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PROPERTIES OF ANNEALED JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM.) SEED STARCH

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Jackfruit seed starch was annealed by single stage and double stage processes and characterised for changes in properties. Single-stage annealing gave higher crystalline order than double-stage annealed starch. No major change in the granular morphology was observed. Annealing resulted in notably altered pasting properties. Increased peak viscosity was indicative of molecular rigidity developed in the granules due to annealing. The increased thermal stability in DSC and specific peak sharpening in the XRD patterns of single-stage annealed starches indicated development of 'site specific' crystallinity. The new crystallites formed during the first stage of double-stage annealing were heat labile as crystallinity lowered after the second stage. Single stage annealed jackfruit seed starch can be used for noodle making.

Keywords: starch, annealing, morphology, crystallinity, thermal properties

Starch is used in the industries in modified form (Jayakody & Hoover, 2008). Annealing of starch is a modification technique involving mild heat treatment of the polymer in excess water (Tester & Debon, 2000). The temperature employed is above the glass transition temperature but below the gelatinization temperature (GT) of the starch. Starch annealing has been described as diffusion controlled non-equilibrium process leading to crystal growth and perfection (Tester et al., 2000) that results in more homogeneous crystallites. Jacobs and co-workers (1998) suggested that this increase in crystalline perfection may result from interaction of amylose-amylose and/or amylose-amylopectin chains during the process. Annealing changes the physicochemical properties of the starch, however with the least effect on the native granular structure and crystallinity patterns. Annealing initially enhances the order in the amorphous lamellae and gradually affects the order in the amylopectin double helices. Annealed starches have higher GT (Tester & Debon, 2000). However, difference in source of starch, amylose content, and molecular organization was found to have variable effect on the properties after annealing (Dias et al., 2010; Vamadevan et al., 2013). Single step, double- and multi-step annealing involving higher temperature after an initial low temperature step are followed (Jayakody & Hoover, 2008; Shi, 2008). The GT was found to increase with successive repetition of annealing with controlled increase in temperature.

Jackfruit seed cotyledons contain more than 73% starch (d.b.) (Dutta et al., 2011). The starch has been studied for its native and modified properties in recent times (Kittipongpatana & Kittipongpatana, 2011; Rengsutthi & Charoenrein, 2011). With 27.1% apparent amylose content, it may have food and non-food applications. In the present study, isolated jackfruit seed starch was annealed by single and double-step annealing processes and the physico-chemical changes were studied to assess its industrial use. Further, effect of α-amylase on the morphology of native and annealed jackfruit seed starch granules was observed.

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1. Materials and methods

1.1. Sample collection

Jackfruits were collected from a single tree near Tezpur University campus in the month of June, 2013. The ripened fruit were cut open manually with a sharp knife and the seeds were taken out from the pods. The seeds were washed to remove the outer cohesive material and the arils were manually removed. The seeds were then lye peeled to remove spermoderms. The peeled seeds were thoroughly rinsed in distilled water and then dried at 30 ºC for 48 h and stored at 4 ºC.

1.2. Isolation of starch

Starch was isolated by a method described by Dutta and co-workers (2011).

1.3. Annealing

For single-stage annealing, 10 g native starch was heated with 50 ml distilled water at 45, 50, 55, and 60 ºC for 72 h in a shaking hot water bath (Dubnoff, model 304/D). Double-stage annealing involved an additional initial heating step at a temperature that was 10 ºC below the above stipulated temperature for the same duration. The annealed samples were centrifuged at 5000 r.p.m. for 5 min and the residue that was collected was dried at 40 °C, ground in a mill (Fritsch Pulverisette 14, Germany), passed through a 100 µm sieve, and stored at 4 °C for further analysis. The single-stage annealed samples were coded as ‘S’ and suffixed with the treatment temperature and the double-stage annealed were coded as ‘D’ and suffixed with the treatment temperature applied during the second stage. The native sample was coded as ‘N’.

1.4. Study of granule morphology after annealing and enzyme treatment

Annealed starches were subjected to enzymatic hydrolysis, according to the method described by Dias and co-workers (2010). The native, annealed, and enzyme treated samples were observed under a Scanning Electron Microscope (SEM, JEOL 6993V, Japan) operating at an acceleration voltage of 15 kV and at a magnification of × 2000.

1.5. Swelling power and solubility

Swelling power and solubility of the annealed samples were determined as per Dutta and co-workers (2011).

1.6. Light transmittance

Light transmittance was determined according to Dutta and co-workers (2011).

1.7. Pasting properties

A rapid viscosity analyser (RVA, Newport Scientific Instruments, Starchmaster2, Australia) was employed to record the pasting profiles of the native and annealed starch samples. Starch suspension in distilled water (12%, w/w; 28 g total weight) was prepared and the STD1 profile of Newport Scientific was run. The peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), breakdown (BD), and setback (SB) were recorded. The gel obtained after each RVA cycle was cooled to room temperature, covered to prevent moisture loss and stored overnight at 4 ºC for texture analysis.

1.8. Gel hardness

A texture analyser (Stable Micro Systems, TA. HD. plus, UK) with a 5 kg load cell fitted with a Bakelite cylindrical probe of 5 mm diameter was employed for performing the test. The gel (obtained in subsection 1.7) was compressed at three points up to 10 mm at 1 mm sec^{-1}. Hardness, defined as the maximum force in kg to fracture the gel was recorded.

1.9. X-ray diffraction

Wide angle X-ray diffractograms of native and annealed jackfruit seed starch samples (11–13% moisture, db) were obtained with an X-ray diffractometer (Rigaku Miniflex, Japan) with a λ value of 1.54, operating at 30 kV acceleration potential, and 15 mA current with a copper target. The scanning range was 2–40º of 2θ at a scan speed of 5º 2θ/min. The diffractograms were evaluated according to ZOBEL (1964) and percentage crystallinity was determined according to SINGH and co-workers (2006).

% Crystallinity=(Area under the peaks/Total area under the curve)×100

1.10. Thermal properties

Differential Scanning Calorimetry (DSC) of the starch samples were carried out (Shimadzu, DSC-60, Japan). The machine was periodically calibrated with pure indium for heat flow and temperature. Briefly, 2 mg starch slurry samples (1:2, starch to water ratio, w/w) were weighed in aluminium pans. The pans were then hermetically sealed and heated against empty reference pans from 20 ºC to 150 ºC at a heating rate of 5 ºC min^{-1} under nitrogen atmosphere. The thermal transition patterns were studied and melting transition points, viz., onset temperature (T_o), peak temperature (T_p), final temperature (T_f), and the range of endotherms (T_f–T_o) were determined using TA-60WS software. The enthalpy change (ΔH) was calculated as the area under the endothermic peak.

1.11. Statistical analysis

For statistical analyses, SPSS 11.5 (SPSS Inc., USA) software was employed. All the experiments were carried out in multiple replicates and the mean values are reported. Duncan's multiple range test was carried out for determining the significance of difference amongst the values for each parameter.

2. Results and discussion

2.1. Granule morphology

Figure 1 A, B, and C shows the morphology of the native, S55, and D55 samples, respectively. Four different shapes of native Jackfruit seed starch granules were reported in our earlier study (DUTTA et al., 2011). The structure of the granules did not change upon annealing, which was in agreement with STUTE (1992), HOOVER and VASANTHAN (1994), JACOBS and co-workers (1998), and WADUGE and co-workers (2006). However, some swelling of a few granule edges could be observed in the D55 sample. KISELEVA and co-workers (2005) also reported partial and permanent deformation of the lens-shaped waxy wheat starch granules that did not reform even after drying. Significant segmental mobility of starch fractions during prolonged annealing might have created space for inward penetration of water into the granules causing this swelling in D55 sample. The native starch granules were severely affected by α amylase (Fig. 1D). Enzyme attacked the surface of the granules (Fig. 1E and F), similar to acid-alcohol treated starch (Dutta et al., 2011). Surface etching was less in both single and double annealed samples. Jacobs and co-workers (1998) reported similar non-uniform granule digestion of annealed wheat starch by pancreatin, which may be due to the formation of enzyme resistant fractions (Hoover & Vasanthan, 1994). The crystalline regions developed on annealing were perhaps less affected by the enzyme (Chung et al., 2010). Digestibility of annealed starch reportedly varies with the source of the starch (Chung et al., 2009; Chung et al., 2010). A detailed research on the digestibility pattern will be useful for targeted application of the single and double annealed jackfruit seed starch.

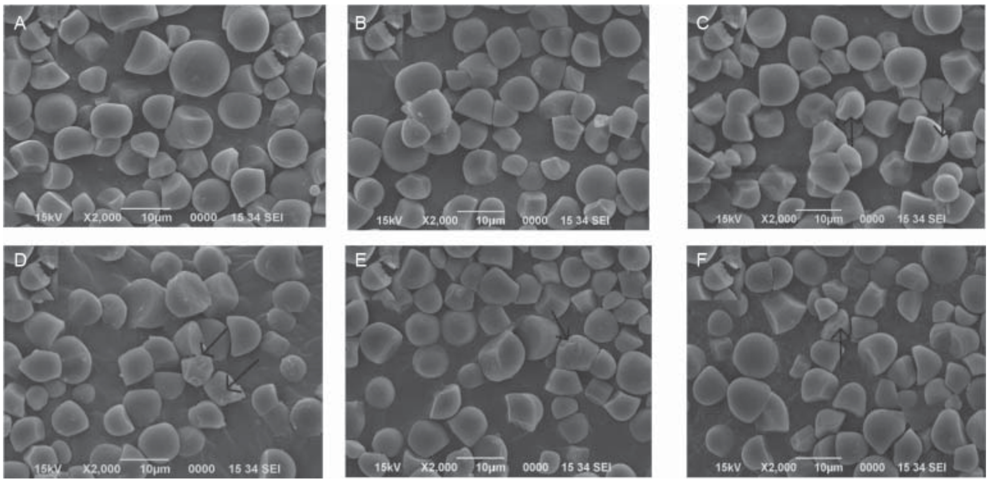

Fig. 1. SEM micrographs of (A) N, (B) S55, and (C) D55. (D): enzyme treated N; (E): enzyme treated S55, and (F): enzyme treated D55. Arrow marks (↑) indicates altered granule morphology on annealing and enzyme treatment

2.2. Swelling power and solubility

Native jackfruit seed starch showed the highest swelling power (Fig. 2A). Swelling power decreased upon annealing across all temperatures due to a corresponding increase in crystallinity. Solubility was also found to be lower in annealed starches (Fig. 2B). At a given temperature post-annealing the granules swell less than non-annealed starch granules that restrain the leaching of the amorphous leachate (Morrison et al., 1993). The lower availability of soluble fractions in the annealed samples may possibly be due to leaching out of a portion of amylose during annealing (Tester et al., 2000). The greater extent of lowering of swelling power and solubility at different temperatures in single step annealed samples indicated higher degree of order in it.

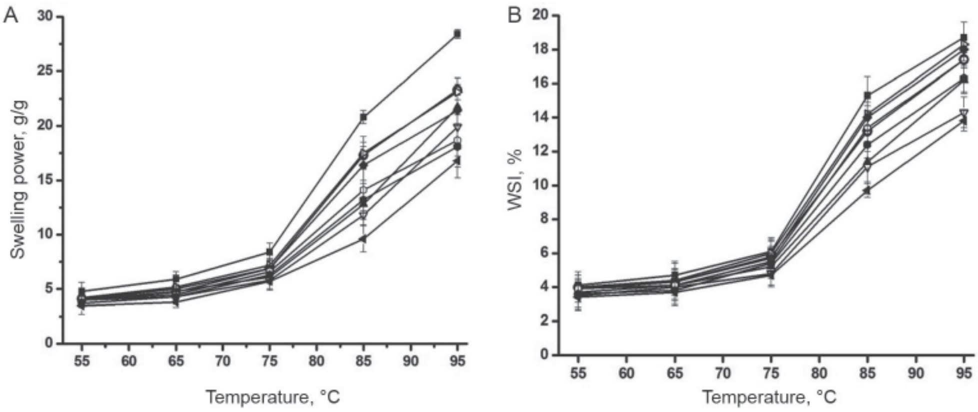

*Fig. 2.*A: Swelling power and B: Solubility of the native and annealed samples.
N: ; S45: ; S50: ; S55: ; S60: ; D45: ; D50: ; D55: ; D60:

2.3. Light transmittance

The % transmittance at 640 nm of the native starch sample was the lowest of all (Fig. 3). The annealed samples exhibited progressively higher transmittance than native, indicating increased tendency towards retrogradation due to their greater molecular stability against degradation into soluble fractions (Jayakody & Hoover, 2008). The transmittance of the cooked slurry increased irregularly yet progressively with refrigerated storage (Fig. 3). Native starch paste required longer time to attain clarity. Probably leached fractions in annealed samples formed larger stable fractions that could sediment easily on storage after cooking.

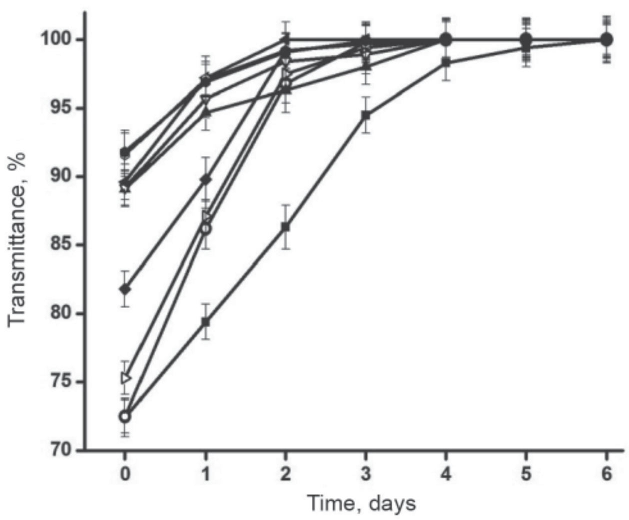

Fig. 3. Transmittance of cooked slurries of the native and annealed samples with storage time.
N: ; S45: ; S50: ; S55: ; S60: ; D45: ; D50: ; D55: ; D60:

Table 1. Pasting properties and gel hardness of the raw and annealed starch samples

	Native	S45	S50	S55	S60	D45	D50	D55	D60
Pasting properties									
PV (cP)	3895 ± 3.3^{e}	4137 ± 2.9^{f}	4226 ± 1.6^{h}	4230 ± 3.2^{h}	4207 ± 2.8^{g}	3528 ± 1.9^{cd}	3449 ± 2.4^{b}	3444 ± 1.7^{a}	3527 ± 2.5^{cd}
HPV (cP)	3067 ± 2.2^{e}	3107 ± 2.1^{f}	3130 ± 1.4^{h}	3175 ± 2.9^{i}	3115 ± 3.1^{g}	2645 ± 2.6^{cd}	2582 ± 2.7^{b}	2560 ± 1.9^{a}	2643 ± 2.2^{cd}
CPV (cP)	5239 ± 3.1^{e}	5630 ± 2.2^{f}	5666 ± 1.9^{g}	5748 ± 2.3^{h}	6138 ± 2.1^{i}	4819 ± 3.1^{d}	4685 ± 2.0^{c}	4604 ± 1.8^{a}	4668 ± 3.4^{b}
BD (cP)	828 ± 2.4^{a}	1030 ± 2.6^{d}	1096 ± 2.1^{f}	1055 ± 3.5^{e}	1092 ± 1.9^{f}	883 ± 2.5^{c}	867 ± 0.8^{b}	884 ± 3.1^{c}	884 ± 2.6^{c}
SB (cP)	2172 ± 1.3^{d}	2523 ± 1.7^{e}	2536 ± 1.6^{f}	2573 ± 3.3^{g}	3023 ± 1.4^{h}	2174 ± 2.6^{d}	2103 ± 2.7^{c}	2044 ± 2.1^{b}	2025 ± 2.7^{a}
Gel hardness (kg)	44.64 ± 1.1^{a}	46.22 ± 1.3^{b}	47.17 ± 0.7^{bc}	48.19 ± 1.1^{cd}	48.96 ± 1.2^{cd}	48.68 ± 1.1^{cd}	49.43 ± 1.0^{d}	51.89 ± 0.8^{e}	52.81 ± 1.3^{e}

Means followed by a common letter in a row are not significantly different by Duncan's Multiple Range Test at $P<0.05$

2.4. Pasting properties

RVA pasting properties of starch are very important for its targeted use. The single-stage annealed samples (Table 1) showed higher PV than the native sample. The double-stage annealed samples exhibited lower PV than the native sample, indicating weaker molecular arrangement in them that did not allow swelling during cooking. JACOBS and co-workers (1998), however, had attributed the reduced granular swelling and amylose leaching to increased interaction between starch crystallites during annealing, which developed granular rigidity and resistance to shear. S60, the highest crystalline sample as revealed by XRD and DSC studies, exhibited the highest CPV and SB. This meant that the single stage annealed jackfruit seed starch molecules had greater tendency to retrograde on cooling in the RVA cycle. The results may prove to be useful for utilization of the native and annealed jackfruit seed starch.

2.5. Gel hardness

The hardness values of cooked and cooled gels of raw and annealed jackfruit seed starch are given in Table 1. Annealing caused distinct increase in cooked gel hardness (CHAM & SUWANNAPORN, 2010). Hardness was higher in double-annealed samples than single annealed samples and the native starch gave the softest gel. CHUNG and co-workers (2000) reported that annealing causes rearrangement of starch molecules, resulting in reduction of swelling power and solubility of cooked starch that increases in gel hardness.

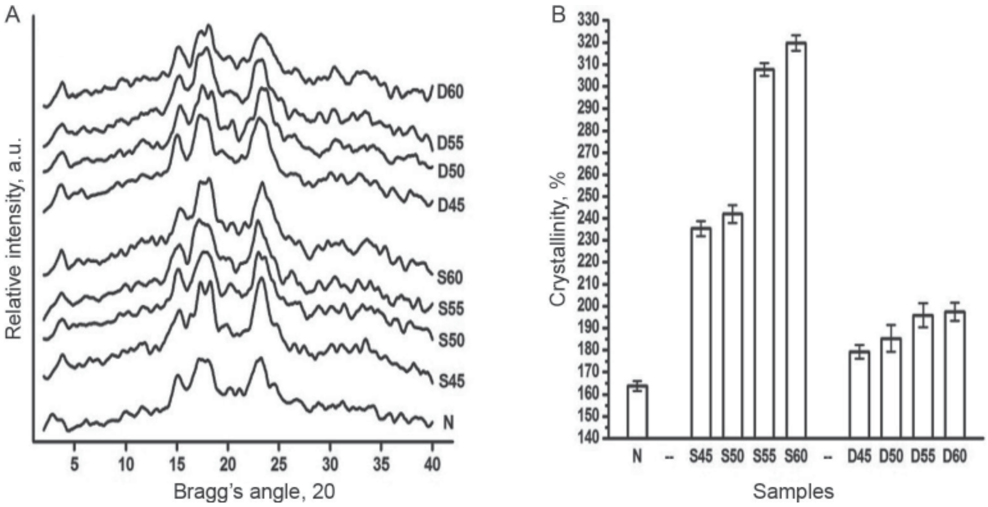

*Fig. 4.*A: X-ray diffractograms of native and annealed starch samples. Arrow marks (↑) indicate developments in the diffraction peaks at 2θ2Θ near 18 and 23 on annealing. B: % Crystallinity of the samples

2.6. X-ray diffraction

The native A-type crystallinity with distinct peaks near 2θ values of 15, 18, and 23 (Zobel, 1964) remained unchanged with both types of annealing (Fig. 4A). However, a clear increase in crystallinity was observed in the annealed samples (Fig. 4B), with double-stage annealed starch showing lower crystallinity than single-stage annealed starch. Probably, fractions of the crystallites formed during first annealing stage were heat labile, remained intermediate between the amorphous and crystalline forms of the polymer, and lost the acquired crystallinity during the second stage. As explained in the SEM studies (section 2.1), after the first stage annealing, the granules tend to absorb more water in the second stage to lose native shapes, and during this, the newly coiled chains that exhibited crystallinity tend to decoil and regain amorphous mobility, thereby resulting in lowered crystallinity after the second stage.

2.7. Thermal properties

DSC endotherms of the native and annealed starch samples revealed distinct shift of T_o, T_p and T_f towards higher values (Fig. 5 and Table 2) along with a narrowing of the endothermic peak as demonstrated by the T_f–T_o values (Genkina et al., 2007). The increase in stability of the crystalline zones must have required higher temperature to melt the crystallites. S60 exhibited a marked shift in endotherm, suggesting that the highest degree of annealing occurred in it. No endotherm for amylose-lipid complex melting was observed in any of the samples. The DSC results were in accordance with the XRD patterns of the samples (section 2.6). The double-stage annealed samples were less crystalline than the single-stage annealed samples and also exhibited lower melting enthalpy, which indicated that the second step in the double-stage annealing brought the degradation of a few crystallites formed during the first stage of annealing.

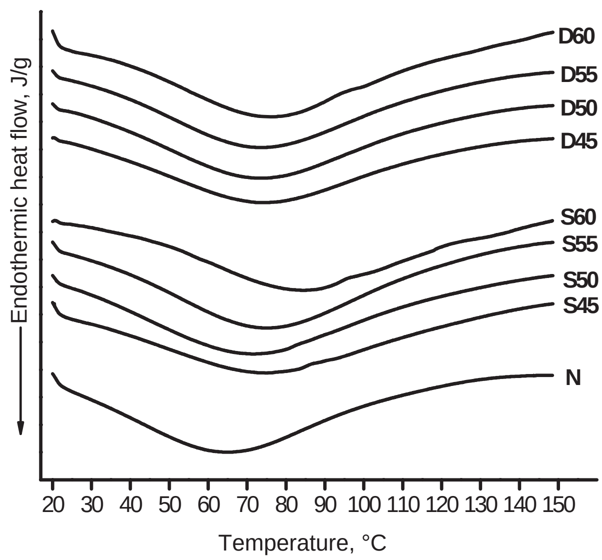

Fig. 5. DSC endothermic curves of native and annealed jackfruit seed starch samples

Table 2. Values of thermal parameters of native and annealed starch samples

Parameter	N	S45	S50	S55	S60	D45	D50	D55	D60
T_o (ºC)	44.7±0.9^a	48.7±0.7^d	49.9±0.4^e	50.5±0.7^e	51.7±0.7^f	44.9±0.9^b	45.1±0.5^{ab}	45.7±0.4^{ab}	46.3±0.5^c
T_p (ºC)	63.7±0.8^a	75.2±0.4^{cd}	75.9±0.5^d	75.9±0.7^d	83.4±0.7^e	73.9±0.5^b	74.2±0.4^{bc}	74.6±0.4^{bc}	75.8±0.2^d
T_f (ºC)	94.3±0.3^a	98.3±0.5^b	99.1±0.6^b	99.4±0.6^{bc}	100.2±0.4^c	94.3±0.9^a	94.4±0.2^a	94.9±0.6^a	95.5±0.3^c
T_f–T_o (ºC)	49.6±0.1^e	49.6±0.2^e	49.2±0.2^c	48.9±0.1^b	48.5±0.1^a	49.4±0.2^d	49.3±0.1^d	49.2±0.1^c	49.2±0.2^c
ΔH (J/g)	19.7±0.2^a	21.8±0.2^d	23.8±0.1^f	24.8±0.2^g	27.2±0.3^h	20.0±0.2^b	21.3±0.1^c	23.2±0.3^e	24.9±0.2^g

T_o: onset temperature; T_p: peak temperature; T_f: conclusion temperature; ΔH is enthalpy of the crystallite melting endotherm evaluated from the DSC curve. The means followed by a common letter in a row are not significantly different by Duncan's Multiple Range Test at $P<0.05$.

3. Conclusions

Significant differences in the physico-chemical properties of single and double-stage annealing on jackfruit seed starch were noted. Single-stage annealing was found to increase the crystalline order of jackfruit seed starch and hence could find use in noodle making.

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