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Studies on tuber starches. II. Molecular structure, composition and physicochemical properties of yam (*Dioscorea* sp.) starches grown in Sri Lanka

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Abstract

Starch from varieties of *Dioscorea esculenta* (Lour.) Burkill (kukulala, java-ala, nattala) and *Dioscorea alata* L. (hingurala, raja-ala) tubers grown in Sri Lanka was isolated and its morphology, composition, structure and physicochemical properties were studied. The yield of starch was in the range 12.2–18.0% on a whole tuber basis. The shape and size of *D. esculenta* and *D. alata* starch granules were round to polygonal (3–10 μm) and spade shaped (30–45 μm), respectively. There was considerable variation in total amylose, phosphorous, bound lipid, amylose leaching, swelling factor, gelatinization parameters, extent of retrogradation, pasting characteristics, susceptibility towards enzyme and acid hydrolysis crystallinity and lipid complexed amylose chains between and among the starches belonging to the two species. The chain length distribution of debranched amylopectins of the starches showed that the proportion of short branched chains (dp 6–12), medium chains (dp 25–36) and average chain length of amylopectin were significantly ($P < 0.05$) higher in the *D. esculenta* starches. However, variations in amylopectin structure among varieties of both species were not significant ($P < 0.05$). *D. esculenta* starches exhibited a 'B'-type powder X-ray diffraction pattern. However, in the *D. alata* starches, hingurala and raja-ala displayed 'C'- and 'B'-type patterns, respectively. The results showed that some of the starches from the above species could be used in foods subjected to thermal processing and frozen storage, respectively, with minimal physical or chemical modification.

Keywords: *Dioscorea* starches; Molecular structure; Composition; Properties

1. Introduction

Starchy tubers and root crops are important subsidiary or subsistence food in tropical and sub-tropical countries. Although, a wide range of tuber crops are grown worldwide, only five species account for almost 99% of the total world production. These are potato (*Solanum tuberosum*, 46%), cassava (*Manihot esculenta*, 28%), sweet potato (*Ipomea batatas*, 18%), yams (*Dioscorea* spp., 6%) and taro (*Colocassia*, *Cytosperma*, *Xanthosoma* spp., 1%). *Dioscorea* (Family Dioscoreaceae) is a large genus consisting of about 630 species, nearly 40 *Dioscorea* varieties are grown in Sri Lanka (Jayasuriya, 1995; Senevirathne & Appadurai, 1966). Tubers of *Dioscorea* are commonly called true yams. *Dioscorea alata* and *Dioscorea esculenta* are the two major yam species widely cultivated in Sri Lanka. The stem of the trailing vine of *D. esculenta* is cylindrical, spiny and twines in an anticlockwise direction. The base of the stem contains a large number of tubers. Tubers are generally ovoid and cylindrical (Fig. 1). Among the *D. esculenta* varieties (kukulala, java-ala, nattala), kukulala is widely cultivated

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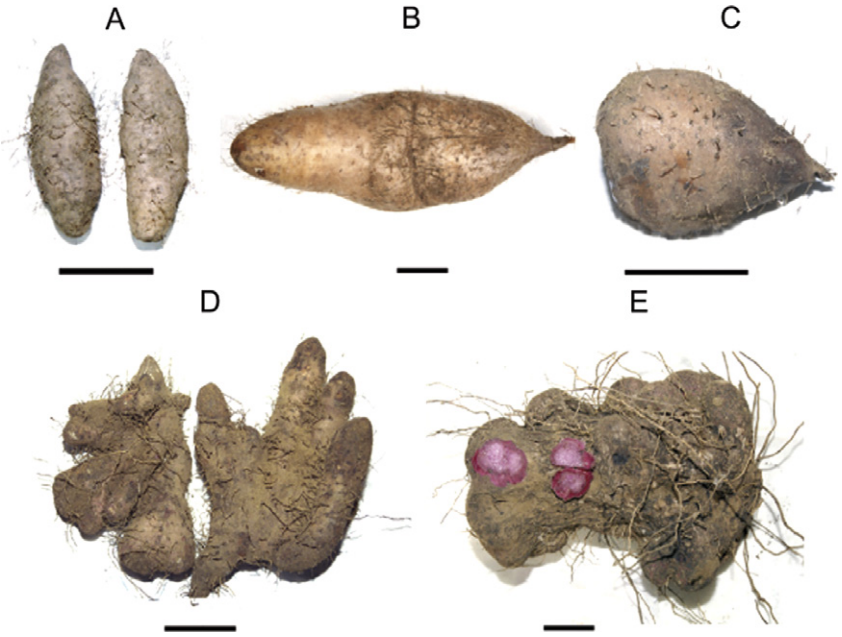

Fig. 1. Morphology of *D. esculenta* (kukulala [A], java-ala [B], nattala [C]), and *D. alata* (hingurala [D], raja-ala [E]) tubers. The scale bar represents 5 cm.

in Sri Lanka, due to its flavor and shorter maturity period. However, the other two varieties remain as 'wild types'. The average tuber size of kukulala, java-ala and nattala has been shown to vary from 100 to 300 g, 2 to 3 kg and 3 to 5 kg, respectively. The yield of kukulala varies from 28–30 t/ha. *D. alata* is a climber, an aerial stem that is square in cross section and twines in a clockwise direction. The fleshy underground stem contains only a large solitary tuber per vine. Raja-ala and hingurala are the two varieties of *D. alata* cultivated in Sri Lanka. The tubers of raja-ala and hingurala generally vary from 2 to 5 kg and 300 to 600 g, respectively. The yield of these two tubers vary from 20 to 24 t/ha (hingurala) and 16–20 t/ha (raja-ala). *Dioscorea* are well adapted to most tropical climates with temperatures of 30–34 °C and annual rainfall of 1500 mm. The "seed plant" of *Dioscorea* is normally planted with the first rains from the end of March to April (in early *Yala* season). The mature crop is usually ready for harvest around December–February (in late *Maha* season) with the signs of yellowing leaves and withered vines. *Dioscorea* tubers form an important component in the diet of individuals living in rural areas of Sri Lanka as a source of carbohydrates, protein, vitamins and minerals. The tubers are eaten boiled or as a vegetable dish. As starch is the major component of these tubers (60–85% dry basis) its properties would be expected to have major influence in the food, textile, paper and pharmaceutical industries in Sri Lanka. Furthermore, the small granule size of *D. esculenta* starches (<10 μm) makes them ideal as fillers in biodegradable plastics and talcum powders.

There has been several reports on true yam starches (Rasper & Coursey, 1967; Emiola & Delarossa, 1981; Gallant et al., 1982; Moorthy, 1999; Gebre-Mariam & Schmidt, 1998; McPherson & Jane, 1999; Farhat, Oguntona, & Neale, 1999; Afoakwa & Sefa-Dedeh, 2002; Alves et al., 2002; Rolland-Sabate, Amani, Dufour, Guilois, & Colonna, 2003; Amani, Buleon, Kamenan, & Colonna, 2004; Freitas, Paula, Feitosa, Rocha, & Sierakowski, 2004; Riley et al., 2004; Daiuto, Cereda, Sarmento, & Vilpoux, 2005; Brunnschweiler et al., 2005). However, there is a dearth of information on amylopectin chain length distribution, crystallinity, thermal stability, retrogradation characteristics and granule susceptibility towards acid and enzyme hydrolysis. A detailed knowledge of amylopectin structure and properties of true yam starches is essential in order to find food and industrial applications for these under utilized starches.

It is well known that genetic variations and environmental conditions influence starch structure and properties (Debon & Tester, 2000). Thus, structure–property relationships that have been established for starches from varieties of *D. alata* and *D. esculenta* grown in other countries may not be applicable to the varieties grown in Sri Lanka. Therefore, our objective was to determine the amylopectin structure, granule crystallinity, composition and physicochemical properties of different varieties of starches from *D. esculenta* (kukulala, java-ala, nattala) and *D. alata* (hingurala, raja-ala) grown in the same location (Algama) in Sri Lanka. It is hoped that this study would enable food processors to tailor the properties of *Dioscorea* starches by physical and/or chemical modification. Adding value by modification would enable Sri Lanka to compete more effectively in the markets in both food and non-food sectors. In addition, the results of this study may be beneficial to food processors in other countries where *Dioscorea* species are widely cultivated and consumed. Further, publications based on starches from the above varieties of *D. alata* and *D. esculenta* will deal with the effect of hydrothermal treatment on starch structure and functional properties.

2. Materials and methods

2.1. Materials

Tubers from *D. esculenta* (kukulala, java-ala, nattala) and *D. alata* (raja-ala, hingurala) were grown under the same field conditions in Algama, Sri Lanka. Crystalline porcine pancreatic α-amylase (type 1A, 790 U/mg protein), fungal α-amylase (157 U/mg protein) from *Aspergillus oryzae* were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Isoamylase (68,000 μ/mg protein) from *Pseudomonas amylodermosa* were purchased from Hayashibana Biochemical Laboratories Ltd. (Okayama, Japan). All chemicals and solvents were of ACS certified grade.

2.2. Methods

2.2.1. Starch isolation

2.2.1.1. Sampling. For starch isolation, well matured tubers from each variety of *D. alata* and *D. esculenta* were collected from three separate vines in late *Maha* season.

2.2.1.2. Starch extraction and purification. Dioscorea tubers were washed, peeled and fibrous roots removed. Immediately after peeling, the tubers were sliced into 2–3 cm cubes, soaked in potassium metabisulfite (50 mg/L) for 1 h, and then shredded in a Waring blender (model 33BL73, New Hartford, CT, USA) for 10 s at high speed and then at low speed for 15 s. The starch in the slurry was separated from the cell debris by vacuum filtration through a muslin cloth. The filtrate containing the starch was allowed to stand [~2 h (*D. alata*), ~12 h (*D. esculenta*)] at room temperature until a dense firm starch layer was obtained. The supernatant was siphoned and discarded and the precipitate was suspended in excess 0.02% sodium hydroxide. After standing (~4 h) the supernatant was removed. The washing-sedimentation process with alkali was repeated until the supernatant layer was almost free of color and suspended haze. The final sediment was suspended in deionized water, passed through a 70 μm polypropylene screen (Spectrum Laboratory Products, California, USA), neutralized to pH 7.0, filtered through a Buchner funnel and thoroughly washed on the filter with deionized water. The starch was air dried at room temperature and then passed through a 250 μm test sieve (Fisher Scientific Company, Mentor, OH, USA) to obtain a free flowing powder, which was weighed and the yield was calculated as the percentage of the initial tuber weight.

2.2.2. Granule morphology

2.2.2.1. Light microscopy. The size and shape of native starches were examined by a Leica Gallen III (Buffalo, New York, USA) light microscope. The range of granule size was determined by measuring the length and width of approximately 75 granules from a 1.0% (w/v) starch suspension (stained with 0.01 M iodine) at 1000× measured with an occular micrometer.

2.2.2.2. Scanning electron microscopy. The granule surface of the starches were examined by a FEI Quanta 400 environmental scanning electron microscope (Brno, Czech Republic). Starch samples were freed of granule clumps by sieving through a 250 μm mesh. The samples were then mounted on Cambridge type circular aluminum stubs with carbon electro-conductive adhesive tape (Electron Microscopy Science, Hatfield, PA, USA). The starches on the stubs were spread evenly by viewing the granules through a stereoscopic microscope (Carl Zeiss, Stemi 2000-C, Wek Gottingen, Germany), and then coated with gold (10 nm) for 60 s at 50 mA using a EMS500 sputter coater (Electron Microscopy Science, Hatfield, PA, USA). The granules were then examined under the following conditions: accelerating voltage of 5.0 kV, emission current 100 μA, high vacuum mode (10^{-4} Pa), spot size 2 (range of 1–10), working distance 10.5–10.7 mm, Mode 300 V. Granules within a horizontal field width of 54.08 μm were photographed at a magnification of 5000× using an image integration mode of 128 frames (2 frames/s or 50 μs/pixel/frame) and a Everhart–Thornley detector (ETD).

2.2.3. Proximate analysis

Quantitative estimation of moisture, ash and nitrogen contents were determined by the standard AACC methods (2000). Starch lipids (surface, bound) were determined by procedures outlined in an earlier publication (Vasanthan & Hoover, 1992). Amylose content (apparent and total) and starch damage was determined according to the method of Jayakody, Hoover, Liu, and Weber, 2005. Total starch phosphorus was determined by a slight modification of the method of (Jayakody et al., 2005). Starch (5 mg db) was placed into screw-capped tubes (calibrated at the 5 mL level) and digested with concentrated H_2SO_4 (0.3 mL) for 12 h at room temperature before charring. The partially digested samples were heated using a micro-Bunsen burner until charring was completed, and the climbing film of acid on the walls of the tubes was no longer viscous at the end of the digestion process. After the contents of the tubes had slightly cooled, hydrogen peroxide (30 μL, 30% [w/v]) was added (15 μL at a time) to hit the walls of the tube just above the digested mixture, and the tubes well shaken. The tubes were then boiled for 1 min. The solutions were allowed to cool to room temperature and the volume was made up to 3.6 mL with deionized water. For assay, anhydrous sodium sulfite [Na_2SO_3] (0.1 mL, 2.62 M) was added with stirring followed by addition of ammonium paramolybdate [$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$] (1.0 mL, 0.0162 M) and ascorbic acid (0.01 g). The contents of the tubes were vortexed vigorously and then heated for 10 min in a boiling water bath. After cooling to room temperature, the contents of the tubes were adjusted to 5.0 mL with deionized water, and then absorbance read at 822 nm using a UV–visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA). Four replicate and control samples were used in each determination.

2.2.4. Amylopectin branch chain length distribution

Isoamylase debranching of whole starch accompanied by high pressure anion exchange chromatography with pulsed amperometric detection (HAPAEC-PAD) was used to determine the branch chain length distribution of the *Dioscorea* starches following the procedure of Jayakody et al. (2005).

2.2.5. X-ray diffraction and crystallinity

Starches for X-ray diffraction measurements were kept in a desiccator (at 25 °C) over saturated K_2SO_4 (aw = 0.98) up to sorption equilibrium (3 weeks). X-ray diffractograms were obtained with a Rigaku RPT 300 PC X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan). The hydrated samples (0.5 g db) were packed tightly into an elliptical aluminum holder. The operating conditions were: target voltage 40 kV, target current 100 mA, aging time 5 min; scanning range 3–35°, scan speed 2.000° min; step time 4.55, divergence slit width 1.00; scatter slit width 1.00 and receiving slit width 0.6. Crystallinity of the starches was quantitatively estimated following the method of Nara and Komiya (1983) by using a software package (Orion-version 6.0 Microcal Inc., Northampton, MA, USA). A line connecting peak baselines was computer-plotted on the diffractogram. The area above the smooth curve was considered as the crystalline portion and the lower area between the smooth curve and a linear baseline was taken as the amorphous portion. The ratio of the upper area to the total diffraction area was calculated as the crystallinity. The moisture content of the samples was determined before and after scanning.

2.2.6. Differential scanning calorimetry

2.2.6.1. Gelatinization characteristics. Gelatinization parameters of native starches were measured using a Seiko differential scanning calorimeter (DSC 210) (Seiko Instruments Inc., Chiba, Japan) equipped with a thermal analysis data station and data recording software. Water (11 μL) was added with a microsyringe to starch (3.0 mg db) in the DSC pans, which were then sealed, reweighed and allowed to stand overnight at room temperature before DSC analysis. The scanning temperature range and the heating rates were 25–130 °C and 10 °C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference. During the scans, the space surrounding the sample chamber was flushed with dry nitrogen to avoid condensation. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c). The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed in terms of joules per gram of dry starch. Four replicates per sample was analyzed.

2.2.6.2. Retrogradation characteristics. Melting of retrograded amylopectin was also determined by DSC. The samples were prepared with a starch to water ratio of 1:1. After the initial DSC run, sample pans containing the gelatinized starch were covered in a single thin layer with Teflon7 film and then with a double layer of saran film. The covered pans were first immersed in a water bath at 4 °C for 24 h and then immersed in a water bath at 40 °C for periods ranging from 0 to 168 h. At the end of each time period, the covering films were removed and the stored samples were equilibrated at 25 °C for 1 h in a desiccator before reweighing and rescanning. The scanning temperature range and heating rate were identical to that used for the study of gelatinization parameters. Three replicates per sample was analyzed.

2.2.7. Swelling factor

The swelling factor was determined by a slight modification of the method of Tester and Morrison (1990). Starch samples (50 mg db) were weighed into 25 mL screw-capped tubes, deionized (5 mL) water added and heated in the range 60–90 °C in a constant temperature water bath for 30 min (the tubes were vortexed every 5 min to resuspend the starch slurry). The tubes were then cooled rapidly to 20 °C, blue dextran (0.5 mL) [Pharmacia, average MW 2×10^6, 5 mg/mL] was added and the contents gently mixed. The tubes were then centrifuged at 4500 rpm/10 min (IEC, Centra MP4 centrifuge, Madison, MA, USA). The absorbance of the supernatant was measured at 620 nm (Milton Roy, Spectronic-601, Rochester, NY, USA) against a reference without starch. The SF is reported as the ratio of the volume of swollen starch granule to the volume of the dry starch. Four replicate and controls samples were used in this determination.

2.2.8. Amylose leaching

Starches (20 mg db) in water (10 mL) were heated at 60–90°C in volume calibrated sealed tubes for 30 min. The tubes were then cooled to room temperature and centrifuged at 2000 rpm for 10 min. Supernatant was diluted 25 mL with deionized water and 2 mL was withdrawn and its amylose content determined according to the method of Jayakody et al. (2005). Percentage amylose leaching was expressed as milligram of amylose leached per 100 g of dry starch. Three replicate starch samples were used in this determination.

2.2.9. Pasting properties

A Rapid Visco™ Analyser RVA-4 (Newport Scientific Pty, Ltd., Warriewood, NSW, Australia) was employed to measure the pasting properties of starches (7% db, 27 g total weight). Experiments were performed using AACC method 76–21 (AACC, 2000), in which the sample is equilibrated at 50 °C for 1 min, heated at 6 °C/min to 95 °C, held at 95 °C for 5 min, cooled at 6 °C/min to 50 °C and held at 50 °C for 2 min. The speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment. Peak viscosity, final viscosity and pasting temperature of starches were compared from pasting curves. The reported values are the means of duplicate measurements.

2.2.10. Acid hydrolysis

Starches were hydrolyzed in triplicate with 2.2 M HCl (1 gdb, starch/40 mL, 2.2 M HCl) at 35 °C in a water bath (New Brunswick Scientific, G76D, Edison, NJ, USA) for periods ranging from 0 to 15 days. The starch slurries were vortexed daily to resuspend the deposited starch granules. Aliquots taken at specific time intervals were neutralized with 2.2 M NaOH and centrifuged (2000 rpm/10 min). The amount of total reducing sugar in the supernatant was determined by the method of Somogyi–Nelson method (Nelson, 1944; Somogyi, 1952). The extent of hydrolysis was calculated as shown below:

$$\text{Hydrolysis}(\%) = \frac{\text{Reducing sugar(as glucose)} \times 0.9 \times 100}{\text{Initial starch wt(g)}}$$

2.2.11. Enzymatic digestibility

Enzymatic digestibility studies on native starches were conducted using a crystalline suspension of porcine pancreatic α-amylase in 2.9 M sodium chloride containing 3 mM calcium chloride (Sigma Chemical Co., St. Louis, MO, USA) in which the concentration of α-amylase were 32 mg protein/mL and the specific activity was 1122 U/mg protein.

Starch granules (20 mg db) were suspended in 10 mL of 0.02 M phosphate buffer (pH 6.9) containing 0.006 M NaCl. A 5.5 μL of α-amylase suspension was added, the mixture gently mixed and digested at 37 °C in a water bath (New Brunswick Scientific, G76D, Edison, NJ, USA) for 72 h. The reaction mixtures were vortexed on a daily basis to resuspend the deposited granules. The digestion reaction was terminated by adding 5 mL of absolute ethanol to the digestion mixture. The hydrolysate was recovered by centrifugation (at 2000 rpm/5 min, IEC HN-SII centrifuge, Madison, MA, USA) of the mixture. Aliquots of the supernatant were analyzed for reducing sugar content (Nelson, 1944; Somogyi, 1952) starch. Controls without enzyme but subjected to the above experimental conditions were run concurrently. The reported values are the means of four replicates. The extent of hydrolysis was calculated as shown below:

$$\text{Hydrolysis}(\%) = \frac{\text{Reducing sugar (maltose)} \times 0.95 \times 1001}{\text{Initial starch weight}}$$

2.3. Statistical analysis

Analysis of variance (ANOVA) was performed by Tukey's HSD test ($P < 0.05$) using Statistical Software SPSS 12.0 for windows (SSPS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Granule morphology

Starch granules of *D. esculenta* and *D. alata* ranged in size from 3 to 10 μm and 30–50 μm, respectively (Table 1). Starch granules of *D. esculenta* were polygonal in shape (Figs. 2 and 3) whereas those of *D. alata* were truncated and spade shaped (Figs. 2 and 3). The granule surfaces of all starches appeared to be smooth and showed no evidence of fissures (Fig. 3).

Table 1
Chemical composition (%) and granule morphology of native *Dioscorea* starches

Characteristics (%)	*D. esculenta*			*D. alata*	
	Kukulala	Java-ala	Nattala	Hingurala	Raja-ala
Starch yield (based on initial tuber weight)	16.81	10.21	12.22	14.25	18.80
Moisture	10.61 ± 0.11^{a}	9.90 ± 0.13^{b}	10.97 ± 0.11^{c}	8.25 ± 0.20^{d}	8.75 ± 0.10^{e}
Ash	0.17 ± 0.00^{a}	0.22 ± 0.00^{b}	0.32 ± 0.00^{c}	0.13 ± 0.00^{d}	0.17 ± 0.00^{a}
Nitrogen	0.01 ± 0.00^{a}	0.03 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{c}	0.01 ± 0.00^{d}
Phosphorous	0.05 ± 0.00^{a}	0.07 ± 0.00^{b}	0.10 ± 0.00^{c}	0.05 ± 0.00^{a}	0.04 ± 0.00^{d}
Lipid: Solvent extracted					
Chloroform–methanol[1]	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.03 ± 0.00^{b}	0.05 ± 0.00^{c}	0.08 ± 0.00^{d}
n-propanol–water[2]	0.39 ± 0.01^{a}	0.35 ± 0.00^{b}	0.44 ± 0.03^{c}	0.25 ± 0.02^{d}	0.20 ± 0.00^{e}
Amylose content					
Apparent[3]	20.38 ± 0.26^{a}	16.19 ± 0.20^{b}	15.58 ± 0.45^{c}	24.73 ± 0.00^{d}	29.29 ± 0.24^{e}
Total[4]	23.97 ± 0.12^{a}	20.07 ± 0.12^{b}	19.98 ± 0.28^{b}	26.98 ± 0.00^{c}	31.02 ± 0.00^{d}
Amylose complexed with lipids[5]	14.98 ± 0.00^{a}	19.33 ± 0.00^{b}	22.02 ± 0.00^{c}	8.34 ± 0.00^{d}	5.58 ± 0.00^{e}
Starch damage	*	*	*	*	*
Granule size range (μm)	8–10	4–5	3–4	30–40	35–45
Granule morphology	Polygonal	Polygonal	Polygonal	Truncated oval	Truncated spade

All data reported on dry basis and represent the mean of at least four replicates. Values followed by the same superscript in each row are not significantly different ($P < 0.05$) by Tukey's HSD test.

*Not detected.

[1] Lipids extracted by chloroform–methanol 2:1 (v/v) at 25 °C (mainly unbound lipids).

[2] Lipids extracted by hot-*n*-propanol–water 3:1 (v/v) from the residue left after chloroform–methanol extraction (mainly bound lipids).

[3] Apparent amylose determined by iodine binding without removal of free and bound lipids.

[4] Total amylose determined by iodine binding after removal of free and bound lipids.

[5] $\frac{\text{Total amylose-apparent amylose}}{\text{Total amylose}} \times 100$.

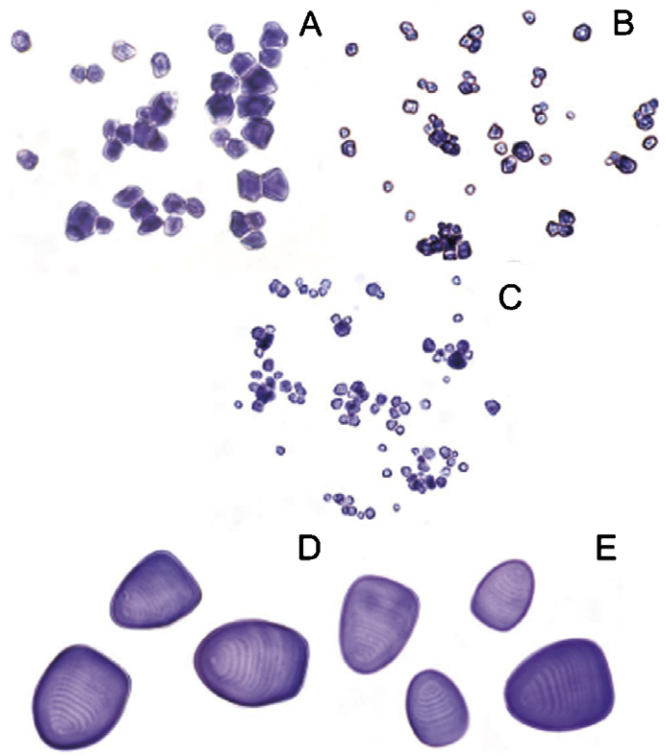

Fig. 2. Light micrographs (500×) of native *D. esculenta* (kukulala [A], java-ala [B], nattala [C]), and *D. alata* (hingurala [D], raja-ala [E]) starches.

3.2. Chemical composition

The data on yield and composition are presented in Table 1. The purity of the starches was judged on the basis of composition [low ash (0.17–0.32%) and low nitrogen content (<0.03%)] and microscopic examination. The average yield of starch from *D. esculenta* and *D. alata* varieties was 19.62 and 11.52%, respectively. This was within the range reported for most tuber starches (Hoover, 2001; Moorthy, 2002). The average total phosphorus content of the starches from *D. esculenta* and *D. alata* was 0.071 and 0.045%, respectively. These values were higher than those reported for sweet potato (0.012%), taro (0.021%), cassava (0.010) kuzu (0.005%) and innala (0.015%) starches (Soni & Agarawal, 1983; Lim, Kasemsuwan, & Jane, 1994; Gunaratne & Hoover, 2002; Jayakody et al., 2005). It was interesting to observe that the phosphorous content (0.10%) of one of the varieties (nattala) of *D. esculenta* was higher than that reported (Lim et al., 1994; McPherson & Jane, 1999; Hoover, 2001) for potato starches (0.069–0.089%). The total starch lipids (surface and bound) in varieties of *D. esculenta* and *D. alata* starches ranged from 0.36% to 0.47% and 0.28% to 0.30%, respectively. These values were generally higher than those reported (Hoover, 2001) for most other tuber starches (0.02–0.19%), but were comparable to that reported for kuzu (0.46%), arrowroot (0.31%), new cocoyam (0.30%) and canna (0.30%) starches (Soni & Agarawal, 1983; Erdman, 1986; Soni, Sharma, Srivastiva, & Gharia, 1990; Gunaratne & Hoover, 2002). The total amylose content of *D. esculenta* and *D. alata* varieties ranged from 19.98% to 23.97% and 24.73 to 29.29%, respectively. The above values were within the range (10–38%) reported for the amylose content of *Dioscorea* starches and for other tuber starches (Hoover, 2001; Moorthy, 2002). Similar differences in amylose content between starches from *D. esculenta* and *D. alata* has also been reported by other researchers (Rasper & Coursey, 1967; Gallant et al., 1982; Farhat et al., 1999; Riley et al., 2004; Amani et al., 2004; Freitas et al., 2004). In tuber starches, the amount of lipid complexed amylose chains has been shown to range from 8.3% to 15.5% (Vasanthan & Hoover, 1992; Gunaratne & Hoover, 2002; Jayakody et al., 2005). In this study the lipid complexed amylose chains in varieties of *D. esculenta* and *D. alata* ranged from 14.98% to 22.02% and 5.58% to 8.34%, respectively. Among the varieties of *D. esculenta*, kukulala showed the lowest content of lipid complexed amylose chains, in spite of its higher amylose and bound lipid content. This suggests that most of the bound lipid in kukulala is probably trapped between amylose helices and/or between amylose and amylopectin chains.

3.3. Amylopectin unit chain length distribution and average chain length

The chain length distribution and the average chain length ($\overline{CL}$) of amylopectins are presented in Table 2. The *D. esculenta* starches exhibited marginal differences in their chain length distribution and $\overline{CL}$. A similar trend was also evident between the varieties of *D. alata* starches. However, both species differed significantly from each other with respect to the proportion of short A chains (dp 6–12), medium chains (dp 25–36) and $\overline{CL}$ (Table 2). Cultivars of both species did not exhibit the amylopectin chain length distribution characteristics of tuber (B-type) starches. Tuber starches have been shown to have a higher proportion of chains with dp > 37, a smaller proportion of chains with dp 6–12, and a larger $\overline{CL}$. For instance, in potato starch, dp > 37, dp 6–12 and $\overline{CL}$ have been reported to 38.6%, 13.7% and 28.6%, respectively (McPherson & Jane, 1999). There has been only one study (McPherson & Jane, 1999) on the amylopectin chain length distribution of *Dioscorea* starch [dp 6–12 (19.1%), dp 13–24 (44.8%), dp 25–36 (14.3%), dp > 37 (21.8%), $\overline{CL}$ (26.3)]. Unfortunately, the species was not specified. Hence, no comparison is possible.

3.4. Powder X-ray diffraction and crystallinity

Tuber starches have been shown to exhibit a 'B' type X-ray pattern with reflections centered at 5.5–5.6°, 14.1°, 15.0°, 17.0°, 19.7°, 22.2° and 24° 2θ angles. Whereas, 'A' type starches (cereals) exhibit reflections at 15.3°, 17.0°, 18.0°, 20.0° and 23.4° 2θ angles (Buléon, Bizot, Delage, & Pontoire, 1987; Hizukuri, Kanebo, & Takeda, 1983; Zobel, 1988). Starches of both species showed no significant change in moisture content during scanning. All three

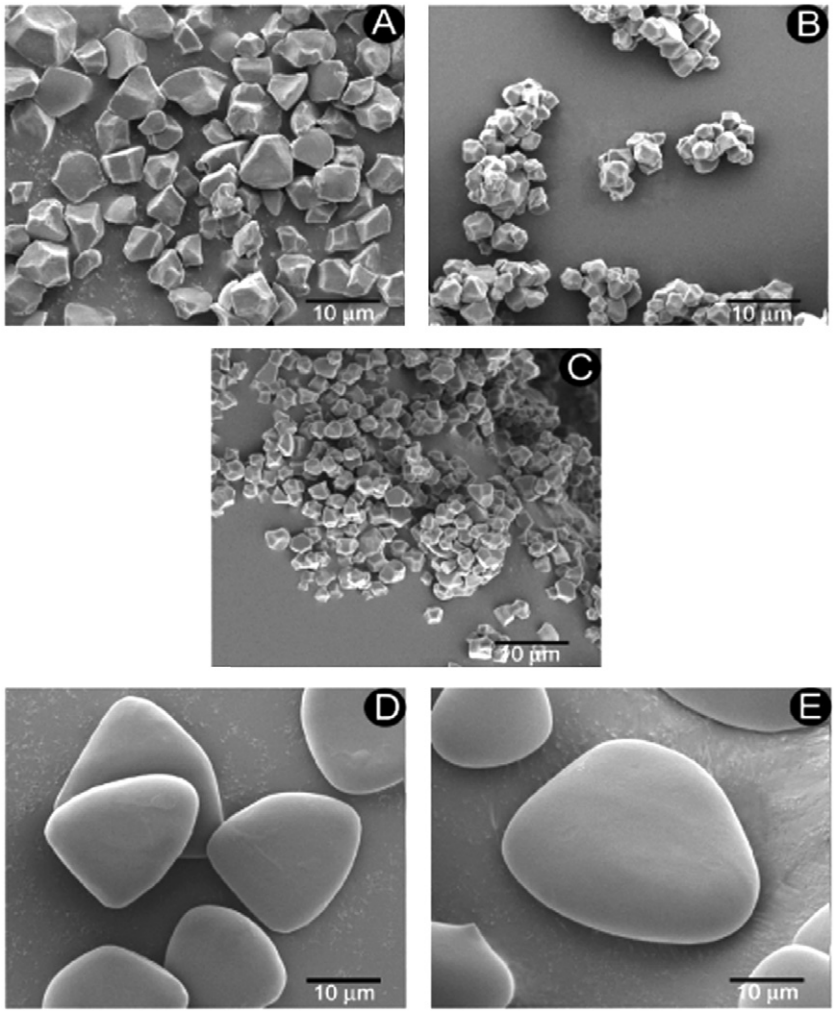

Fig. 3. Scanning electron microscopy (5000×) of native *D. esculenta* (kukulala [A], java-ala [B], nattala [C]), and *D. alata* (hingurala [D], raja-ala [E]) starches.

Table 2
Branch chain length distribution and average chain length ($\overline{CL}$) of native *Dioscorea* starches

Starch source	% Distribution (dp_n)[1]				
	6–12	13–24	25–36	37–54	$\overline{CL}$[2]
D. esculenta					
Kukulala	29.06 ± 0.13^{a}	53.78 ± 0.55^{a}	13.07 ± 0.29^{a}	4.09 ± 0.14^{a}	17.95 ± 0.07^{a}
Java-ala	28.95 ± 1.85^{a}	$55.46 \pm 2.04^{a,b}$	11.75 ± 0.22^{b}	3.84 ± 0.41^{a}	17.65 ± 0.00^{b}
Nattala	27.85 ± 2.94^{a}	$56.46 \pm 2.43^{a,b,c}$	$11.93 \pm 0.80^{b,c}$	$3.75 \pm 0.29^{a,b}$	$17.75 \pm 0.18^{a,b}$
D. alata					
Hingurala	23.09 ± 0.93^{b}	$57.34 \pm 0.71^{b,c,d}$	15.33 ± 0.28^{d}	$4.24 \pm 0.06^{a,c}$	18.81 ± 0.10^{c}
Raja-ala	$21.06 \pm 1.21^{b,c}$	59.26 ± 0.80^{d}	$15.16 \pm 0.41^{d,e}$	$4.52 \pm 0.00^{c,d}$	19.03 ± 0.14^{d}

All data reported on dry basis and represent the mean of triplicates. Values followed by the same superscript in each column are not significantly different ($P < 0.05$) by Tukey's HSD test.

[1] Indicates degree of polymerization.

[2] Average chain length ($\overline{CL}$) calculated by $\sum(dp_n \times \text{peak area}_n)/\sum(\text{peak area}_n)$.

varieties of *D. esculenta* starches, exhibited the 'B' type X-ray pattern (Fig. 4). The intensities of the peaks followed the order: nattala > java-ala–kukulala (Fig. 4). The percentage crystallinity followed the order: nattala> java-ala–kukulala. Of the two varieties of *D. alata* starches, only raja-ala exhibited a 'B' type X-ray pattern. Whereas, hingurala exhibited a 'C'-type pattern. Hizukuri, Fujii, and Nikuni (1960) classified the 'C'-type spectrum into C_a, C_b and C_c based on the extent of their resemblance to 'A' and 'B' type or between the two types, respectively. On this

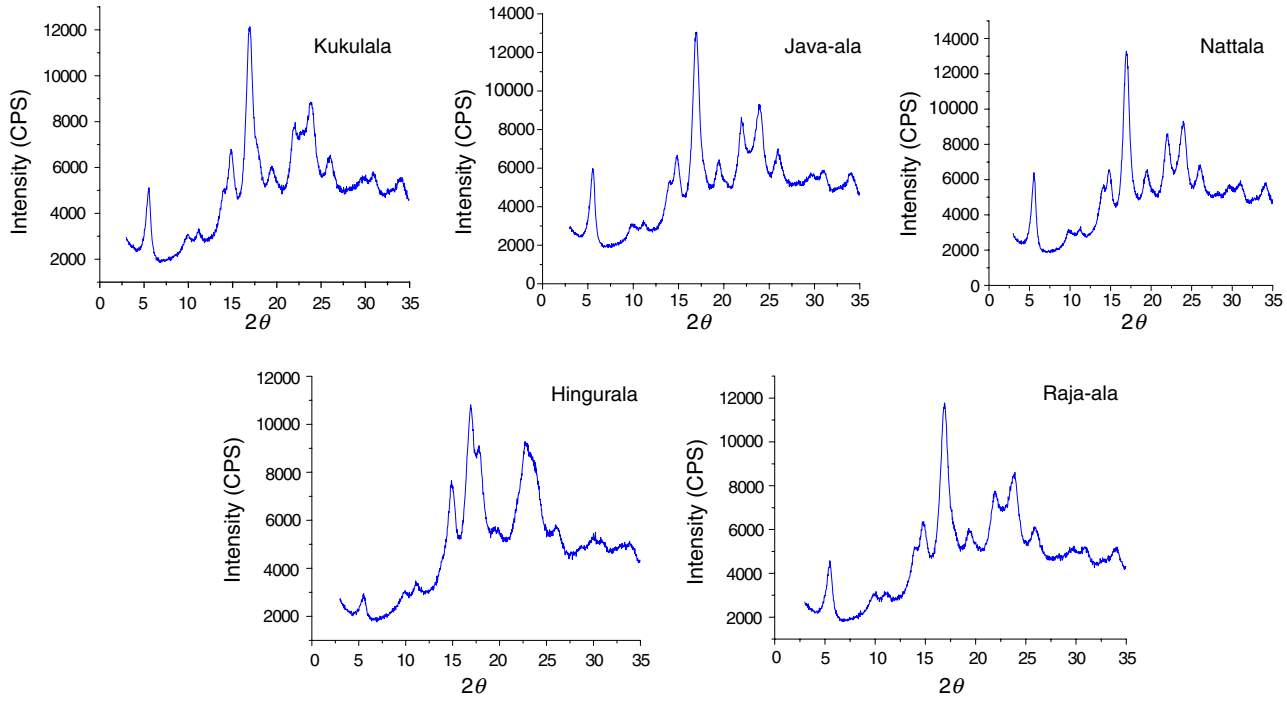

Fig. 4. X-ray diffraction patterns of native *Dioscorea* starches. *D. esculenta* (kukulala, java-ala and nattala), *D. alata* (hingurala, raja-ala).

basis, the X-ray spectra of hingurala could be classified as a 'C_a' type. Both hingurala and raja-ala exhibited the same level of crystallinity (Table 3). Crystallinity of starches has been shown to increase with increase in hydration (Buleon, Colonna, Planchot, & Ball, 1998; Cheetham & Tao, 1998). In this study, granule crystallinity was determined at their maximum water absorption capacity, which was different for each species (*D. esculenta* [33–35%] and *D. alata* [24–28%]). Therefore, a meaningful comparison of the crystallinity of the two species cannot be made. All starches exhibited a peak at $\sim 2\theta = 19.4°$, which has been shown to be indicative of the presence of crystalline V-amylose lipid complexes (Hoover & Hadziyev, 1981; Biliaderis & Galloway, 1989; Zobel, 1988). It was interesting to observe, that the intensity of this peak increased with increase in the amount of lipid complexed amylose chains (Table 1) in varieties of both *D. esculenta* and *D. alata* starches (Fig. 4). However, it must be borne in mind, that differences in the intensity of the V-amylose–lipid complex peak among the starches may also reflect the extent to which the V-amylose–lipid complexes are organized into three dimensional structures (long range order).

Table 3
X-ray diffraction patterns and crystallinity (%) of native *Dioscorea* starches

Starch source	Moisture content (%)	Crystallinity (%)	Crystalline type
D. esculenta			
Kukulala	34[a]	49[a]	B-type
Java-ala	35[b]	50[a]	B-type
Nattala	33[c]	53[b]	B-type
D. alata			
Hingurala	24[d]	43[c]	C-type
Raja-ala	28[e]	43[c]	B-type

All data reported on dry basis and represent the mean of at least four replicates. Values followed by the same superscript in each column are not significantly different ($P < 0.05$) by Tukey's HSD test.

3.5. Gelatinization parameters

The gelatinization temperatures, [onset (T_o), mid point (T_p) and conclusion (T_c)], gelatinization temperature range (T_c–T_o) and gelatinization enthalpy (ΔH) are presented in Table 4. T_o, T_p, T_c, T_c–T_o and ΔH of *D. esculenta* starches were lower than those of *D. alata* starches (Table 4). Among *D. esculenta* starches, T_o, T_p, T_c, T_c–T_o and ΔH was marginal. Whereas, in *D. alata* starches, the above parameters were higher in hingurala (Table 4). It is not possible to compare the above results with published data, due to differences in starch: water ratio, heating rate and differences in methodology (Hoover, 2001). Furthermore, in many cases the variety used in the study has not been specified. Tester (1997) postulated that gelatinization parameters are controlled in part by the molecular structure of amylopectin, starch composition and granular architecture. Noda, Takahata, Sato, Ikoma, and Mochida (1996) showed by studies on wheat and sweet potato starches, that DSC parameters are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (dp 6–11) and not by the proportion of crystalline region which corresponds to the amylose to amylopectin ratio. The above authors showed that a low T_o, T_p, T_c and ΔH reflects the presence of abundant short amylopectin chains.

Table 4
Gelatinization parameters[1] of native *Dioscorea* starches

Starch source	Gelatinization parameters[1]				
	T_o (°C)[2]	T_p (°C)[2]	T_c (°C)[2]	T_c–T_o (°C)[3]	ΔH (J/g)[4]
D. esculenta					
Kukulala	72.30 ± 0.20^a	75.73 ± 0.15^a	85.40 ± 0.50^a	13.10 ± 0.56^a	18.07 ± 0.10^a
Java-ala	72.55 ± 0.07^a	75.00 ± 0.00^b	81.65 ± 0.21^b	9.10 ± 0.14^b	17.32 ± 0.00^b
Nattala	72.45 ± 0.07^a	75.60 ± 0.15^a	82.25 ± 0.35^b	9.80 ± 0.28^b	17.90 ± 0.06^c
D. alata					
Hingurala	78.17 ± 0.06^b	85.13 ± 0.06^c	92.87 ± 0.38^c	14.70 ± 0.35^c	18.98 ± 0.09^d
Raja-ala	75.45 ± 0.07^c	78.40 ± 0.14^d	85.70 ± 0.28^d	12.25 ± 0.35^d	18.60 ± 0.00^e

All data reported on dry basis and represent the mean of at least four replicates. Values followed by the same superscript in each column are not significantly different ($P < 0.05$) by Tukey's HSD test.
[1] Starch: water ratio = 1:3 (w/w dry basis).
[2] T_o, T_p and T_c indicate the temperature of the onset, midpoint and end of gelatinization, respectively.
[3] T_c–T_o indicates the gelatinization temperature range.
[4] Enthalpy of gelatinization ΔH (J/g).

This suggests, that the higher proportion of dp 6–12 chains (Table 2) in the *D. esculenta* starches may have been mainly responsible for their gelatinization parameters being lower than those of the *D. alata* starches (Table 4). This seems plausible, since the difference in gelatinization parameters (*D. alata* > *D. esculenta*) cannot be explained in terms of differences in the amount of lipid complexed amylose chains, amylose content, total phosphorus content or granule crystallinity. The influence of the proportion of dp 6–12 chains on gelatinization parameters was also evident among varieties of *D. esculenta* starches (Table 4). For instance, in spite of large differences in total phosphorus and lipid complexed amylose chains (Table 1), differences in T_o, T_p, T_c and ΔH among varieties of *D. esculenta* was only marginal. This could be attributed to the absence of significant differences in the proportion of dp 6–12 chains among these starches (Table 2). Whereas, in the *D. alata* starches, the large difference in T_o, T_p, T_c and ΔH between hingurala and raja-ala (Table 4) indicates that interactions between starch chains (amylose–amylose, amylose–amylose–amylopectin and amylopectin–amylopectin) are stronger in hingurala. This seems plausible, since differences in composition, crystallinity and proportion of dp 6–12 chains between these starches was not significant (Tables 1–3).

3.6. Amylose leaching (AML) and swelling factor (SF)

The extent of AML and SF in the temperature range 60–90 °C are presented in Figs. 5 and 6, respectively. AML has been shown to be influenced by total amylose content, extent of interaction between amylose–amylose (AM–AM) and amylose–amylopectin (AM–AMP) chains within the native granule and on the amount of lipid complexed amylose chains (Hoover & Vasanthan, 1994; Nakazawa & Wang, 2004). In all starches, AML increased with temperature. *D. alata* starches exhibited AML only at temperatures exceeding 80 °C (Fig. 5). Whereas, AML occurred at lower temperatures (<65 °C) in the *D. esculenta* starches (Fig. 5). This suggests that interaction between AM–AM and/or AM–AMP chains are stronger in the *D. alata* starches. At temperatures beyond 82 °C (Fig. 5), the extent of AML in *D. alata* starches (raja-ala > hingurala) was higher than in the *D. esculenta* (java-ala > kukulala > nattala) starches (Fig. 5). This is indicative of the lower amylose content (Table 1) in the latter. The extent of AML among the *D. esculenta* and between the *D. alata* starches (Fig. 5), is probably influenced by the interplay of difference in: (1) percentage of lipid complexed amylose chains (Table 1), (2) total amylose content (Table 1) and (3) the extent of interaction between AM–AM and/or AM–AMP chains.

SF also increased with increase in temperature (Fig. 6). At all temperatures, SF was lower in the *D. alata* starches. SF has been shown to be influenced by: (1) phosphate monoester content (Galliard & Bowler, 1987; Suzuki, Shibanuma, Takeda, Abe, & Hizurkuri, 1994; Noda et al., 2004; Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005), amount of lipid complexed amylose chains (Hoover & Hadziyev, 1981; Swinkels, 1985; Tester & Morrison, 1990; Hoover & Manuel, 1995) and (3) granule crystallinity (Jayakody et al., 2005). The higher SF shown by the *D. esculenta* starches (Fig. 6) reflects the interplay of differences in: (1) crystallinity (*D. esculenta* > *D. alata* [Table 3]); (2) total phosphorous content (*D. esculenta* > *D. alata* [Table 1]) and (3) extent of interaction between AM–AM and/or AM–AMP chains (*D. esculenta* > *D. alata* [Fig. 5]).

SF differences among the *D. esculenta* starches reflects the interplay of differences in: (1) total phosphorous content (nattala > java-ala > kukulala [Table 1]), (2) crystallinity (nattala > java-ala > kukulala [Table 3]) and (3) extent of interaction between AM–AMP and/or AM–AMP chains (nattala > kukulala > java-ala [Fig. 5]). Lipid complexed amylose chains have been shown to be resistant to granular swelling (Hoover & Hadziyev, 1981; Tester & Morrison, 1990). However, it is unlikely that the observed differences in SF among the *D. esculenta* starches is due to differences in the amount of lipid complexed amylose chains (Table 1), since nattala, having the higher content

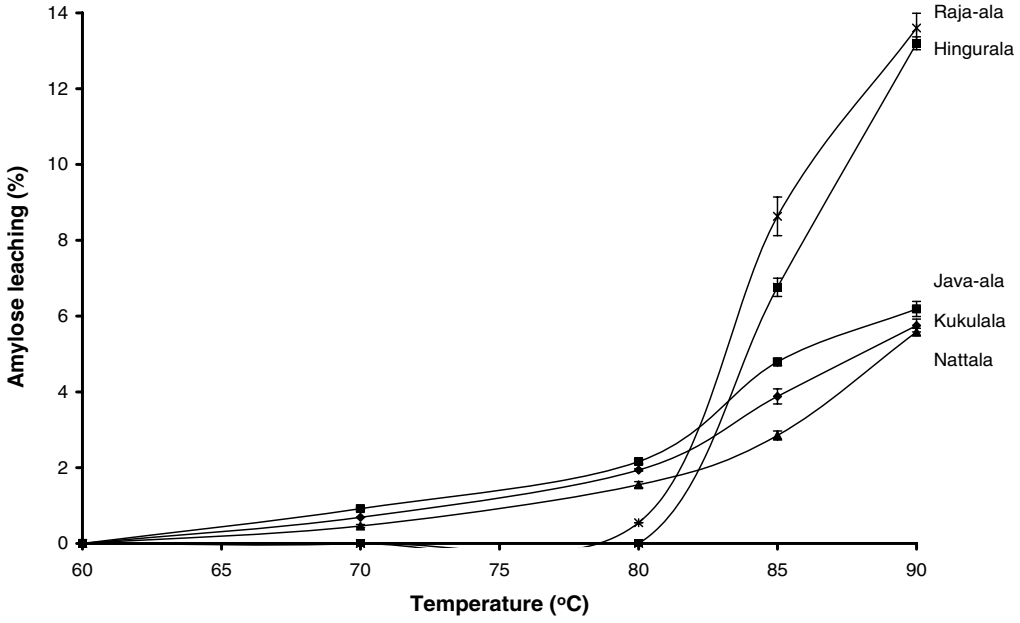

Fig. 5. Effect of temperature on amylose leaching of native *Dioscorea* starches.

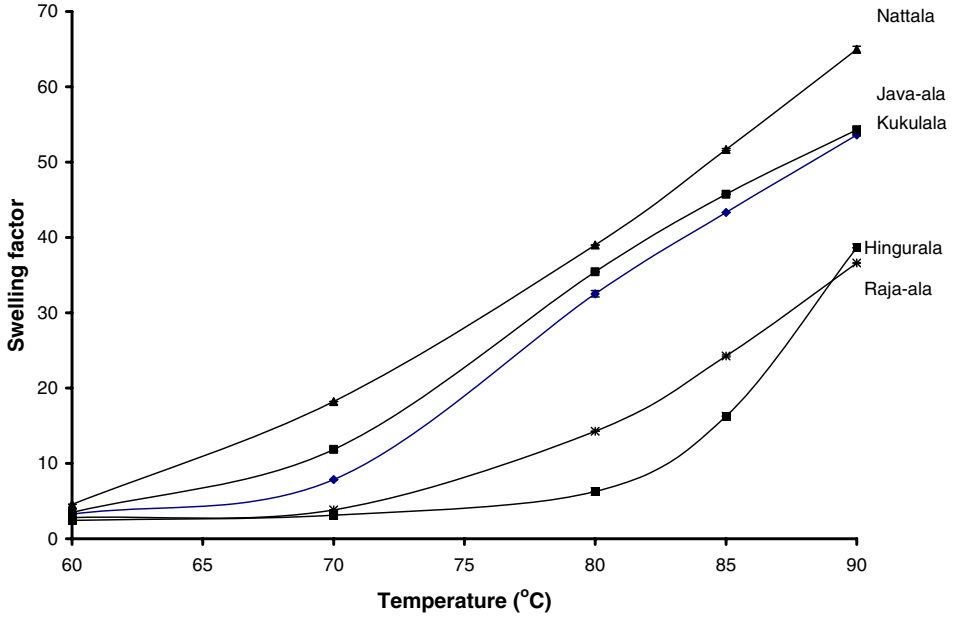

Fig. 6. Effect of temperature on swelling factor of native *Dioscorea* starches.

of lipid complexed amylose chains (Table 1) exhibited the highest SF at all temperatures (Fig. 6). This suggests that the influence of amylose–lipid complexes on SF is negated by factors 1–3. Differences in SF between the *D. alata* (raja-ala > hingurala) (Fig. 6) can be attributed to the higher content of lipid complexed amylose chains (Table 1) and to the higher extent of interaction between AM–AM and/or AM–AMP chains (Fig. 5) in hingurala. This seems plausible, since differences in crystallinity (Table 3) and total phosphorous content (Table 1) between these starches were only marginal.

3.7. Acid hydrolysis

The extent of hydrolysis (2.2 N HCl, 35 °C) of *D. esculenta* and *D. alata* starches are presented in Fig. 7. Starches from both of the above species did not exhibit the typical biphasic pattern (a faster rate of hydrolysis followed by a slower rate) during the time course (15 days) of hydrolysis (Fig. 7). The faster rate has been attributed to the hydrolysis of the amorphous domains (amorphous background and the thin amorphous lamella within the crystalline region) of the starch granule, whereas during the second stage, the

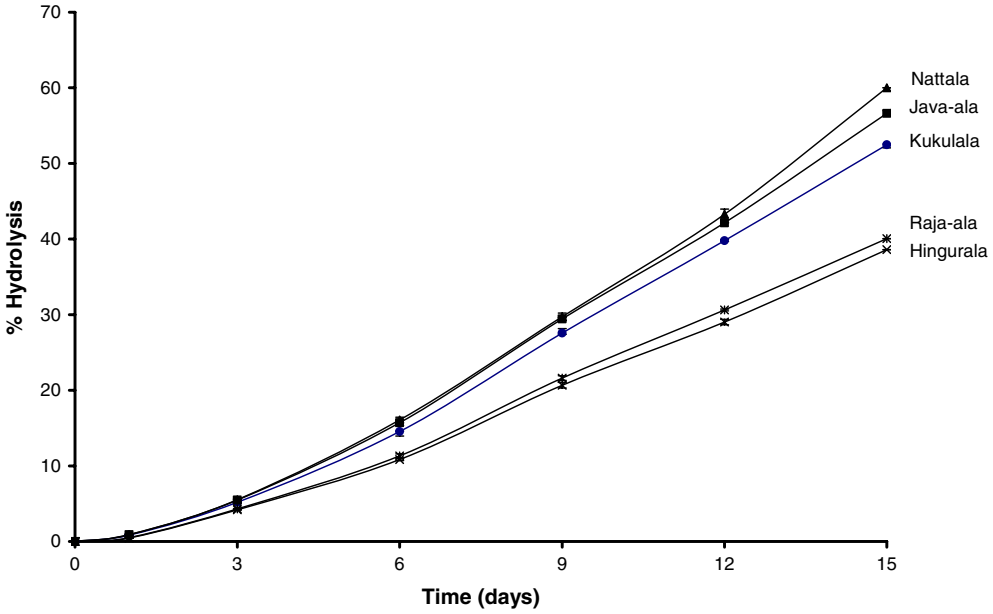

Fig. 7. Acid hydrolysis (2.2 M HCl) of native *Dioscorea* starches at 35 °C.

crystalline regions are slowly degraded (Jayakody et al., 2005; Hoover, 2000; Jayakody & Hoover, 2000). Several researchers have shown that the time taken for the degradation of the crystalline region by H_3O^+ can vary widely (9–25 days) depending on the starch source (McPherson & Jane, 1999; Hoover, 2000). The data suggests that during the time course of hydrolysis, only the amorphous regions were degraded by H_3O^+. This could be attributed to the high level of crystallinity (43–50%) in the *Dioscorea* starches (Table 3). The rate and extent of hydrolysis of *D. alata* starches were lower than those of *D. esculenta* starches (Fig. 7). Significant differences in the extent and rate of hydrolysis between *D. alata* starches (raja-ala > hingurala) and among *D. esculenta* starches (nattala > java-ala > kukuala) were evident only after the 9th and 12th day, respectively (Fig. 7). Differences in the rate and extent of acid hydrolysis among starches has been attributed to differences in: (1) granule size (Vasanthan & Bhatty, 1996); (2) amount of lipid complexed amylose chains (Morrison, Tester, Gidley, & Karkalas, 1993a; Waduge, Hoover, Vasanthan, Gao, & Li, 2006), (3) extent of interaction between starch chains (Hoover & Manuel, 1996); (4) amylopectin chain length distribution (Srichuwong et al., 2005) and (5) phosphorus content (Hoover, 2000). The slower rate and extent of hydrolysis of *D. alata* starches (Fig. 7) could be attributed to the interplay of the following factors: (1) stronger interaction between AM–AM and/or AM–AMP chains within the granule interior, (2) larger granule size (Fig. 2), (3) lower phosphorus content (Table 1) and (4) a lower proportion of dp 6–12/dp 6–24 chains (Table 2). Amylose–lipid complexes have been shown to be resistant to acid hydrolysis (Morrison et al., 1993a, Morrison, Tester, Snape, Law, & Gidley, 1993b; Waduge et al., 2006). However, in this study, the resistance of amylose–lipid complexes to acid hydrolysis was not evident (Fig. 7), since *D. esculenta* starches having a much higher content of amylose–lipid complexes (14.9–22.0%) were hydrolyzed to a greater extent than the *D. alata* starches (5.6–8.3%) (Table 1). It is likely, that the above four factors may have negated the effect of amylose–lipid complexes on acid hydrolysis. The difference in the extent of hydrolysis between the *D. alata* starches (raja-ala > hingurala) (Fig. 7) can be attributed to stronger interaction between AM–AM and AM–AMP chains and to a higher content of lipid complexed amylose chains in hingurala (Table 1). This seems plausible, since the starches did not differ significantly with respect to granule size (Table 1) or amylopectin structure (Table 2). Differences in hydrolysis among the *D. esculenta* starches (nattala > java-ala > kukulala) (Fig. 7) could be attributed to the interplay of differences in: (1) granule size (kukulala > java-ala > nattala) (Table 1), (2) phosphorus content (nattala > java-ala > kukulala) (Table 1), (3) interaction between AM–AM and or AM–AMP chains (java-ala > kukulala > nattala) and (4) amylose–lipid complexes (nattala > java-ala > kukulala) (Table 1). The observed difference in the extent hydrolysis among the *D. esculenta* starches (Fig. 7) suggest that the combined effect of factors 1 and 2 probably negates the effect of factors 3 and 4. The extent of hydrolysis in the *Dioscorea* starches (Fig. 7) was much lower than that reported for potato, cassava, yam (species not specified) and sweet potato (McPherson & Jane, 1999; Gunaratne & Hoover, 2002). In these starches, hydrolysis exceeded 70% after 12 days. However, in the same time period, hydrolysis ranged from 25% to 40% in the *Dioscorea* starches (Fig. 7). This could be attributed to the crystallinity of the *Dioscorea* starches being much higher (43–50%) (Table 3) than that reported for potato (28%), cassava (38%) and sweet potato (38%) (Zobel, 1988; Gunaratne & Hoover, 2002) starches.

3.8. Enzyme hydrolysis

The susceptibility of *Dioscorea* starches towards hydrolysis by porcine pancreatic α-amylase are presented in Table 5. After 72 h of hydrolysis, *D. esculenta* and *D. alata* starches were hydrolyzed to the extent of 66.0–76.6% and 53.6–56.1%, respectively (Table 5). Among *D. esculenta* starches, the extent of hydrolysis followed the order: java-ala > kukulala > nattala. Whereas, in *D. alata* starches, hingurala was hydrolyzed to a greater extent (Table 5). Differences in the *in vitro* digestibility of starches among and within species have been attributed to the interplay of many factors such as starch source (Snow & O'Dea, 1981), granule size (Snow & O'Dea, 1981), amylose/amylopectin ratio (Hoover & Sosulski, 1985); extent of molecular association between starch chains (Dreher, Berry, & Dreher, 1984); degree of crystallinity (Hoover & Sosulski, 1985); amylose–lipid complexes (Holm et al., 1983; Hoover & Manuel, 1995), and unit cell structure (Jane et al., 1992). Several researchers (Colonna, Buleon, & Lemarie, 1988; Lauro, Forssell, Suortti, Hulleman, & Poutanen, 1999; Leach & Sohoch, 1961) have shown that α-amylase can simultaneously solubilize both amorphous and crystalline regions of starch granules. The difference in hydrolysis between *D. esculenta* and *D. alata* starches is probably influenced by differences in granular size (*D. alata* [30–45 μm] > *D. esculenta* [3–10 μm]) and extent of interaction between AM–AM and AM–AMP chains (*D. alata* > *D. esculenta*), since differences in amylose content (*D. alata* > *D. esculenta*), crystallinity (*D. esculenta* > *D. alata*) or the content of lipid complexed amylose chains (*D. alata* > *D. esculenta*) cannot account for the observed differences in hydrolysis. In the *D. alata* starches, differences in granular size (raja-ala > hingurala) is the main causative factor responsible for the higher susceptibility of hingurala towards α-amylase. Among the *D. esculenta* starches, the lower susceptibility of nattala towards α-amylase can be attributed to its higher crystallinity (Table 3) and more extensive interaction between AM–AM and/or AM–AMP chains. Whereas, the difference in susceptibility between kukulala and java-ala starches can be attributed to the smaller granule size (Table 1) and a lower degree of interaction between starch chains in the latter.

A meaningful comparison with regard to variations in the extent of hydrolysis between the *Dioscorea* and other tuber starches cannot be made due to differences in α-amylase source, reaction times and quantity of enzyme used.

Table 5
Hydrolysis (%)[1] of native *Dioscorea* starches by porcine pancreatic α-amylase

Starch source	Hydrolysis (%)[2]
D. esculenta	
Kukulala	68.84 ± 0.60^{a}
Java-ala	76.58 ± 0.26^{b}
Nattala	66.08 ± 0.40^{c}
D. alata	
Hingurala	56.14 ± 0.78^{d}
Raja-ala	53.63 ± 0.66^{e}

[1] After 72 h.
[2] All data reported on dry basis and represent the mean of at least four replicates. Values followed by the same superscript in each column are not significantly different ($P < 0.05$) by Tukey's HSD test.

3.9. Pasting characteristics

The pasting properties of the *Dioscorea* starches measured using a rapid viscoanalyzer (RVA) are presented in Fig. 8. The *D. esculenta* starches exhibited lower pasting temperatures, a greater degree of viscosity breakdown and lower set-back values than *D. alata* starches (Fig. 8). The *D. alata* starches differed significantly from each other with respect to peak viscosity (raja-ala > hingurala), viscosity breakdown (hingurala > raja-ala) set-back (hingurala > raja-ala) and pasting temperature (hingurala > raja-ala). The difference in pasting temperatures between the hingurala and raja-ala suggests that bonding forces between starch chains are stronger in the former. The increase in viscosity during the heating cycle is influenced by the extent of amylose leaching, granular swelling and the extent of friction between swollen granules. The higher peak viscosity exhibited by raja-ala could be attributed to its higher degree of amylose leaching (Fig. 5) greater SF (Fig. 6) and larger granule size (Table 1). Srichuwong et al. (2005) have postulated that starches with larger granules might occupy more volume and thus enhance viscosity. The higher resistance of granules of raja-ala to viscosity breakdown (due to granule fragmentation) during the holding cycle at 95 °C, can be attributed to its larger granule size (Table 1) and more extensive amylose leaching (Fig. 5). It is likely, that the highly swollen raja-ala granules may have become resistant to shear due to granules becoming embedded within the amylose network during the holding cycle. The higher set-back (mainly due to amylose gelation) shown by raja-ala could be attributed to its greater degree of amylose leaching and/or to more rigid unfragmented granules embedded within the amylose network. The *D. esculenta* starches differed only marginally (kukulala > java-ala–nattala) with respect to their pasting properties. However, they differed significantly with respect to peak viscosity (nattala > kukulala > java-ala), viscosity breakdown during the holding cycle (nattala > java-ala > kukulala) and degree of set-back (kukulala > nattala > java-ala). Amylose leaching (Fig. 5) and swelling factor (Fig. 6) measurements showed that among the *D. esculenta* starches, interaction between starch chains was stronger in nattala. This would then explain the ability of nattala to swell to a higher degree and attain a higher degree of viscosity during the heating cycle (Fig. 8). The extent of viscosity breakdown during the holding cycle (at 95 °C) is more pronounced in nattala (Fig. 8) due to susceptibility of the highly swollen granules to shear. Between kukulala and java-ala starches, the extent of amylose leaching (Fig. 5) and granular swelling (Fig. 6) was

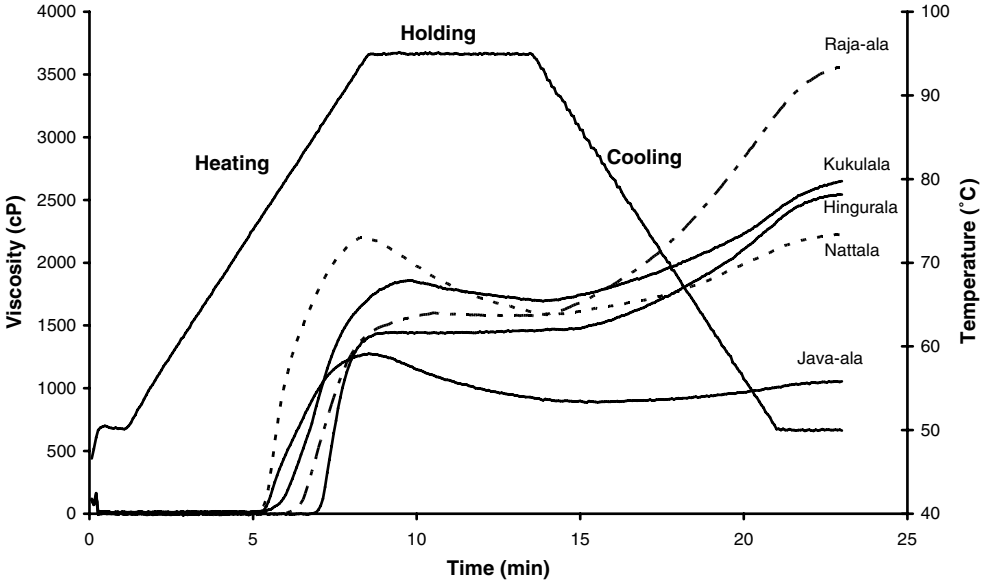

Fig. 8. RVA-Viscograms (7% [w/w]) of native *Dioscorea* starches.

more pronounced in the latter. Therefore, theoretically, the viscosity rise during the heating cycle (Fig. 8) should have been higher in java-ala. It is likely, that the causative factor influencing the viscosity differences between java-ala and kukulala (kukulala > java-ala) may have been due to the large difference in granular size between the starches (kukulala > java-ala) (Table 1). The viscosity rise during the heating cycle is higher in kukulala due to greater friction between swollen granules. The lower extent of viscosity breakdown and the high degree of set-back shown by kukulala can be attributed to its larger granules becoming embedded in the leached amylose network. It is difficult to compare the RVA results obtained in this study with those reported for other tuber starches, due to differences in starch concentration and to the methodology (Brabender viscoamylogram, micro viscoanalyzer) used for determination of pasting characteristics. There is only one report in the literature (Amani et al., 2004) where the RVA pasting characteristics of *D. alata* and *D. esculenta* starches (4% w/v) have been compared. This study also showed that

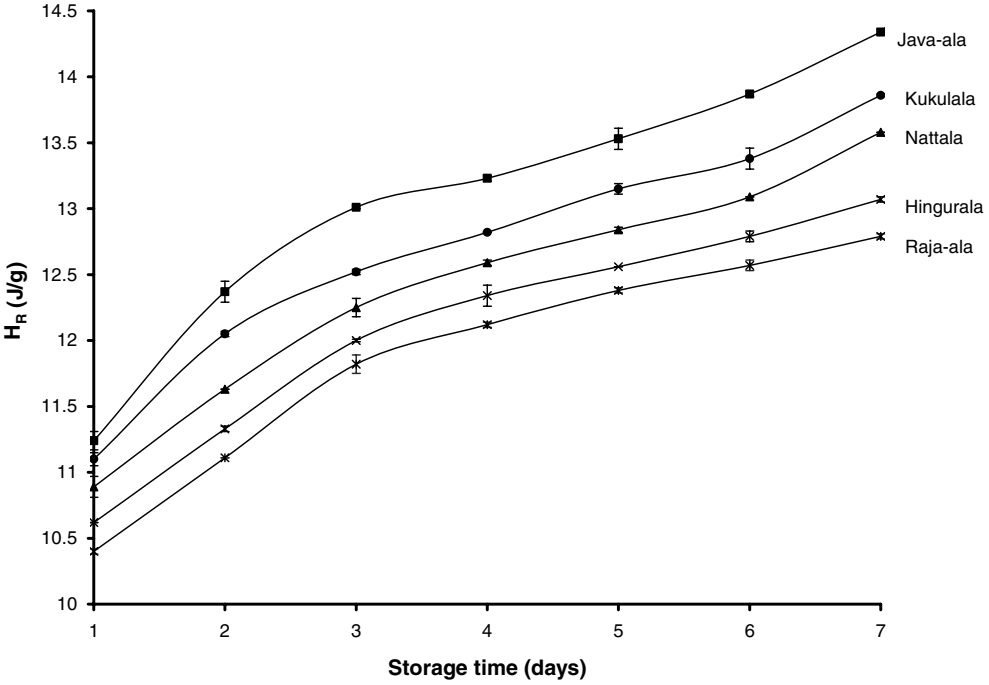

Fig. 9. Enthalpy of retrogradation (ΔH_R) of native *Dioscorea* starch gels during storage at 40 °C (0–7 days).

pasting temperatures, peak viscosity and final viscosity (at 50 °C) of *D. alata* starches were higher than those of *D. esculenta* starches.

3.10. Retrogradation

The melting enthalpies (ΔH_R) of amylopectin recrystallization are presented in Fig. 9. ΔH_R reflects the extent of retrogradation during the storage period (7 days at 40 °C). ΔH_R of *D. esculenta* (java-ala > kukulala > nattala) starches were higher than those of the *D. alata* (hingurala > raja-ala) starches. In all starches, ΔH_R increased rapidly during the first 3 days of storage. Thereafter, the increase was gradual. Differences in ΔH_R among starches have been explained on the basis of amylopectin unit chain length distribution (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998; Kalichevsky, Orford, & Ring, 1990; Lai, Lu, & Lii, 2000; Shi & Seib, 1992; Ward, Hoseney, & Seib, 1994), the number of branch points in close proximity in the amylopectin clusters (Liu & Thompson, 1998) and phosphate monoester content (Jane, Kasemsuwan, Chen, & Juliano, 1996). The main difference in amylopectin unit chain length distribution between *D. esculenta* and *D. alata* starches was in the proportion of short (dp 6–12) chains (*D. esculenta* > *D. alata*) (Table 2). Ward et al. (1994) and Würsch and Grumy (1994) postulated that an increase in molar proportion of short chains with dp 6–9 inhibits retrogradation. Whereas, an increased molar proportion of unit chains with dp 14–24 increases the extent of retrogradation. On this basis, the *D. alata* starches should have shown a higher ΔH_R than the *D. esculenta* starches. Thus the observed differences in the extent of retrogradation between the two species (Fig. 8) cannot be attributed to amylopectin unit chain length distribution.

Studies have shown that in tuber and root starches, phosphorus is primarily in the form of starch phosphate monoester derivatives (Lim et al., 1994; Hizukuri et al., 1983; Lim & Seib, 1993), and they are mainly found on amylopectin. Jane et al. (1996) have shown that starch phosphate monoesters slow retrogradation due to repulsion between negative charges. On this basis, the ΔH_R (enthalpy of retrogradation) of *D. esculenta* starches should have been lower than those of the *D. alata* starches. The results (ΔH_R *D. esculenta* > ΔH_R *D. alata*) indicate that the higher amylopectin content of the *D. esculenta* starches (Table 1) negates the effect of phosphorous content (Table 1) on ΔH_R. Among the *D. esculenta* starches differences in ΔH_R between nattala and java-ala (Fig. 8) is mainly due to differences in phosphorous content (nattala > java-ala [Table 1]), since there was no significant difference in amylopectin content between these starches (Table 1). In kukulala, the amount of phosphorous and amylopectin were lower than in nattala and java-ala (Table 1). Therefore, theoretically, kukulala should have either exhibited a higher ΔH_R (if phosphorous content had been the main causative factor influencing ΔH_R) or a lower ΔH_R (if amylopectin content had been the main causative factor influencing ΔH_R) than nattala and java-ala. Liu and Thompson (1998) have shown by DSC studies that dull waxy maize starch retrogrades faster than waxy maize starch. They attributed this difference to the presence of a large number of branch points in close proximity, in the amylopectin clusters of dull waxy maize starch, which hinders large scale motion of the outer a chains during retrogradation. This would then favor formation, proper alignment and organization of the double helices. This suggests that the observed extent of retrogradation in the *D. esculenta* starches (java-ala > kukulala > nattala) may have been influenced by differences in the number of branch points in close proximity to amylopectin clusters. The results indicate, that the number of branch points in close proximity to amylopectin clusters in kukulala are probably higher than in nattala, but lower than in java-ala. The difference in ΔH_R between the *D. alata* starches (hingurala > raja-ala) can be attributed to the interplay between amylopectin content (hingurala > raja-ala) and phosphorus content (hingurala > raja-ala).

4. Summary and conclusion

The results showed major differences in molecular structure, composition and physicochemical properties between *D. alata* and *D. esculenta* starches. Variations in physicochemical properties between the two species and among varieties were influenced by the interplay of factors such as: granule size, crystallinity, magnitude of interaction between starch chains (within the native granule), phosphorus content, amylopectin chain length distribution, amylose/amylopectin ratio and the number of branch points in close proximity to amylopectin clusters. Some of the *Dioscorea* starches exhibited high thermal stability and low retrogradation rates. Consequently, these starches can be used in foods with minimal modification. Research is underway to modify the physicochemical properties of the above starches by annealing and heat-moisture treatment.

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