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Studies on tuber starches III. Impact of annealing on the molecular structure, composition and physicochemical properties of yam (*Dioscorea* sp.) starches grown in Sri Lanka

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ABSTRACT

This study reports the impact of one step annealing on the composition, molecular structure, granule morphology and physicochemical properties of starches isolated from cultivars of *Dioscorea esculenta* (kukulala, java-ala-nattala) and *Dioscorea alata* (hingurala, raja-ala), yam tubers grown in Sri Lanka. In all starches, granule morphology (shape, size, surface appearance), birefringence patterns, acid hydrolysis profile and X-ray patterns remained unchanged on annealing. Crystallinity remained unchanged on annealing in hingurala, kukulala and java-ala. However, crystallinity of raja-ala and nattala increased and decreased, respectively, on annealing. In all starches, annealing decreased the gelatinization temperature range (kukulala ~ hingurala > nattala ~ raja-ala > java-ala), amylose leaching (raja-ala > nattala > hingurala > kukulala > java-ala), granular swelling (raja-ala ~ hingurala > kukulala > java-ala > nattala), peak viscosity (raja-ala > hingurala > kukulala > java-ala > nattala), enthalpy of retrogradation (kukulala ~ java-ala ~ nattala ~ hingurala ~ raja-ala) and susceptibility towards acid hydrolysis (java-ala > raja-ala > hingurala ~ nattala > kukulala). However, annealing increased gelatinization temperatures (kukulala ~ java-ala ~ nattala ~ raja-ala ~ hingurala) and the enthalpy of gelatinization (kukulala > hingurala > java-ala > nattala > raja-ala). Set-back viscosity increased in nattala, but decreased in the other starches (raja-ala > hingurala > kukulala > java-ala) on annealing. The study showed that the different responses shown by the cultivars of the *Dioscorea* starches towards annealing were to a large extent influenced by their composition and molecular structure.

1. Introduction

The world's leading tuber and root starch sources are potato, cassava, sweet potato, taro and yams (*Dioscorea*). In Sri Lanka, *Dioscorea* is commonly known by its vernacular name, 'Vel-ala'. It plays an important role in the diet (consumed in the form of a vegetable dish, boiled, or roasted) of individuals living in suburban and rural areas of the Island. *Dioscorea alata* (variety: Hingurala & raja-ala) and *Dioscorea esculenta* (variety: Kukulala, java-ala, & nattala) are the two popular yam species widely cultivated in Sri Lanka. Starch is the major component of these tubers (60–85%, dry basis). Recently, we reported a detailed study on the molecular structure and physicochemical properties of the above varieties of *D. alata* and *D. esculenta* starches (Jayakody, Hoover, Liu, & Donner, 2007). The above study showed that there were major differences in molecular structure, composition and physicochemical properties between *D. alata* and *D. esculenta* starches. In addition, the above differences were also seen among cultivars of each species. Variations in physicochemical properties between the two species and among cultivars 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 and amylose/amylopectin ratio. Some of the varieties exhibited high thermal stability and low retrogradation rates. Whereas, others exhibited poor functional properties. Therefore, there is a need to modify these starches, so that their properties would be comparable to those of modified cereal starches. Presently, starches utilized in the food industry are modified chemically by cross-linking, substitution and acid hydrolysis to alter their functionality. However, chemical methods are cost prohibitive. Physical modification by hydrothermal treatment such as annealing and heat–moisture treatment would fit in with current societal trends towards natural products and offers the potential to alter starch functionality in a low cost and environmentally friendly way. Annealing is a process whereby starch granules in excess (>60% w/w) or at intermediate water content (40% w/w) are held at a temperature above the glass transition temperature (T_g) but below the onset (T_o) temperature of gelatinization for a set period of time (Hoover & Vasanthan, 1994a; Jacobs

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& Delcour, 1998; Jayakody et al., 2007; Nakazawa & Wang, 2004; O'Brien and Wang, 2008; Qi, Tester, Snape, & Ansell, 2005; Tester & Debon, 2000; Vermeylen, Goderis, & Delcour, 2006; Waduge, Hoover, Vasanthan, Gao, & Li, 2006; Jacobs et al., 1998a; Hoover & Manuel, 1996; Hoover & Vasanthan, 1994a, 1994b). Annealing has been shown to cause changes to starch structure (increase in granule stability, perfection of starch crystallites, formation of new double helices, interaction between glucan chains and an increase in contrast between crystalline and amorphous lamella) and properties (elevation of gelatinization temperature, narrowing of the gelatinization temperature range, decrease in swelling factor and amylose leaching, increase in hot and cold paste viscosities and an increase or decrease in susceptibility towards acid and enzyme hydrolysis). Annealing of cereal starches has been well documented. However, among tuber starches, annealing has been studied in detail only in potato starch (Genkina et al, 2004a; Genkina, Wasserman, & Yuryev, 2004b; Hoover & Vasanthan, 1994a, 1994b; Jacobs, Earlingen, Rouseu, Colonna, & Delcour, 1998a; Jacobs, Eerlingen, Charwart, & Delcour, 1995; Jacobs, Eerlingen, & Delcour, 1996; Jacobs, Eerlingen, Spaepen, Grobet, & Delcour, 1998c; Jacobs et al, 1998b; Muhrbeck & Svensson, 1996; Stute, 1992; Vermeylen et al., 2006). There is also some limited information on the effect of annealing on cassava (Gomez, Mendes da Silva, Ricardo, Sasaki, & Germani, 2004) and sweet potato (Genkina et al., 2004b) starches.

Native starch composition and molecular structure have been shown to have a significant impact on the structural changes that occur within the amorphous and crystalline domains during annealing (Lan et al., 1998; Jayakody et al., 2007; Qi et al., 2005; Jacobs and Delcour, 1998; Hoover & Vasanthan, 1994a). Tuber starches have been shown to differ significantly from cereal starches with respect to molecular structure (average chain length of amylose and amylopectin, amylopectin chain length distribution, co-crystallization of amylose with amylopectin, crystallinity, polymorphic composition) and composition (bound lipids, phosphate monoester content). Furthermore, significant variations in structure and composition have also been shown to exist among tuber starches and among cultivars of the same species (Hoover, 2001). This suggests that the extent of starch chain mobility (influences interaction between starch chains and realignment of double helices) on annealing may differ between cereal and tuber starches and also among cultivars of the above species. Thus, the objective of this study was to determine how variations in composition and structure among cultivars of *D. esculenta* and *D. alata* starches influence changes to physicochemical properties on annealing.

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. Fungal α-amylase (157 U/mg protein) from *Aspergillus oryzae* was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Isoamylase (68,000 μ/mg protein) from pseudomonas amylodermosa was purchased from Hayashibana Biochemical Laboratories Ltd. (Okayama, Japan). All chemicals and solvents were of ACS certified grade.

2.2. Methods

2.2.1. Extract

Starch extraction and purification, granule morphology, proximate composition, amylopectin chain length distribution, swelling factor, amylose leaching, gelatinization and retrogradation parameters and acid hydrolysis and pasting characteristics were determined using the same procedures and conditions outlined in our previous publication on native *D. esculenta* and *D. alata* starches (Jayakody et al. 2007).

2.2.2. Annealing

Starches were subjected to one step annealing. Native starch samples (30 dry basis) were weighed into glass containers. Starch moisture content was brought to 75% by adding the appropriate amount of distilled water. The samples were heated at 55 °C for 72 h in a thermostatically controlled water bath. Samples were centrifuged (2000*g*) and supernatant was decanted (soluble carbohydrates were not detected in the supernatant). The annealed starches were air dried at room temperature (25 °C) and then passed through a 250 μm test sieve to obtain a free flowing powder.

2.2.3. Statistical analysis

Analysis of variance (two way ANOVA) was performed by Tukey's HSD test ($P < 0.05$) using Statistical Software SPSS version 14.0 for Microsoft Windows (SSPS Inc. Chicago, IL, USA).

3. Results and discussion

3.1. Impact of annealing on granule morphology

The shape, size, granular surface, concentric growth rings and birefringence of *D. alata* and *D. esculenta* starches remained unchanged on annealing. This is indicative that the granular and lamellar structures of the starches were not altered on annealing.

3.2. Impact of annealing on amylopectin structure

The amylopectin branch length distribution in both *D. alata* and *D. esculenta* starches remained unchanged on annealing (Table 2).

3.3. Impact of annealing on the extent of amylose leaching (AML)

The extent of AML in native and annealed starches in the temperature range 60–90 °C is presented in Table 3. In all starches, AML decreased on annealing (Table 3). Decreased AML on annealing has been attributed to: (1) interaction involving amylose chains, (2) decrease in granular swelling and (3) increase in V-amylose-lipid content (Tester & Debon, 2000; Hoover & Vasanthan 1994a, 1994b). The extent of decrease in AML was more pronounced in the *D. alata* starches (Table 3). This suggests that on annealing, interactions between AM–AM and/or AM–AMP may have been much stronger in the *D. alata* starches. It is likely that due to their higher amylose and lower bound lipid content (Table 1), amylose chains of *D. alata* starches are probably more compactly packed and/or are more flexible than those of *D. esculenta* starches. Consequently, during annealing, interactions between AM–AM and/or AM–AMP chains would be stronger in the *D. alata* starches. The extent of reduction in AML in the *Dioscorea* starches was much higher than that reported for potato starch (Hoover & Vasanthan, 1994a). For instance, at 80 °C, AML was zero (decreased by 100%) in all annealed *Dioscorea* starches (Table 3). Whereas, at the same temperature, the difference in AML between native and annealed potato starch has been reported (Hoover & Vasanthan, 1994a) to be only 2.1%. This was rather surprising, since the extent of interaction between AM–AM and/or AM–AMP chains during annealing should have been more pronounced in potato starch due to its lower bound lipid (0.11%, Hoover & Vasanthan, 1994a) content, and also due to its amylopectin average chain length ($\overline{\text{CL}} = 28.1$, Gunaratne & Hoover, 2002) and degree of polymeriza-

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
Ash	0.17 ± 0.00a	0.22 ± 0.00b	0.32 ± 0.00c	0.13 ± 0.00d	0.17 ± 0.00a
Nitrogen	0.01 ± 0.00a	0.03 ± 0.00b	0.01 ± 0.00b	0.02 ± 0.00c	0.01 ± 0.00d
Phosphorus[a]	0.05 ± 0.00a	0.07 ± 0.00b	0.10 ± 0.00c	0.05 ± 0.00a	0.04 ± 0.00d
Lipid: Solvent extracted					
Chloroform–methanol[b]	0.01 ± 0.00a	0.01 ± 0.00a	0.03 ± 0.00b	0.05 ± 0.00c	0.08 ± 0.00d
n-propanol–water[c]	0.39 ± 0.01a	0.35 ± 0.00b	0.44 ± 0.03c	0.25 ± 0.02d	0.20 ± 0.00e
Amylose content:					
Apparent[d]	20.38 ± 0.26a	16.19 ± 0.20b	15.58 ± 0.45c	24.73 ± 0.00d	29.29 ± 0.24e
Total[e]	23.97 ± 0.12a	20.07 ± 0.12b	19.98 ± 0.23b	26.98 ± 0.00c	31.02 ± 0.00d
Amylose complexed with lipids[f]	14.98a	19.33b	22.02c	8.34d	5.58e
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 alphabets in each row are not significantly different ($P < 0.05$) by Tukey's HSD test.

Starch damage was not detected for the native and annealed starches.

[a] Phosphorus—Phosphorus content remained the same before and after defatting. (This signifies the absence of phospholipids). The phosphorus content includes starch phosphate monoester and inorganic phosphate.

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

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

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

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

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

tion (DP) of amylose (DP = 4850, Gunaratne & Hoover, 2002) being longer than that of the native *Dioscorea* starches ($\overline{CL}$ = 17.6–19.5 (Table 2), DP = 1800–2000, Gunaratne & Hoover, 2002; Suzuki, Kaneyama, Takeda, & Hizukuri 1986). This suggests that it is the arrangement of amylose chains (loose or compactly packed) rather than the $\overline{CL}$ and DP of amylopectin and amylose, respectively, that has the greatest influence on the extent of interaction between AM–AM and/or AM–AMP chains during annealing. The greater relative reduction in AML in the annealed *Dioscorea* starches is indicative of amylose chains in the native *Dioscorea* starches being more compactly packed than in native potato starch.

3.4. Impact of annealing on X-ray pattern and crystallinity

The X-ray pattern of all starches remained unchanged on annealing. However, on annealing, X-ray crystallinity decreased in nattala (*D. esculenta*) and increased in raja-ala (*D. alata*), but remained unchanged in the other starches (Fig. 1). In potato starch, X-ray crystallinity has been reported to remain unchanged on annealing (Hoover & Vasanthan, 1994a; Tester, Ansell, Snape, & Yusuph, 2005). Lim, Kasemsuwan, and Jane (1994) showed by ^{31}P NMR measurements that phosphorus in cereal and some legume starches occur as monophosphates and phospholipids. However, no phospholipids were detected in tuber and root starches. McPherson and Jane (1999) also showed by ^{31}P NMR measurements that in potato, waxy potato, yam and sweet potato, that phosphorus is mainly in the form of phosphate monoesters with minor amounts (<0.001%) as inorganic phosphate. In this study, the total phosphorus content remained unchanged after defatting (see footnote Table 1). This suggests that phosphorus in the *Dioscorea* starches are not in the form of phospholipids, but occur as starch phosphate monoesters and as inorganic phosphate. The ester phosphate groups have been shown to occur exclusively on amylopectin (Gracza, 1965).

Posternak (1951) and Lim et al. (1994) showed that large amounts of starch phosphates were on C-6 than on C-3 of amylopectin. The decrease in crystallinity observed in nattala starch on annealing could be attributed to its total phosphate content (0.10%) being higher than those of the other *Dioscorea* starches (0.04–0.07%). It is likely, that movement of amylopectin double

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

Starch source		% Distribution (DP_n)[a]				
		6–12	13–24	25–36	37–50	$\overline{CL}$[b]
D. esculenta						
Kukulala	Native	25.85 ± 0.94	57.78 ± 0.02	13.19 ± 0.76	3.17 ± 0.16	17.94 ± 0.20
	Annealed	26.16 ± 0.96	57.63 ± 0.46	13.17 ± 0.60	3.04 ± 0.10	17.87 ± 0.11
Java-ala	Native	25.46 ± 2.33	56.55 ± 1.55	13.58 ± 1.23	4.41 ± 0.44	18.33 ± 0.20
	Annealed	25.90 ± 2.52	56.19 ± 1.55	13.53 ± 1.02	4.38 ± 0.06	18.27 ± 0.31
Nattala	Native	24.57 ± 1.95	59.64 ± 0.10	12.61 ± 1.64	3.17 ± 0.22	17.93 ± 0.41
	Annealed	25.33 ± 1.34	59.66 ± 0.73	12.05 ± 0.68	2.96 ± 0.08	17.73 ± 0.18
D. alata						
Hingurala	Native	20.68 ± 2.45	57.40 ± 1.22	17.46 ± 1.01	4.46 ± 0.21	19.29 ± 0.39
	Annealed	20.97 ± 2.30	57.10 ± 1.11	17.30 ± 1.26	4.63 ± 0.08	19.31 ± 0.31
Raja-ala	Native	17.89 ± 3.41	59.76 ± 1.55	17.47 ± 1.72	4.87 ± 0.14	19.61 ± 0.52
	Annealed	18.32 ± 3.12	59.66 ± 1.57	17.03 ± 1.55	5.00 ± 0.01	19.58 ± 0.45

[a] DP_n: Indicates degree of polymerization.

[b] Average chain length ($\overline{CL}$) calculated by $\Sigma(DP_n \times \text{peak area}_n)/\Sigma(\text{peak area}_n)$.

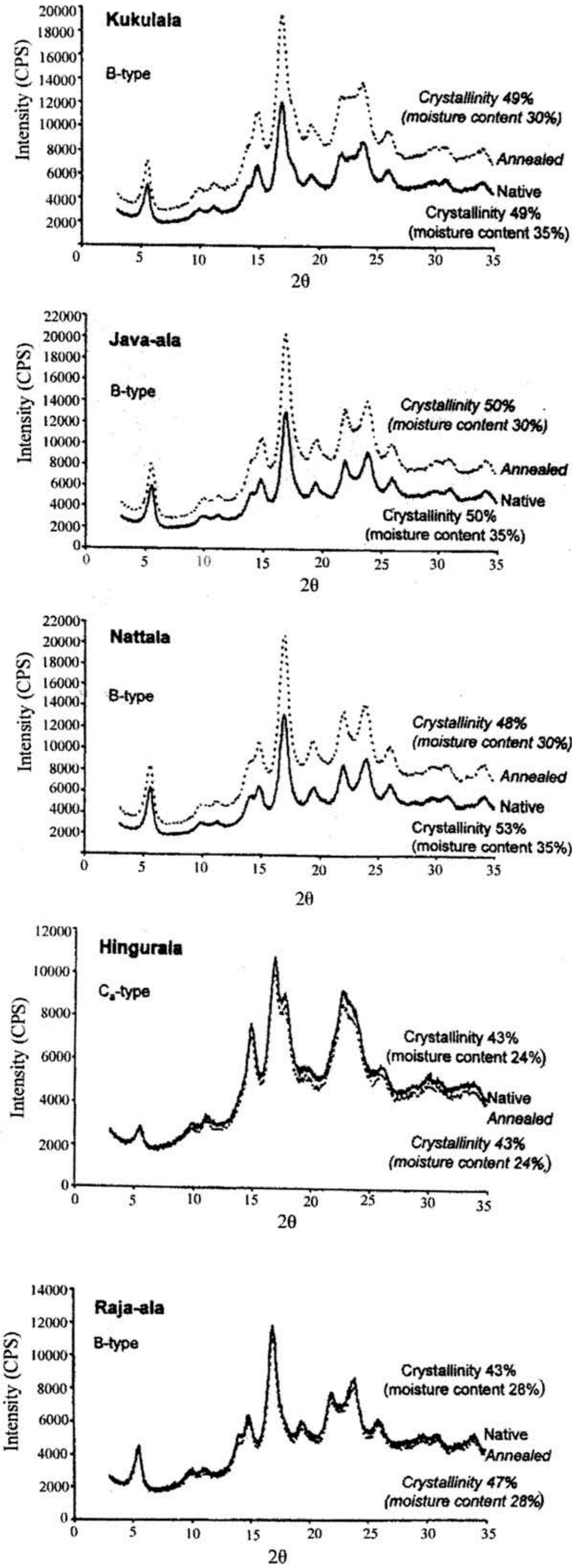

Fig. 1. X-ray diffusion patterns and crystallinity of native and annealed yam starches.

helical chains on annealing, may have caused phosphate groups on adjacent amylopectin chains being in closer proximity to each other than in the native granule. Consequently, repulsion between the negativity charged phosphate groups may have resulted in double helical chains (forming the starch crystallites) being oriented in a crystalline array, that may have been different to that in native starch. This could then account for the decreased crystallinity in nattala starch on annealing (Fig. 1). AML studies (Table 3) showed that interactions involving AM–AM and/or AM–AMP chains on annealing were more extensive in raja-ala than in the other starches. This suggests that the increase in crystallinity in raja-ala on annealing is due to the formation of additional crystallites (resulting from interactions between AM–AM and/or AM–AMP chains). The unchanged crystallinity exhibited by java-ala, kukulala and hingurala starches on annealing (Fig. 1) could be attributed to their low phosphate monoester content (Table 1) and to the lack of formation of new crystallites on annealing.

3.5. Impact of annealing on gelatinization parameters

Annealing increased the onset (T_o), mid-point (T_p) and conclusion (T_c) temperatures and decreased the gelatinization temperature range ($T_c{-}T_o$) in all starches (Table 4). Similar changes on annealing has also been reported for other tuber starches such as potato (Genkina et al. 2004a; Hoover & Vasanthan, 1994a; Jacobs et al., 1998a, 1998b, 1998c; Tester et al., 2005), sweet potato (Genkina et al., 2004b) and cassava (Gomez et al., 2004). The increase in T_o, T_p, T_c on annealing has been attributed to perfection of pre-existing crystallites (Hoover & Vasanthan, 1994a). Crystalline perfection on annealing has been attributed to lengthening of amylopectin double helices caused by coiling of previously uncoiled ends (Genkina et al., 2004a; Tester et al., 2005) and to improved alignment of the double helices forming the crystalline structure (Hoover & Vasanthan, 1994a). In general, the extent of increase in T_o, T_p and T_c on annealing was nearly the same for all *Dioscorea* starches (Table 4). $T_c{-}T_o$ reflects variations in crystalline stability. Annealing has been shown to minimize these variations (Hoover & Vasanthan, 1994a). Annealing decreased $T_c{-}T_o$ in all starches (Table 4). The extent of this decrease followed the order: Hingurala > kukulala > raja-ala > nattala > java-ala (Table 4). This was expected, since the variations in crystallinity stability ($T_c{-}T_o$) within the native starches followed the same order: Hingurala > kukulala > raja-ala > nattala > java-ala (Table 4). The enthalpy of gelatinization (ΔH) of all *Dioscorea* starches increased slightly on annealing (kukulala > hingurala > java-ala > nattala > raja-ala) (Table 4). The extent of this increase although marginal was significant ($P < 0.05$). Marginal increases in ΔH on annealing has also been reported for potato (Genkina et al., 2004a; Hoover & Vasanthan, 1994a; Tester et al., 2005), sweet potato (Genkina et al., 2004b) and cassava (Gomez et al., 2004) starches. The increase in ΔH could be the result of crystalline perfection and/or to melting of additional crystallites that may have formed as a result of interaction between AM–AM and/or AM–AMP chains.

3.6. Impact of annealing on swelling factor (SF)

In all starches, SF decreased on annealing (Table 5). The extent of this decrease was more pronounced in the *D. alata* than in the *D. esculenta* starches. This suggests that the higher extent of AML reduction in the *D. alata* starches (Table 3) on annealing may have also been influenced by their larger decrease in SF (Table 5). The results showed that the decrease in SF on annealing is influenced to a large extent by the interplay between the extent of crystalline perfection (Table 5) and on the extent of interaction involving AM–AM and/or AM–AMP chains (Table 3). Both crystalline perfection and interactions involving amylose chains would decrease hydration of the amorphous regions, thereby decreasing granular swelling. Decreased granular swelling on annealing has

Table 3
Amylose leaching (%) of native and annealed *Dioscorea* starches in the temperature range 60–90 °C.

Variety		60 °C	70 °C	80 °C	85 °C	90 °C
D. esculenta						
Kukulala	Native	0.00 ± 0.00a	0.69 ± 0.00a	1.94 ± 0.03a	3.88 ± 0.02a	5.75 ± 0.07a
	Annealed	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00b	1.69 ± 0.05b	2.35 ± 0.09b
Java-ala	Native	0.00 ± 0.00a	0.92 ± 0.02c	2.16 ± 0.06c	4.97 ± 0.01c	6.19 ± 0.06c
	Annealed	0.00 ± 0.00a	0.00 ± 0.00b	0.00 ± 0.00b	2.29 ± 0.00d	3.29 ± 0.02d
Nattala	Native	0.00 ± 0.00a	0.46 ± 0.04d	1.55 ± 0.08d	2.85 ± 0.02e	5.58 ± 0.01e
	Annealed	0.00 ± 0.00a	0.00 ± 0.00b	0.00 ± 0.00b	1.16 ± 0.08f	2.05 ± 0.06f
D. alata						
Hingurala	Native	0.00 ± 0.00a	0.00 ± 0.00b	0.00 ± 0.00b	6.76 ± 0.04g	13.20 ± 0.07g
	Annealed	0.00 ± 0.00a	0.00 ± 0.00b	0.00 ± 0.00b	1.94 ± 0.03h	5.92 ± 0.00h
Raja-ala	Native	0.00 ± 0.00a	0.00 ± 0.00b	0.55 ± 0.01e	8.63 ± 0.05i	13.60 ± 0.09i
	Annealed	0.00 ± 0.00a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00j	1.17 ± 0.00j

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

Table 4
Gelatinization parameters of native and annealed *Dioscorea* starches.

Starch source		Gelatinization parameters[a]				
		T_o (°C)[b]	T_p (°C)[b]	T_c (°C)[b]	T_c–T_o (°C)[c]	ΔH (J/g)[d]
D. esculenta						
Kukulala	Native	72.30 ± 0.20a	75.73 ± 0.15a	85.40 ± 0.50a	13.10	18.07 ± 0.10a
	Annealed	75.15 ± 0.06b	78.18 ± 0.17b	86.33 ± 0.10b	11.18	19.09 ± 0.05b
Java-ala	Native	72.55 ± 0.07a	75.00 ± 0.00c	82.00 ± 0.00c	9.45	17.32 ± 0.00c
	Annealed	74.50 ± 0.25c	77.11 ± 0.15d	83.48 ± 0.38d	8.98	7.57 ± 0.04d
Nattala	Native	72.45 ± 0.07a	75.60 ± 0.15a	82.25 ± 0.35c	9.80	17.90 ± 0.06e
	Annealed	74.51 ± 0.25c	77.79 ± 0.47e	83.07 ± 0.32d	8.56	18.05 ± 0.05a
D. alata						
Hingurala	Native	78.17 ± 0.06d	85.13 ± 0.06f	92.70 ± 0.06e	14.53	18.98 ± 0.09f
	Annealed	80.50 ± 0.16e	85.58 ± 0.05g	93.00 ± 0.08e	12.50	19.45 ± 0.19g
Raja-ala	Native	75.45 ± 0.07b	78.40 ± 0.14b	85.70 ± 0.28a	10.25	18.60 ± 0.00h
	Annealed	78.55 ± 0.13f	80.68 ± 0.15h	87.25 ± 0.55f	8.70	18.74 ± 0.05i

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

[a] Starch:Water ratio = 1:3 (w/w dry basis).

[b] T_o, T_p, T_c, indicate the temperature of the onset, mid-point and end of gelatinization, respectively.

[c] T_c–T_o indicates the gelatinization temperature range.

[d] Enthalpy of gelatinization ΔH (J/g).

Table 5
Swelling factor of native and annealed *Dioscorea* starches in the temperature range 60–90 °C.

Variety		60 °C	70 °C	80 °C	85 °C	90 °C
D. esculenta						
Kukulala	Native	3.27 ± 0.20a	7.68 ± 0.00a	32.52 ± 0.42a	43.31 ± 0.00a	53.61 ± 0.11a
	Annealed	0.00 ± 0.00b	1.99 ± 0.34b	19.77 ± 0.00b	24.88 ± 0.37b	31.97 ± 0.19b
Java-ala	Native	3.50 ± 0.00c	11.85 ± 0.22c	35.45 ± 0.00c	45.73 ± 0.00c	54.29 ± 0.33c
	Annealed	0.00 ± 0.00b	3.73 ± 0.54d	26.67 ± 0.75d	32.43 ± 0.47d	41.31 ± 0.30d
Nattala	Native	4.55 ± 0.00d	18.22 ± 0.00e	39.00 ± 0.00e	51.69 ± 0.12e	64.97 ± 0.42e
	Annealed	0.00 ± 0.00b	8.59 ± 0.54f	29.62 ± 0.63f	32.20 ± 0.65f	44.87 ± 0.70f
D. alata						
Hingurala	Native	2.45 ± 0.35e	3.13 ± 0.00g	6.26 ± 0.19g	16.27 ± 0.13g	38.64 ± 0.00g
	Annealed	0.00 ± 0.00b	0.00 ± 0.00h	3.99 ± 0.36h	7.57 ± 0.35h	21.68 ± 0.44h
Raja-ala	Native	2.81 ± 0.50e	3.83 ± 0.00i	14.28 ± 0.00i	24.27 ± 0.13i	36.60 ± 0.00i
	Annealed	0.00 ± 0.00b	0.00 ± 0.00h	6.16 ± 0.00j	11.65 ± 0.32j	20.55 ± 0.50j

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

also been observed in potato starch (Hoover & Vasanthan, 1994a; Kuge & Kitamura, 1985). However, in potato starch (Hoover & Vasanthan, 1994a) the decrease in SF (24.7% at 80 °C) is much lower than that observed for the *Dioscorea* starches (29–54%) at the same temperature (Table 5). This indicates that crystalline perfection and/or interactions involving amylose chains on annealing is/are less pronounced in potato starch.

3.7. Impact of annealing on pasting characteristics

In the *D. esculenta* starches, annealing decreased peak viscosity (kukulala > java-ala > nattala), but increased peak time (kukulala > nattala > java-ala), pasting temperature (kukulala > nattala ~ java-ala) and thermal stability (kukulala > nattala > java-ala). Set-back decreased in kukulala, remained the same in java-ala, but increased in nattala (Fig. 2) on annealing. In the *D. alata* starches, annealing decreased peak viscosity (raja-ala > hingurala) and setback (raja-ala > hingurala), but increased peak time (raja-ala > hingurala), pasting temperature (raja-ala > hingurala) and thermal stability (raja-ala > hingurala). Changes in peak viscosity, thermal stability and pasting temperature on annealing were generally higher in the *D. alata* starches (Fig. 2). Similar changes in pasting properties on annealing has also been observed in potato starch (Hoover & Vasanthan, 1994a; Jacobs et al., 1995; Stute, 1992). However, the magnitude of these changes cannot be compared with those of the *Dioscorea* starches due to differences in concentration and on the type of instrument (RVA vs Brabender Viscoam-

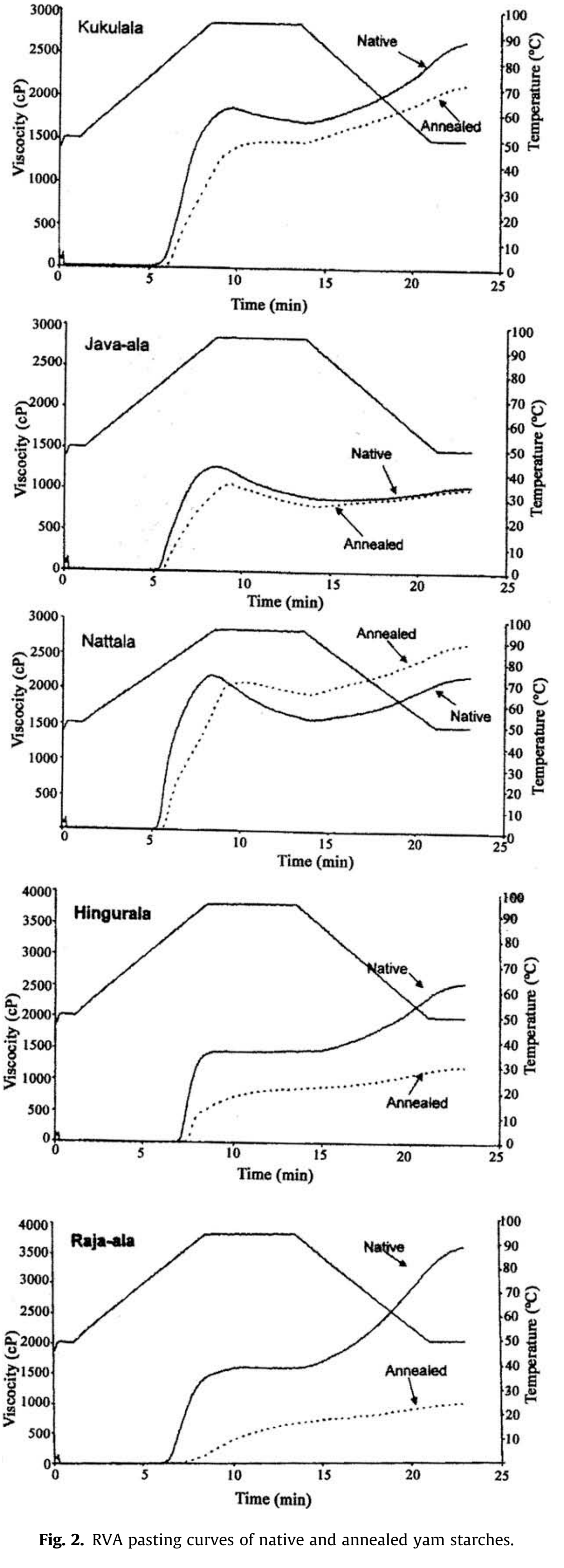

Fig. 2. RVA pasting curves of native and annealed yam starches.

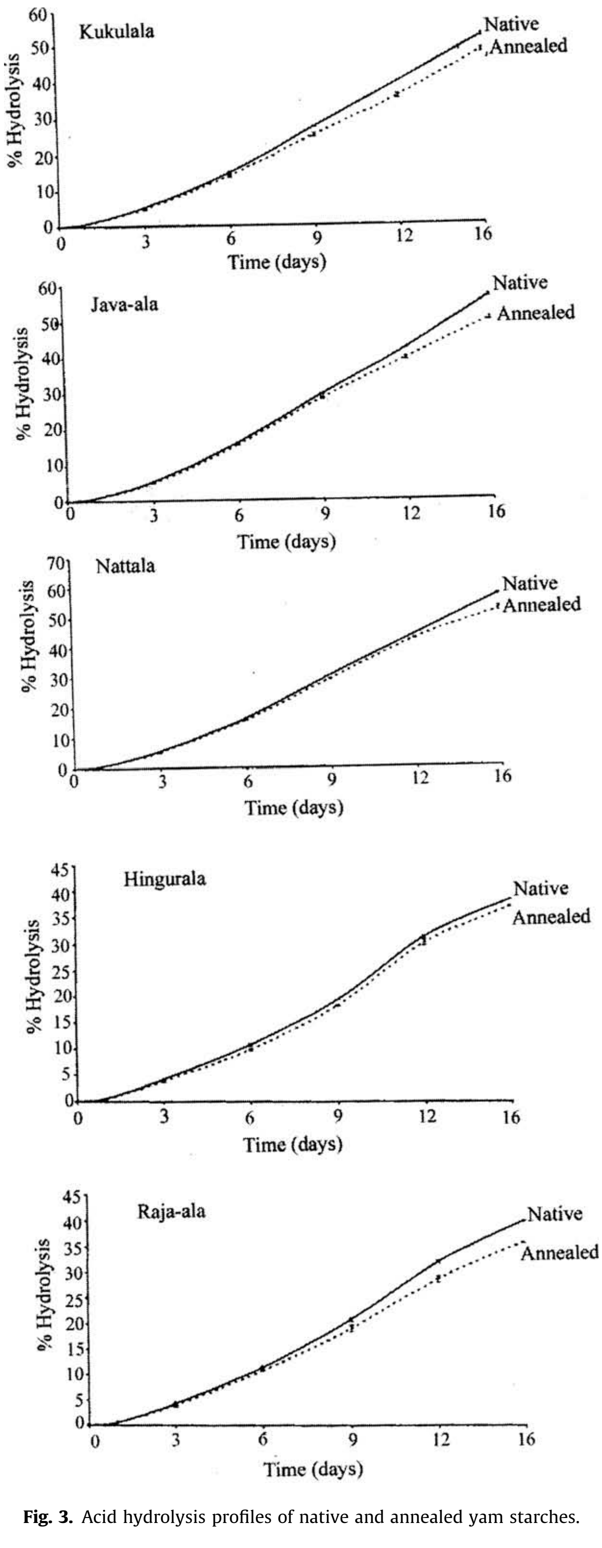

Fig. 3. Acid hydrolysis profiles of native and annealed yam starches.

ylogram) used for viscosity measurements. Viscosity development during heating of starch granules with water under shear has been attributed to friction between swollen granules, amount of leached amylose and amylopectin content (Hoover & Vasanthan, 1994b; Jacobs et al., 1995; Sasaki, Yasui, Matsuki, & Satake, 2002). The viscosity rise during cooling of a heated starch suspension has been attributed to the amount of leached amylose, extent of interaction between leached amylose chains, molecular weight of leached amylose, and to the presence of intact swollen granules embedded in the leached amylose network (Hoover & Vasanthan, 1994a; Jacobs et al., 1995; Loh, 1992). In all *Dioscorea* starches, the decrease in peak viscosity on annealing can be attributed to decreased SF (Table 5) and AML (Table 3). The effect of annealing on peak viscosity is more pronounced in the *D. alata* than in the *D. esculenta* starches (Fig. 2) due to a greater reduction in SF (Table 5) and AML (Table 3) in the former. The increase in thermal stability exhibited by all the annealed *Dioscorea* starches (Fig. 2) is mainly due to the decrease in SF (Table 5). This seems plausible since the extent of thermal stability increase closely followed the extent of decrease in SF in both *D. esculenta* (kukulala > nattala > java-ala) and *D. alata* (raja-ala > hingurala) starches.

In both *D. alata* and *D. esculenta* starches, the extent of increase in pasting temperature (Fig. 1) also closely followed the extent of decrease in SF (Table 5). In all starches, the decrease in SF on annealing (Table 5), should have increased the proportion of unfragmented swollen granules embedded in the leached amylose network. Consequently, the extent of set-back during the cooling cycle in the RVA (Fig. 2) should have theoretically increased after annealing. The results suggest that the decreased in set-back (raja-ala > hingurala > kukulu-ala) on annealing largely reflects the decrease in AML (raja-ala > hingurala > kukulu-ala) (Table 3), which negates the influence of increased granule rigidity (due to decrease in SF) on set-back. The marginal difference in set-back between native and annealed java-ala starch (Fig. 1) suggests that the extent of decrease in SF (Table 5) and AML (Table 3) may have not been large enough to have any significant impact on set-back.

In the *D. alata* starches, the extent of decrease in SF on annealing was nearly the same in both raja-ala and hingurala (Table 5). How-

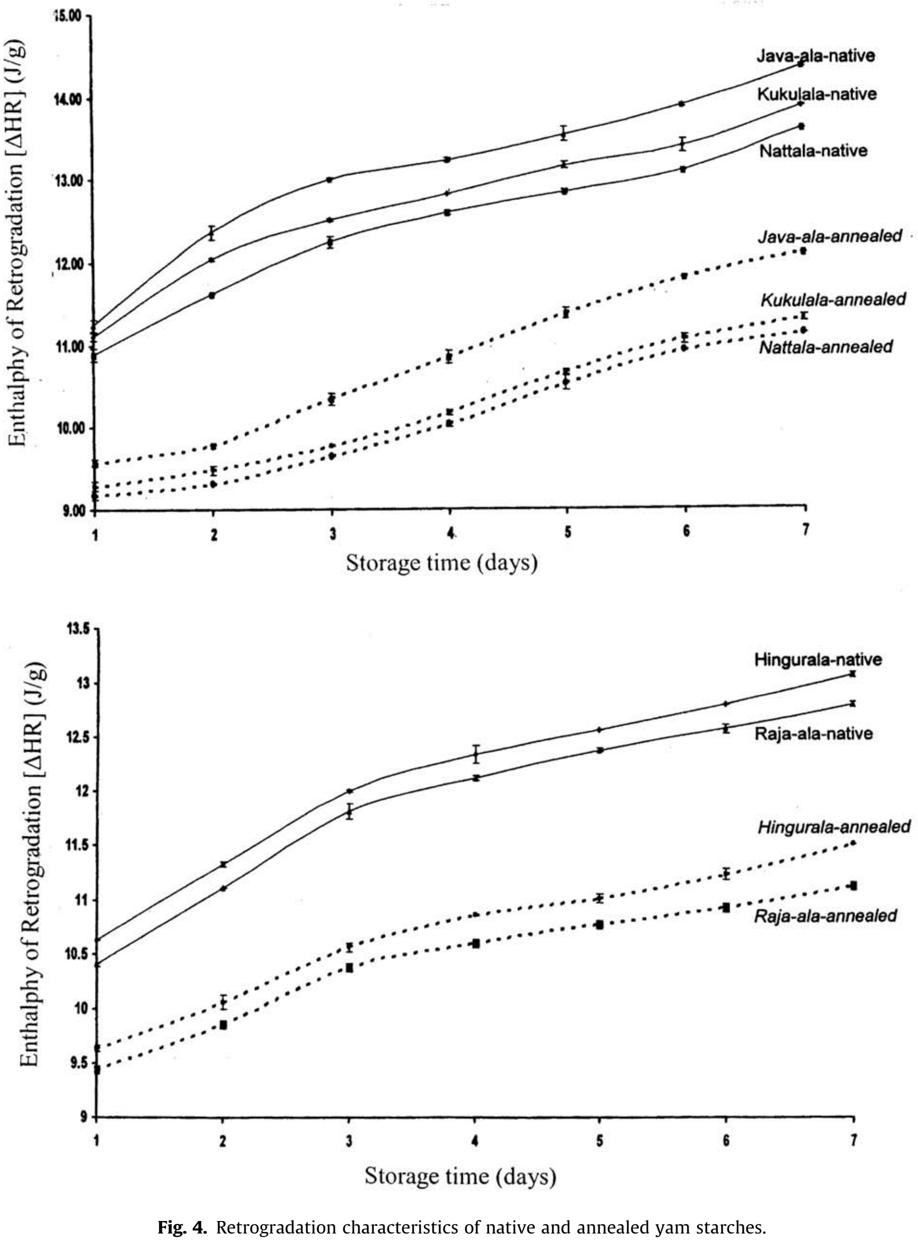

Fig. 4. Retrogradation characteristics of native and annealed yam starches.

ever, the extent of decrease in AML was much higher in raja-ala (Table 3). This would then explain the larger decrease in set-back in annealed raja-ala starch. The set-back increase in nattala starch on annealing (Fig. 2) was rather surprising since the extent of reduction in SF (Table 5) was lower and the extent of reduction in AML (Table 3) was higher than that of kukulala starch. The only explanation that seems plausible is that the large reduction in AML on annealing may have facilitated rapid aggregation of leached amylose chains (due to a less dense leached amylose matrix). This may then account for the set-back in annealed nattala starch being higher than that of its native counterpart (Fig. 2).

3.8. Impact of annealing on acid hydrolysis

The acid hydrolysis patterns of annealed starches (Fig. 3) were similar to those of their native counterparts (Jayakody et al., 2007). However, in all starches, hydrolysis decreased on annealing (Fig. 3). At the end of the 15th day, the difference in hydrolysis between native and annealed *D. esculenta* starches followed the order: Java-ala (9.0%) > kukulala (6.0%) > nattala (4.5%). Whereas, the *D. alata* starches followed the order: Raja-ala (6.0%) > hingurala (1.8%). The corresponding decrease for potato starch being $\sim$ 5.0% (Hoover & Vasanthan, 1994a; Jacobs et al., 1998a). Decreased hydrolysis on annealing has been attributed to the interplay of the following factors: (1) formation of V-amylose-lipid complexes, (2) perfection of starch crystallites and (3) formation of amylose double helices (Hoover & Vasanthan, 1994a; Jacobs et al. 1998a; Waduge et al., 2006). The X-ray diffraction pattern (Fig. 1) showed that additional lipid complexes were not formed on annealing. Jayakody et al. (2007) have shown that the crystalline regions of native *D. esculenta* and *D. alata* starches are not hydrolyzed during the time course of hydrolysis (15 days) used in this study. Therefore, perfection of starch crystallites during annealing cannot be considered as a factor influencing hydrolysis of the annealed starches. Amylose leaching studies (Table 3) showed that interactions involving amylose chains (AM–AM and/or AM–AMP) occur on annealing. Formation of crystallites (within the amorphous regions) resulting from the above interactions could hinder accessibility of H_3O^+ to the glycosidic oxygens. This would then explain the decrease in hydrolysis on annealing. Variations in the extent of reduction in hydrolysis among the starches may be a reflection of differences in the size and/or number of crystallites.

3.9. Impact of annealing on retrogradation

In all starches, annealing decreased the extent of retrogradation (monitored by changes to the enthalpy of retrogradation (ΔH_R) during storage) nearly to the same extent (Fig. 4). For instance, at the end of the 7th day of storage (40 °C), the increase in ΔH_R for the native starches was 2.8 J/g (kukulala), 3.1 J/g (java-ala), 2.7 J/g (nattala), 2.5 J/g (hingurala) and 2.4 J/g (raja-ala). However, the corresponding increase for the annealed starches were 2.1 J/g (kukulala), 2.5 J/g (java-ala), 1.9 J/g (nattala), 1.9 J/g (hingurala) and 1.7 J/g (raja-ala). The decrease in ΔH_R was surprising, since all interactions formed among and between amylose and amylopectin chains should have been destroyed during gelatinization. The decrease in ΔH_R suggests weaker interactions between amylopectin chains and/or decreased amylopectin chain mobility during gel storage of the annealed starches. It is likely that AM–AMP interactions formed during annealing are stronger than those formed between AMP chains (since AMP–AMP interactions involve branched molecules). Consequently, if AM–AMP interactions were not destroyed during gelatinization, then they could hinder (by decreasing their mobility) the ability of adjacent AMP chains to form double helices. This would then explain the difference in ΔH_R between native and annealed starches.

4. Conclusion

The results showed that changes to the physicochemical properties of the *Dioscorea* starches on annealing was influenced by: (1) native starch structure (amylopectin chain length distribution, arrangement of amylose chains in the granule interior), (2) native starch composition (phosphate monoester content, amylose/amylopectin ratio) and (3) structural changes formed during annealing (crystallite perfection, crystallite reorientation, interaction between and among starch chains). This study would help food processors to tailor the properties of annealed *Dioscorea* starches (by different moisture/temperature/time combinations) to a level that is presently met by chemical modification.

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