species of the genus *Pachyrhizus* (yam bean) which is native to South and Central America. Three species of this genus are cultivated: *P. tuberosus* in South America (principally Bolivia, Puru, Equador, and Brazil), *P. erosus* (jacatube or Mexican yam bean) in Central America and the Caribbean, and *P. ahipa* (ahipa) in the Andes of Bolivia and Northern Argentina (*1, 2*). The yields of these species are up to 100 tonnes fresh weight per hectare, with dry matter contents of up to 25% (*1*). They are therefore considered to have potential for cultivation in countries ranging from the humid tropics to southern Europe (*1, 2*).

Previous studies have shown that *Pachyrhizus* tubers contain high amounts of starch and sugars and lower amounts of protein, although the precise values may vary with species and growth conditions. For example, eighteen accessions of *P. ahipa* grown in a glasshouse trial in Denmark contained an average of about 49% starch, 31% sugars, and 12.5% protein (on a dry weight basis), with the corresponding values for two accessions of *P. tuberosus* being about 56%, 16%, and 6%, respectively (*2*). In addition, it has been reported that starches from *P. ahipa* and *P. tuberosus* contain unusually low levels of amylose. In the glasshouse trial discussed above, starch from the *P. ahipa* lines was reported to contain an average of about 1.5% amylose (range about 0−4%), and starch from the *P. erosus* lines contained virtually zero. Similarly, Gruneberg et al. (*3*) reported that starch from thirteen lines of *P. ahipa* grown under glasshouse conditions in Germany contained about 1−6% amylose ,with two lines of *P. tuberosus* containing about 22.5% and 13%, respectively. Similar values of 13% amylose and 23% amylose have been reported for starch from *P. erosus* by Taderra et al. (*4*) and Melo et al. (*5*), respectively. It is notable that the values of 22−23% amylose are within the range of 20−30% reported for starches from seeds and tubers of a range of species (*6*), whereas the low amylose levels reported by other workers could be of considerable value as a replacement for waxy cereal starches in the food industry.

This work studied the composition and properties of *P. ahipa* in relation to its potential as a novel crop for growth in Europe. Therefore, a detailed analysis was carried out on starch fractions from tubers of six accessions of *P. ahipa* grown under glasshouse conditions in the UK, using starch from seeds of *P.*

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ahipa and wheat and from tubers of a single cultivar of *P. erosus* for comparative purposes.

MATERIALS AND METHODS

Plant Material. Seeds of six accessions of *P. ahipa* (AC102 and AC526, Argentinand AC524, National Botanical Garden, Belgium) were provided by Mr. B. Ørting (Department of Botany, Dendrology and Forest Genetics, The Royal Veterinary and Agricultural University (KVL), Denmark). *P. erosus* (cv. Catalina) was supplied by Dr. F. Hosein (Department of Plant Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago, West Indies). These seeds were sown in 10-cm (0.3-L) pots containing a mixture of a peat-based compost (Levington M2) and a slow release fertilizer (Osmocote, 4.1 g/L). The plants were kept at 25−30 °C with a 16-hour day. Once established (about 6 true leaves), the plants were transferred to 20-cm pots (5-L) containing a support and watered automatically via capillary matting. The six accessions of *P. ahipa* were grown in a random block design (5 pots × 6 accessions) with reproductive pruning to encourage tuberization (*7*) and minimize the effect of day length. *P. erosus* was grown under similar conditions, whereas seeds of AC230 were harvested from unpruned plants. Wheat starch was obtained from Sigma (S-5127, Red Hard Wheat).

Starch Granule Preparation. Two methods were used to prepare the starch. Method A was modified from Edwards et al. (1995). Samples of tuber (200 g, peeled and chopped) or seed (600 g, seed coat removed) were homogenized with 500 mL of buffer (50 mM Tris/HCl, pH 7.5, containing 1 mM diaminoethanetetraacetic acid (EDTA), 1 mM dithiothreitol (DTT), and 0.1% (w/v) sodium metabisulfite). The homogenate was filtered through four layers of muslin, and the filtrate was centrifuged for 5 min (10000*g*). The supernatant was discarded, and the starch was re-suspended in buffer (50 mM Tris/HCl, pH 7.5, containing 1 mM EDTA and 1 mM DTT), then centrifuged for 5 min (10000*g*). This was repeated a further three times. After the last wash with buffer, the starch was re-suspended in cold acetone and left to settle at −20 °C, and the supernatant was then discarded. This was repeated twice and the starch was allowed to air-dry. Method B, modified from Banks and Greenwood (*6*), was used to prepare starch suitable for calorimetry and for the preparation of nongranular starch, as it did not include EDTA, DTT, or sodium metabisulfite, which may have interfered with the assays. Samples of tuber (200 g, peeled and chopped) and seed (600 g, seed coat removed) were homogenized with 500 mL of buffer (50 mM Tris/HCl, pH 7.5, containing 1 M NaCl). The homogenate was filtered through 4 layers of muslin and washed with 500 mL of buffer. The filtrate was centrifuged for 5 min (10000*g*), the supernatant was discarded, and the residue was re-suspended in buffer and toluene (8:1, v/v). This was shaken overnight, then allowed to settle; the supernatent was removed, and the residue was re-suspended (buffe/toluene, 8:1). This was repeated until the supernatant and starch were visibly clean (approximately 10 times). After the final wash, the starch was re-suspended in cold acetone and allowed to settle; the supernatant was removed and the starch was air-dried.

Starch Granule Cracking. Starch (0.3 g, Method A) was mixed with 1 mL of water, added to liquid nitrogen in a pre-cooled mortar, and ground hard until the slurry started to defrost. The mortar was refilled with liquid nitrogen and the grinding was repeated. This process was repeated three times. The starch was then allowed to defrost and the mortar was rinsed out with water to ensure all the starch was removed. The resulting starch/water mixture was centrifuged for 5 min (10000*g*), the supernatant was removed, and the starch was re-suspended in 1 mL of water. Buffer (0.5 mL, 100 mM sodium acetate, pH 5.5) was added to 0.5 mL of the re-suspended starch and mixed with 10 units of α-amylase (Roche/Boeringer, pig pancreas). The sample was digested for 5 h at 25 °C, and aliquots were removed at 30-min intervals after 3 h. The aliquots were washed 3 times in acetone and allowed to air-dry.

Determination of Granule Size. Starch (0.3 g) prepared using Method A was mixed with 1 mL of water, placed on a microscope slide, and covered with a coverslip. Individual starch granules were observed using a Zeiss Axiovert 135 microscope connected to a JVC camera. Starch granule measurements were made and recorded using Leica Qwin software. From each sample, 500 granules were measured using granule edge detection across the widest point. Starch granules were also observed under polarizing light using a Leica DMR/B microscope.

SEM of Whole Starch Granules and Cracked Granules. Whole starch granules extracted using Method A were mounted onto stubs, coated with gold (0.1 nm, Polaron sputter coater) and observed using a Philips 501B SEM. Cracked starch granules were observed using a field emission scanning electron microscope (Philips XL 30 FEG-SEM). The samples were coated with 15−20 nm of gold using an Agar high-resolution sputter coater. In both cases images were recorded digitally.

Preparation of Nongranular Starch. Water (1 mL) was added to 100 mg of starch (prepared using Method B) and mixed thoroughly. Once the starch was dispersed, 9 mL of dimethyl sulfoxide was added and the sample was mixed. The sample was placed in a boiling water bath until the starch had dissolved (approximately 90 min), with frequent mixing to aid solubilization. The sample was removed from the water bath and allowed to stand at room temperature for 5 min. The starch was then precipitated by the addition of 96% (v/v) ethanol (40 mL) and placed at 4 °C for 15 min. The sample was centrifuged for 10 min (3000*g*), the supernatant was discarded, and the ethanol was drained off for a further 10 min. The starch was redissolved in 90% DMSO, mixed, and heated for 30 min in a boiling water bath. The starch was reprecipitated by the addition of ethanol; the mixture was centrifuged and allowed to air dry.

Separation of Amylose and Amylopectin. With mixing, 1 M KOH was added to the solubilized starch at 2 °C until the starch was dissolved; and then the mixture was diluted with water to give a 10-mM solution. A 2-mL aliquot of this solution was loaded onto a 0.5 × 100-cm column of Sepharose CL-2B and eluted with water at 20 mL/h. Fractions were collected at 40-minute intervals and were tested for the presence of starch using Lugol solution (iodine/potassium iodide) and a spectrophotometer at 500 nm. Fractions from the first peak (containing amylopectin) were pooled and freeze-dried.

Total Starch Analysis. The total amount of starch present in tubers and seeds was determined using a kit (Sigma STA-20) which utilizes the α-amylase−amyloglucosidase−glucose oxidase−peroxidase enzymic assay based on the AACC (1995) method.

Amylose Determination. The percentage amylose was determined using an amylose/amylopectin assay kit (Megazyme, K-AMYL) which removed any lipids by ethanol precipitation and amylopectin using concavalin A. The remaining amylose was converted to glucose, which was assayed using glucose/peroxidase reagent, and the absorbance was read at 510 nm.

Dry Weight Determination. The amount of moisture in the starch samples (prepared using method B) was calculated after drying at 60 °C overnight over phosphorus pentoxide in a vacuum oven (Heraeus Vacutherm Series 6000 Type M).

Debranching of Amylopectin. Samples (5 mg) of freeze-dried amylopectin were weighed into a Sovirel tube and 0.375 mL of water was added. The tube was capped and heated to 135−140 °C for 15 min until the solution was clear. After the solution cooled, 25 μL of buffer (sodium acetate 1 M, pH 3.6) and 1 μL of isoamylase (Sigma I2758) were added. The sample tube was capped and incubated at 37 °C for 18 h, heated to 100 °C for 10 min, and allowed to cool, and 0.5 mL of water was added. The solution was filtered through a 0.2-μm syringe filter and labeled solution 1.

HPLC Analysis of Amylopectin. Analysis of solution 1 (see above) was carried out using a Carbopac−PA100 ion exchange column using a Gilson gradient chromatography system with a Degas eluent module and a Dionex pulsed amperometric detector (PAD-2). The eluents used were as follows: A, 100 mM NaOH; B, 100 mM NaOH containing NaOAc with an 11−36% gradient over 66 min. The results were recorded using Gilson 715 HPLC software. The pulse potentials and durations were as follows: $E_1 = 0.05$ v 420 milliseconds, $E_2 = 0.8$ v 180 milliseconds, and $E_3 = -0.15$ v 360 milliseconds. The sensitivity detector was set at 1 KnA. Sample volumes of 20 μL (1 mg/mL) were loaded onto the column. A standard containing maltotriose, maltopentaose, and maltoheptaose (Sigma) was run under the same conditions.

Calorimetry. Thermal studies of starch gelatinization were carried out using a Perkin-Elmer robotic system DSC7 RS. Starch samples (5

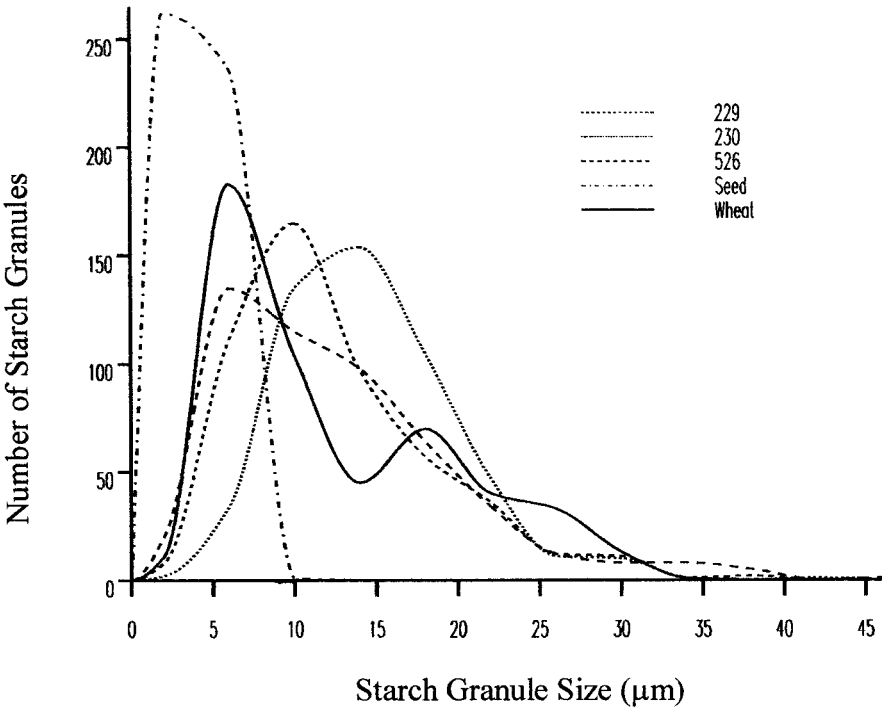

Figure 1. Size distribution of starch granules from *P. ahipa* tubers (AC229, 230, 526), seed (AC230), and wheat.

Table 1. Contents and Characterization of Starch from Tubers of *P. ahipa* and *P. erosus*, and Seeds of *P. ahipa* and Wheat

species	accession/ cultivar	organ	starch (%) (± SD)	amylose (%) (± SD)	X-ray diffraction patterns	T_{max} °C	ΔH (Jg^{-1})	mean granule size (μm)
P. ahipa	102	tuber	57.71 ± 0.66	16.83 ± 0.99	A/C_A	62.79	12.48	12.29
P. ahipa	229	tuber	56.06 ± 0.32	15.64 ± 0.82	A/C_A	60.68	10.89	12.53
P. ahipa	230	tuber	55.94 ± 1.51	17.37 ± 0.40	C_A	58.87	11.88	14.63
P. ahipa	521	tuber	56.13 ± 1.38	14.6 ± 0.96	A	66.29	12.52	13.95
P. ahipa	524	tuber	57.21 ± 1.02	11.57 ± 0.81	A/C_A	61.22	13.03	13.58
P. ahipa	526	tuber	57.94 ± 0.26	13.13 ± 0.63	A	58.78	11.57	12.63
P. erosus	catalina	tuber	58.65 ± 0.12	16.86 ± 0.88	C	65.46	14.23	9.34
P. ahipa	230	seed	3.48 ± 0.23	23.65 ± 0.93	A	68.48	9.14	3.9
wheat		seed	-	23.15 ± 1.30	-	63.67	10.38	12.36

mg, prepared using Method B) were weighed using a Mettler ME30 balance into a 50-μL aluminum pan with a 2-bar pressure limit. Water (20 μL) was added and the moisture was allowed to equilibrate overnight. The sample pan and reference pan containing air were sealed (Perkin-Elmer sealer) and scanned at 10 °C min^{-1} over the temperature interval 5−100 °C. The cells were then rapidly cooled (50 °C/min) to the starting temperature and rescanned (3 replicate measurements were carried out for each sample). The instrument was calibrated for heat capacity measurements with a sample of artificial sapphire (C_p 0.775 $J \cdot g^{-1} \cdot K^{-1}$ at 25 °C). Heat flow calibration was achieved using indium (T_m 156.6 °C), dodecane (T_m −9.65 °C), and octadecane (T_m 28.24 °C)

X-ray Diffraction Analysis. X-ray diffraction measurements were carried out using radiation of wavelength 0.154 nm. The X-ray generator was a Philips Scientific PW 1730, run at 40 kV and 30 mA. The diffractometer was a Philips Scientific PW 1820 vertical goniometer with an Anton Paar TTK camera. All measurements were carried out at ambient temperature and humidity. Samples were scanned from 5 to 42° 2θ at a speed of 0.005° 2θ/sec, with a step size of 0.1° 2θ. Data were collected using a proportional detector, then stored and processed on a PC using Philips (Version 3.6b) PC-APD software. This software was also used to identify the position of each diffraction peak in the patterns obtained.

RESULTS AND DISCUSSION

Plant Material. Tubers were bulked from five replicate pots each of the six accessions of *P. ahipa* and one cultivar of *P. erosus* (cv Catalina), and seeds were bulked from five pots of a single accession of *P. ahipa* (AC230). The total starch content, determined by enzymic digestion, ranged from about 56−58% dry weight for the *P. ahipa* tubers, with 58.6% and 3.5% for the *P. erosus* tubers and *P. ahipa* seeds, respectively (Table 1).

Starch Granule Size, Structure, and Morphology. Starch granules were prepared from the various samples of *Pachyrhizus* tubers and seeds using a method based on Edwards et al. (*8*) and compared with granules of commercial wheat starch. Optical microscopy using polarized light showed that the amount of starch damage was negligible, all granules showing typical "Maltese cross" patterns with no swollen granule ghosts being observed.

The *P. ahipa* preparations contained granules ranging in size from less than 5 μm to about 35 μm, with peaks at about 10 μm for all accessions except 526 (about 6 μm) and 230 (about 15 μm) (Figure 1, Table 1). A similar peak at about 10 μm was obtained for the *P. erosus* granules, but a lower proportion of granules larger than 20 μm was present. In contrast, the granules from *P. ahipa* seed were smaller (below 10 μm), whereas the wheat granules showed the bimodal distribution which is typical for this species (with peaks at about 6 and 18 μm).

Scanning electron microscopy showed that the granules from *Pachyrhizus* tubers had a range of geometric forms (a typical accession of *P. ahipa*, AC229, is shown in Figure 2A and *P.*

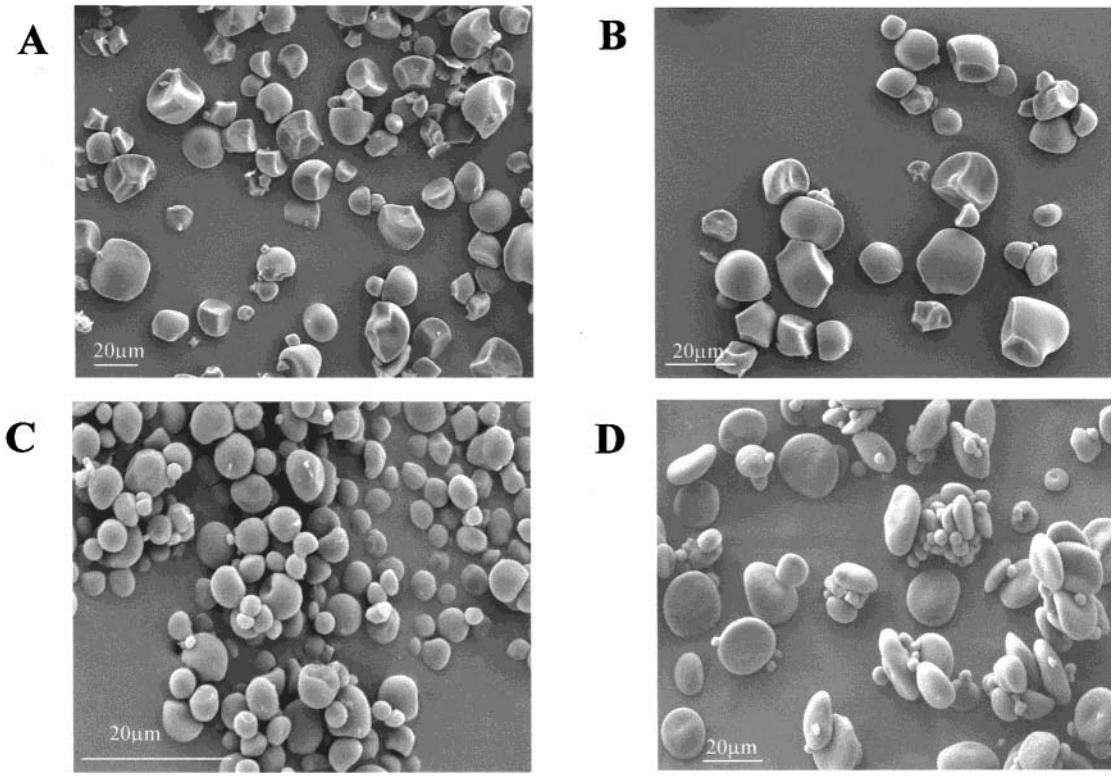

Figure 2. Scanning electron microscopy of starch granules from tubers and seeds. (A) *P. ahipa* (AC229) tuber starch. (B) *P. erosus* (cv. Catalina) tuber starch. (C) *P. ahipa* (AC230) seed starch. (D) Wheat seed starch.

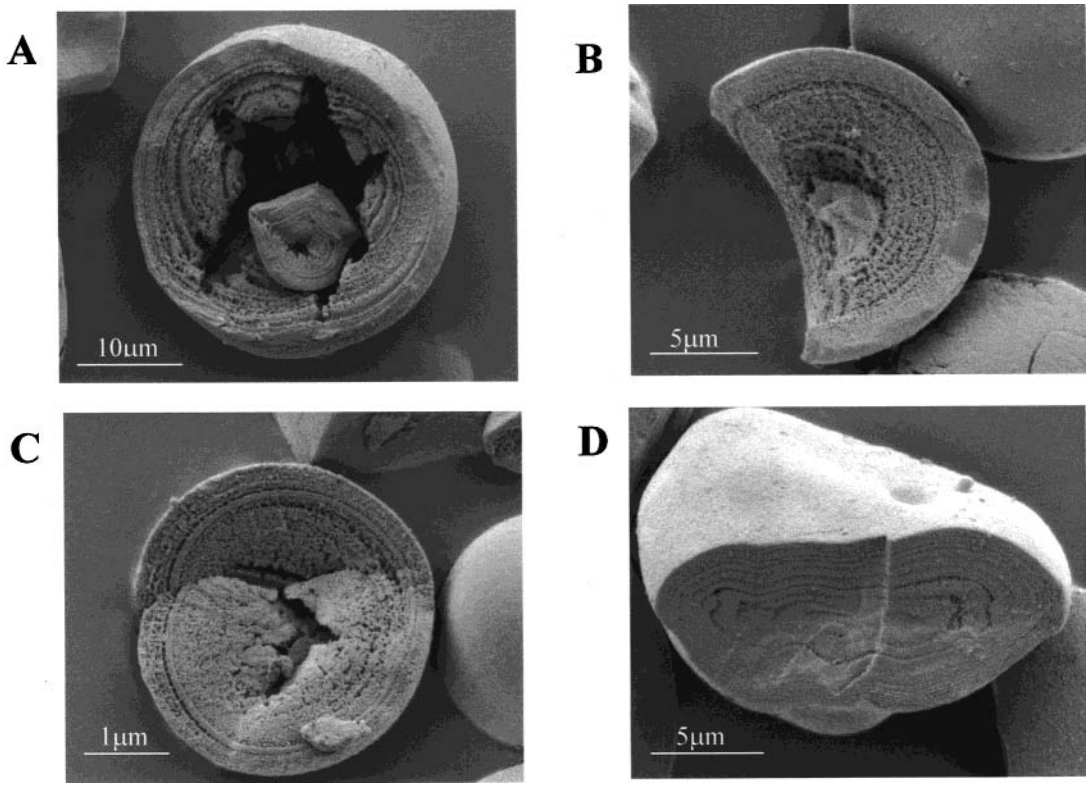

Figure 3. Field emission scanning electron microscopy of cracked starch granules from seeds and tubers. (A) *P. ahipa* (AC230) tuber starch. (B) *P. erosus* (cv. Catalina) tuber starch. (C) *P. ahipa* (AC230) seed starch. (D) Wheat seed starch.

erosus is shown in 2B) compared with the more regular morphology of the granules from seeds of *P. ahipa* and wheat seed granules (Figure 2C and D, respectively).

Granules were also cracked to reveal the ring structure by SEM (Figure 3). The starches from *P. ahipa* (AC230 is shown in Figure 3A) and *P. erosus* (Figure 3B) tubers, *P. ahipa* seeds (Figure 3C), and wheat seeds (Figure 3D) all showed the presence of growth rings.

Starch Composition, Structure, and Properties. Determination of the amylose content by enzymic digestion showed a

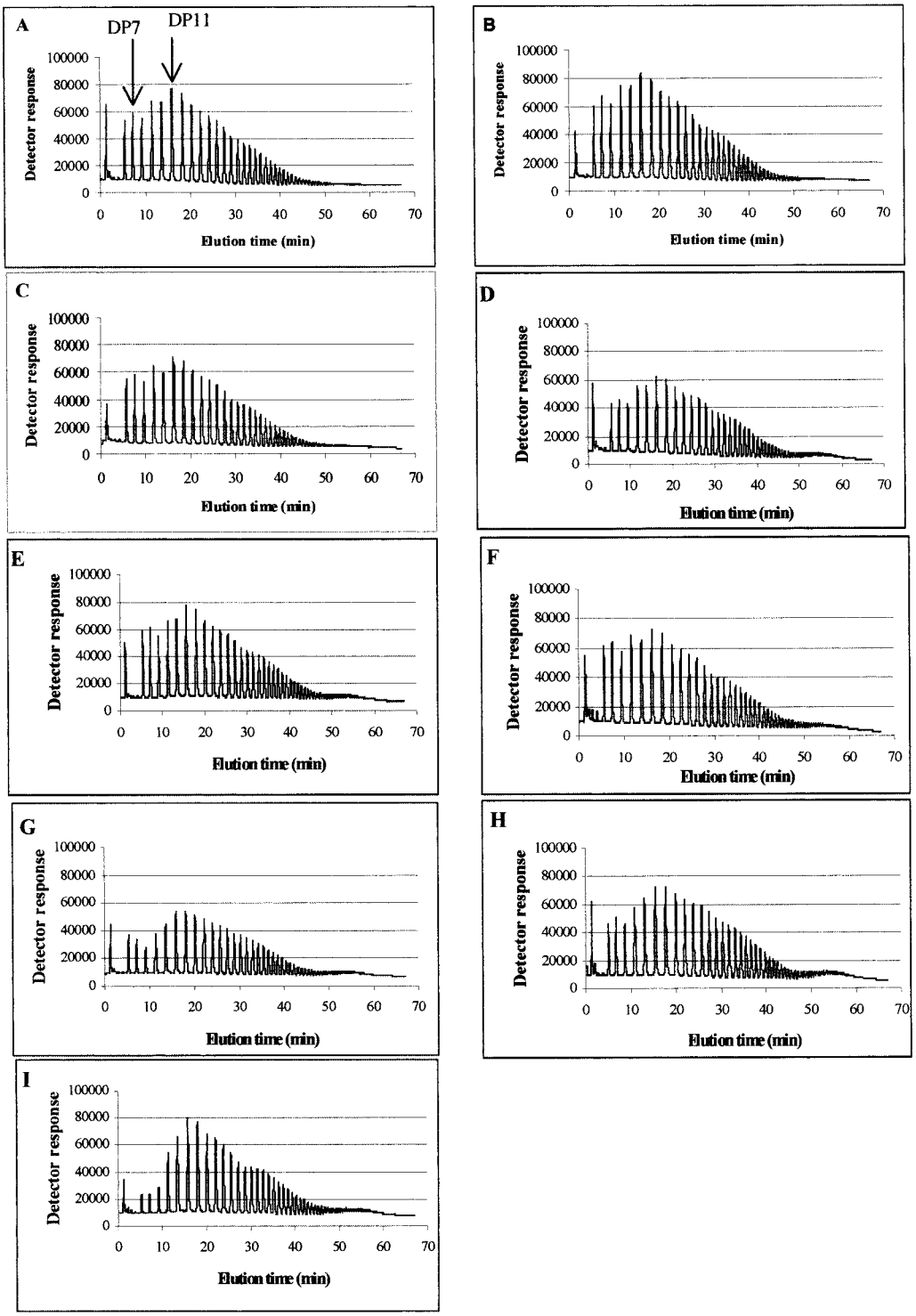

Figure 4. Chain lengths of starches from tubers and seeds of *Pachyrhizus* and seeds of wheat. A–F, from tubers of *P. ahipa* AC 102, 229, 230, 521, 524, and 526 respectively; G, from seed of *P. ahipa* AC230; H from tubers of *P. erosus* cv. Catalina; I, from wheat seed.

range from about 11.6 to 16.8% dry weight in *P. ahipa* tuber starch compared with about 16.9% in *P. erosus* and over 23−24% in the seed starches from *P. ahipa* and wheat (Table 1). The amylose contents of the tuber starches are generally lower than those commonly found in cereal starches, and more particularly legume starches such as smooth-seeded pea where the amylose content is generally in excess of 25% (*9*).

X-ray diffraction analysis was carried out on three separately prepared samples of the seed and tuber starches. Cereal starches generally give an A-type diffraction pattern, whereas most legume starches give a C-type diffraction pattern which is a mixture of the A and the B forms. This description may be further refined by indicating whether the C form has a tendency to A or B. Starches from *P. ahipa* seeds and from tubers of *P. ahipa* accessions 521 and 526 consistently showed A-type diffraction patterns, while starch from tubers of accession 230 consistently showed C_A-type patterns (i.e., C-type consisting of about 90% A-type and 10% B-type), and starch from *P. erosus* tubers showed C-type patterns. However, the tuber samples of the other three *P. ahipa* accessions gave different patterns for the three preparations, being either A-type or C_A-type.

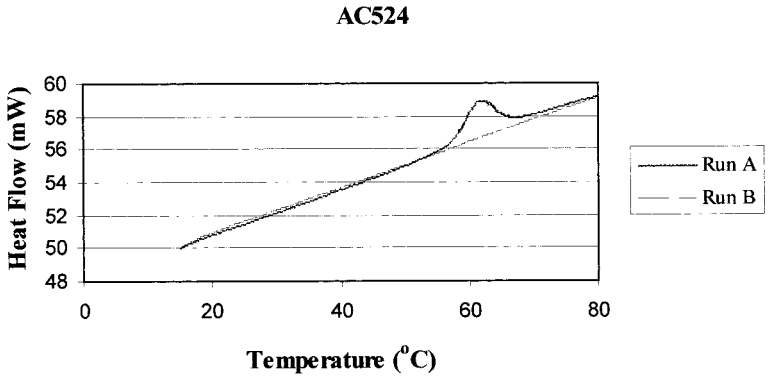

Figure 5. Differential scanning calorimitry of starch from tubers of *P. ahipa* AC524.

Amylopectin is a branched glucan which generally shows a broad range of chain legnths; the amounts and average degrees of polymerization of the chains are dependent on the botanical origin. Ion exchange chromatography was used to separate the constituent chains after debranching. An advantage of this approach is that it allows the separation of individual chain lengths, particularly at low degrees of polymerization (DP). A disadvantage is that the detector response falls with increasing DP. Figure 4 shows the separations achieved for the debranched amylopectins. For all starches, the long-chain fraction with an average DP of 45−50 units elutes between 45 and 60 min and the short-chain fraction elutes between 5 and 45 min. For wheat starch the short-chain fraction contains two populations. The main population has a peak DP of 11 with a marked shoulder at DP 21−22. The peak DP of the short-chain fraction is similar for the yam bean starches (Figure 4), but the shoulder at DP 21−22 is less marked, and chains of this average length form a greater fraction of the short-chain population. There is an association between the average chain length of the short-chain fraction and the crystalline form in the granule, with the form changing from A, to C, to B with increasing chain length (*10*). The chain length of the short-chain fraction is different from that found in starches giving a B-type diffraction pattern, with the observed chain lengths of the short-chain fraction being intermediate between that found for an A-type starch, such as wheat, and a legume starch giving a C-type X-ray diffraction pattern, such as smooth-seeded pea (DP15) (*11*). However, in the present case it is not possible to accurately predict the crystalline form (i.e., A, C_A, or C) simply from a knowledge of the chain-length distribution.

The crystalline form of starch, and the length of chain involved in the crystalline regions of the granule, also has a potential impact on the heat changes associated with gelatinization. A typical differential scanning calorimetry trace of endothermic heat flow versus temperature for the gelatinization of *P. ahipa* (524) starch is shown in Figure 5. An endothermic peak corresponding to gelatinization is observed, with a peak maximum, T_{max}, at 61 °C. In addition to T_{max}, the size of the endotherm, ΔH (Jg^{-1}), was measured and the data are summarized in Table 1. For the tuber starches of *P. ahipa*, T_{max} ranged from 59 to 66 °C. The tuber starch from *P. erosus* gave a T_{max} of 65.5 °C, and the seed starch from *P. ahipa* gave a T_{max} of 68 °C. At a fixed chain length the T_{max} of starch crystallites is dependent on crystalline form, and increases in the order B to C to A. In legume starches having a C-type diffraction pattern the regions of the granule giving a B-type diffraction pattern gelatinize before those regions giving an A-type diffraction pattern (*12*). In the present case two of the starches giving an A-type diffraction pattern (*P. ahipa* seed and tuber 521) have a relatively high T_{max} in agreement with expectation, but a second tuber starch (526) has a much lower T_{max}, suggesting that other factors are also influencing the observed behavior. The size of the gelatinization endotherm is broadly comparable with the observed behavior of wheat starch suggesting similar levels of granular order as assessed by calorimetry.

CONCLUSIONS

Yam bean tuber and seed starch were characterized using a range of physical and chemical techniques. Many of the granular and molecular features of the seed starch are consistent with the known properties of legume seed starches. However, the yam bean tuber starch is unusual in that it has a relatively low amylose content. This would suggest that it could find applications in foods that require low amylose content and low retrogradation after processing.

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