



## A study of the properties of starch isolated from three varieties of *Lablab purpureus* seeds

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### ABSTRACT

Starch isolated from three varieties of *Lablab purpureus*: Rongai white, Rongai brown and Highworth black ranged from 13.2–15.8%. The starch granules were similar in shape (oval) and medium in size (12.51–20.56  $\mu\text{m}$ ) but slightly differed in granule size distribution. The starches exhibited a C-type X-ray diffraction pattern with degree of crystallinity ranging from (37.0–46.3%). The apparent amylose ranged from 23.1–26.0% and absolute amylose was 17.5–23.5% and the two were significantly different ( $p < 0.05$ ). The starches had high onset gelatinization temperatures ( $T_o = 73.5$ – $75.7^\circ\text{C}$ ), the gelatinization range and enthalpy change were 12.9–17.7  $^\circ\text{C}$  and 12.3–18.8 J/g, respectively. The starches had single stage swelling and amylose leaching patterns. The starch pastes exhibited significant shear thinning, low clarity and poor freeze–thaw stability.

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### 1. Introduction

*Lablab purpureus* is an annual fodder legume grown for conservation in tropical environments. It is a vigorously trailing and twining herbaceous plant resistant to disease and insect attacks (Murphy & Colucci, 1999). Of the two hundred types of *Lablab* so far recognized, only two varieties, Rongai and Highworth are available commercially. Additionally, the three subspecies that have been identified are *purpureus*, *benghalensis* and *uncinatus*. The wild forms of *Lablab* are believed to have originated from India (Deka & Sarkar, 1990) and were introduced into Africa from South East Asia during the 18th century (Kay, 1979). *Lablab* is used as a vegetable and grain legume (Adebisi & Bosch, 2004; Shivashankar & Kulkarni, 1989; Smartt, 1985). It has high grain yields of 1–2.5 t/ha (Grain, 2006). The annual production of the bean in Bangladesh was put as 49,000 metric tons during 1997–2000 (Pervin, Islam, & Islam, 2008). Despite its wide distribution in the tropics, *Lablab* is still considered neglected in terms of research and development. Available information showed that the cotyledons of *Lablab* constituted 82.9–90.8% by weight of whole seed, with 45.9–52% of starch (Betancur-Ancona, Chel-Guerrero, Bello-Perez, & Davila-Ortiz, 2002; Chau, Cheung, & Wong, 1998). Salimath and Tharanathan (1982) reported 50% starch and 26% protein in *Lablab* seeds with starch recovery of 49.0%. Information on *Lablab* starch is scanty but some properties such as granule size, amylose content, gelatinization temperature, swelling power and amylograph viscosity have been

reported. The granules of *Lablab* starch have been reported to be oval in shape, with sizes up to 30  $\mu\text{m}$ ; gelatinization temperature ranged from 65 to 80  $^\circ\text{C}$  and Brabender amylograph viscosity was similar to that of cross-linked starch (Rosenthal, Espindola, Serapião, & Silva, 1971). El Tinay, El Hardalou, and Nour (1983) in another study, compared the properties of starches from *Lablab*, Chickpea and Pigeon pea legumes and reported restricted single stage swelling patterns and stabilized Brabender hot paste viscosity for the starches. Starch in legumes vary with the botanical source but is generally in the range of 10.9–44% (Azam-Ali et al., 2001; Powrie, Adams, & Pflug, 1960; Schoch & Maywald, 1968; Stevenson, Doorenbos, Jane, & Inglett, 2006; Velasco, Rascon, & Tovar, 1997). Starch granule sizes are variable, from as small as 0.7  $\mu\text{m}$  in soybean starch (Stevenson et al., 2006) to as large as up to 34  $\mu\text{m}$  in Jack bean (Lawal & Adebawale, 2005). Legume starches generally exhibit a C-type X-ray diffraction pattern with crystallinity usually in the range of 27.2–36.7%; however, an A-type X-ray diffraction pattern (crystallinity, 43.69%) has been reported for Bambara groundnut (Sirivongpaisal, 2008). Legume starches may contain as high as 40% amylose (Sandhu & Lim, 2008; Schoch & Maywald, 1968), the granules gelatinize at fairly high temperatures (62.5–85  $^\circ\text{C}$ ) but a low onset temperature of 52  $^\circ\text{C}$  has been reported (Stevenson et al., 2006). Many legume starches have restricted swelling and solubility with stabilized Brabender hot paste viscosity but a few exceptions have been reported (Schoch & Maywald, 1968). They also present low freeze–thaw stability and poor paste clarity, hence are usually modified for improved properties (Hoover & Ratnayake, 2002; Lawal, 2008; Rege & Pai, 1996). *Lablab purpureus* could be a potential

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source of industrial starch but this will require a thorough appraisal of the starch content and properties of the different varieties.

## 2. Materials and methods

### 2.1. Materials and starch isolation

Three varieties of *L. purpureus*: Rongai white (NAPRI 4), Rongai brown (P1 509114) and Highworth black (GRIF 12293) (Fig. 1) used in this study were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. A known weight (50 g) of whole-seed sample of each variety was soaked in water for 2 h. The swollen seeds were dehulled by pressing the endosperm out of the softened hull and the hulls air dried. The endosperm was wet milled using a Philips blender. The slurry was dispersed in a large quantity of water and sieved with a muslin cloth. The extract was allowed to settle and the supernatant decanted. The brown sediment was dispersed in 0.3% (w/v) sodium hydroxide solution (Schoch & Maywald, 1968) and washed with the same until a white starch was obtained. The resulting starch was washed repeatedly with distilled water until the washing water was neutral to litmus. The starch was air dried and stored in an air tight container. Samples used in this study were oven dried at 105 °C for 12 h.

### 2.2. Microscopy

Granule micrographs were obtained with a JSM 35 Genie Scanning Electron Microscope (SEM). The starch was sprinkled onto a double-backed adhesive carbon tab stuck to a circular aluminum stub. The aluminum stub with the starch sample on it was placed in the vacuum chamber of a Polaron PS 3 sputter coater. After attaining a vacuum of 0.1 to 0.2 torr and plasma current of 42 mA, the gold coating process was carried out for 140 s. The stub with gold coated starch was then placed in the SEM chamber which was evacuated before the electron beam was turned on. A 10 kV/2.05 A setting was used for the subsequent imaging work on starch, the aperture size being fixed at 3. Image particle size analysis was done with a BT-1600 Image particle size analyzer (Bettersize Instruments Ltd.). The starch powder on a microscope slide was viewed with a light microscope (objective magnification X40) (Meiji Techno, Japan). The computer controlled video was processed and the granule characteristics determined.

### 2.3. X-ray diffraction

The starch sample was passed through a 63 µm mesh sieve and placed in the cavity of a disc sample holder of the diffractometer. Diffraction diagrams were recorded using Inel X-ray equipment operating at 40 kV and generator current of 30 mA. CuKα<sub>1</sub> radiation ( $\lambda = 0.15405$  nm) was selected using a quartz monochromator and scanned between 3° 2θ to 30° 2θ. A curved position detector (Inel CSP120) was used to monitor the intensities using 2 h exposure periods. The data was analyzed using PeakFit software (Systat software Inc.) to quantitatively estimate the degree of crystallinity. Erfc Pk type was used in peak fitting analysis of the amorphous area at  $r^2 > 0.99$ . The percentage crystalline area was obtained by difference.

### 2.4. Determination of the blue value and amylose content

Starch (0.1 g) was added to ethanol (1 mL, 95%) in a test tube followed by the addition of NaOH (9 mL, 1 M) and heated in a water bath to gelatinize the starch. This was transferred quantitatively into a 100 mL standard volumetric flask and made up to the mark with distilled water. 5 mL of the solution was taken into a 100 mL volumetric flask to which was added acetic acid (1 mL, 1 M) followed by 2 mL stock iodine (0.2 g I<sub>2</sub>/2 g KI) and made up to mark with distilled water. This was left for 20 min for the colour to fully develop. The solution was put in a 1 cm cuvette and scanned in a Lambda 25 UV/visible Spectrophotometer (wavelength 350–950 nm, scan speed 480) using iodine solution of the same concentration, but without starch, in the reference cell. A calibration curve was prepared with pure potato amylose (10–50 mg) from which the amylose content of the starches was obtained by extrapolation from the absorbance-amylose concentration curve.

The blue value was calculated as

$$\frac{\text{Maximum absorbance} \times 4}{\text{Starch concentration (g/dL)}}$$

Absolute amylose content was determined by the same procedure except that the starch sample was defatted by dissolving in 90% dimethyl sulphoxide (DMSO) solution (Stevenson et al., 2006) overnight, followed by precipitation with propanol.



Fig. 1. Lablab purpureus seeds.

## 2.5. Gelatinization properties

The gelatinization properties of starch were determined using differential scanning calorimetry (Micro DSC III, Setaram Instruments). 10% starch dispersions were placed in the sample cell and an equal mass of water was placed in the reference cell. The samples were heated from 25 to 100 °C at a scan rate of 0.5 °C/min. The DSC was initially calibrated using naphthalene crystals wrapped with aluminum foil placed in the sample cell and an equal weight of aluminum foil in the reference cell.

## 2.6. Swelling power and amylose leaching

Starch (0.1% (w/w) dry starch) was dispersed in distilled water by means of a magnetic stirrer. Dispersion aliquots (10 g) containing 1 mg/mL starch were transferred into pre-weighed tubes, sealed and immersed in a thermostatic water bath fitted with a mechanical shaker for 30 min from 60 to 95 °C at 5° intervals. The samples were left agitated through out the heating period to maintain a starch suspension. The samples were centrifuged at 1500 rpm for 10 min. The supernatant was carefully drawn up. The weight of the paste was determined and used to calculate swelling power as weight of paste divided by the original weight of dry starch. 5 mL of the supernatant was transferred into a 100 mL volumetric flask followed by the addition of acetic acid (1 mL), stock iodine solution (2 mL, 0.2 g I<sub>2</sub>/2 g KI/ 100 mL) and the volume made up to mark. This was shaken and the absorbance measured after 20 min. The amylose concentration was extrapolated from a standard absorbance-amylose curve. The amylose content was expressed as mg amylose/100 mg starch.

## 2.7. Determination of paste clarity

Paste clarity was determined by the method of Singhal and Kulkarni (1990) by measuring the percentage light transmitted by 1.0% (w/v) starch paste at 660 nm on a UV/visible Spectrophotometer. Distilled water was used in the reference cell.

## 2.8. Freeze–thaw stability

The freeze–thaw stability was determined by the method of Singhal and Kulkarni (1990), starch paste prepared by heating the starch dispersion (5% (w/v)) in a water bath maintained at 95 °C for 30 min. The starch paste was stored at 4 °C (18 h) and thawed at 25 °C (3 h), and centrifuged at 2500 rpm for 10 min and the weight of exudates determined over a 6 day period. Freeze–thaw stability was calculated as percentage weight of exudates per weight of paste.

## 2.9. Rheological properties

The rheological properties of 6.0% starch paste were investigated. The starch dispersions were heated in sealed tubes immersed in a water bath maintained at a temperature of 99 °C for 30 min. The samples were agitated during heating until pasting occurred. The pastes were removed and left at 25 °C and the rheological properties examined after 1 h. The flow properties were measured on a controlled stress Rheometer (AR 500, TA Instruments Ltd.) with cone and plate geometry (60 mm, 2° Cone and 50 µm gap). Measurements were carried out at 25 °C at shear rates of 10<sup>−1</sup>–1000 s<sup>−1</sup>. The viscoelastic properties of the starch pastes were determined by carrying out a frequency sweep in the range of 10<sup>−1</sup>–200 rad s<sup>−1</sup> within the viscoelastic region (strain, 0.2%). The linear viscoelastic region was obtained by performing a strain sweep within the range of 0.01–100 at an angular frequency of

1 rad s<sup>−1</sup>. The storage modulus (G′) and loss modulus (G″) of the starch pastes were analyzed by the TA Data Analysis software.

## 2.10. Statistical analysis

Analysis of variance (ANOVA) was used to compare sample means at 95% confidence level ( $p < 0.05$ ) using Microsoft Excel 2003 software.

# 3. Results and discussion

## 3.1. Starch content

Table 1 shows the starch content of Lablab seeds. The weight of the hull as a percentage of whole seed was 15.2 ± 0.18 (Rongai white), 19.6 ± 0.32 (Rongai brown) and 21.5 ± 0.76 (Highworth black). The Lablab seeds had very thick hulls, its percentage of the whole seed varied with variety; values are similar to 18% reported by Salimath and Tharanathan (1982) but higher than 7.7% reported for Navy bean seed hull (Powrie et al., 1960). The isolated starch constituted 13.2–15.8% of the seed. The hull content was significantly different while the starch yield was not with respect to varieties. The high protein content of Lablab endosperm, ~26% (Salimath & Tharanathan, 1982), makes starch isolation very difficult. It took several washings with 0.3% (w/v) NaOH to recover pure starch from the slurry. *Dolichos lablab* was reported to contain 50% starch with recovery of 49.0% (Salimath & Tharanathan, 1982). The starch yield of Lablab is within the range 10.9–44% reported for different legume starches (Sandhu & Lim, 2008; Schoch & Maywald, 1968; Stevenson et al., 2006).

## 3.2. Granule characteristics

The scanning electron micrographs of Lablab starches are shown in Fig. 2a–c. The starch granules were similar in morphology and shape for the three varieties. The granules were oval and medium in size and in the following range: Rongai white (17.77–6.44 µm), Rongai brown (20.56–7.57 µm) and Highworth black (19.76–8.18 µm). Rongai white and Highworth black had similar granule size distribution different from Rongai brown with a higher population of smaller granules. The granule size distribution and image analysis for the starches are presented in Fig. 3 and Table 2. The largest granule (20.56 µm) was found in Rongai brown and the smallest (6.44 µm) in Rongai white. The average granule size was 12.51 µm (Rongai white), 12.59 µm (Rongai brown) and 13.18 µm (Highworth black). The starches were characterized by L/D ratio of 1.26–1.29 and roundness 0.66–0.71. Granule size range of 10–30 µm was reported for Lablab bean starch by Salimath and Tharanathan (1982). Mung bean starch was reported to have oval to round granules with sizes 7–26 µm (Hoover, Li, Hynes, & Senanayake, 1997). Lawal (2008) reported granule diameters of 7–40 µm for pigeon pea starch. Stevenson et al. (2006) reported very small granules (0.7–4 µm) for soybean starch granules. Starch granule size distribution has generated great interest in recent times and several studies have examined the influence of granule

**Table 1**  
Starch content of *Lablab purpureus*.

Parameters	Rongai white	Highworth black	Rongai brown
Hull (%)	15.2 ± 0.18 <sup>a</sup>	21.5 ± 0.76 <sup>b</sup>	19.60 ± 0.32 <sup>b</sup>
Starch (db) (%)	15.4 ± 0.6 <sup>a</sup>	13.2 ± 0.35 <sup>a</sup>	15.8 ± 1.10 <sup>a</sup>
Starch moisture (%)	12.63 ± 0.27 <sup>a</sup>	13.60 ± 0.41 <sup>a</sup>	5.29 ± 0.07 <sup>b</sup>

Values with superscripts are means of two determinations. Different superscripts on the same row are significantly different ( $p < 0.05$ ).



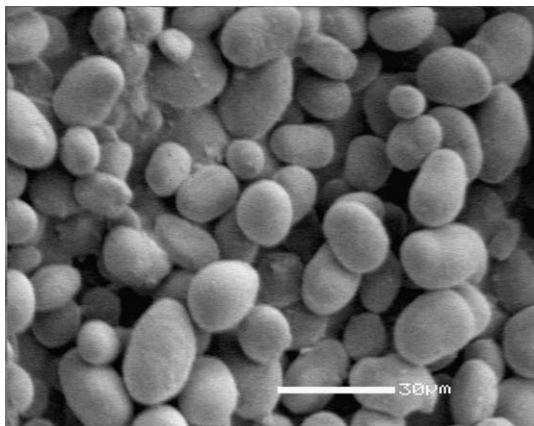


Fig. 2. (a) Rongai white starch granules 1600 $\times$ .

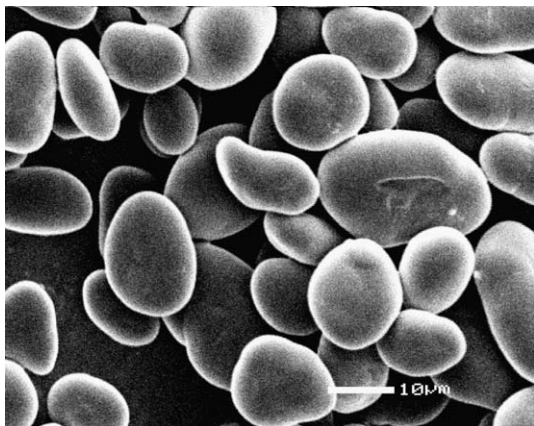


Fig. 2. (b) Rongai brown starch granules 3000 $\times$ . (c) Highworth black starch granules 3000 $\times$ .

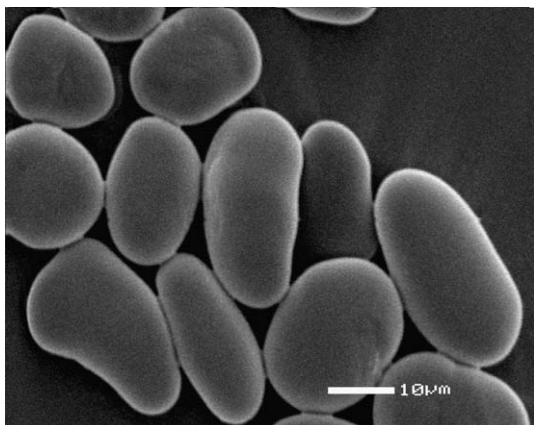


Fig. 2. (c) Highworth black starch granules 3000 $\times$ .

size on starch physicochemical properties. Smaller granules have been reported to show greater swelling, enzyme susceptibility and faster retrogradation compared with larger ones (Fukai, Takaki, & Kobayashi, 1994; Tang, Watanabe, & Mitsunaga, 2002; Vasanthan & Bhatt, 1996).

### 3.3. X-ray diffraction

Native starch is known to display three characteristic X-ray diffraction patterns, A-type, B-type and C-type. An A-type diffraction pattern is common in cereal starches and has characteristic peaks at 15, 17, 18 and 23 while a B-type common in tuber starches is associated with peaks at around 5.8, 15, 17 and two small peaks at 23 and 24. However, a C-type X-ray diffraction pattern which is common in pea starches is a mixture of A- and B-type patterns. The three varieties of Lablab exhibited a C-type diffraction pattern (Fig. 4). The strong peak  $2\theta = 17.3$  is characteristic of a B-type crystallinity, and a very weak peak at  $2\theta = 5.6$ , weaker than in a typical B-type. The single peak at  $2\theta = 23.4$ , instead of two peaks at around  $2\theta$  of 23 and 24, is characteristic of an A-type crystallinity. The analysis of the diffraction peaks gave the following percentage amorphous and crystalline areas: Rongai white (53.7, 46.3), Rongai brown (61.7, 38.3) and Highworth black (63.0, 37.0), respectively. We have not found any data on the X-ray diffraction pattern and crystallinity of Lablab starch for comparison. However, a C-type diffraction pattern has been reported for some other bean starches: mung bean (Hoover et al., 1997), soybean (Stevenson et al., 2006) and pigeon pea (Lawal, 2008). Percentage crystallinities ranging from 27.7% to 36.7% were reported for three varieties of soybean starch (Stevenson et al., 2006) and 43.69% for Bambara groundnut (Sirivongpaisal, 2008).

### 3.4. Blue value and amylose composition

Fig. 5a and b show the starch-iodine absorption spectra for apparent (untreated) and absolute (defatted) amylose for Lablab starches. The wavelength of maximum absorption ( $\lambda_{\max}$ ) was not the same for all the starches (Table 3) and differed for apparent and absolute amylose. This could be due to the difference in the starch composition and structural characteristics. In all the starches, the  $\lambda_{\max}$  increased while the blue value decreased with defatting. The  $\lambda_{\max}$  for absolute Lablab starch was centered around 604 nm. Pure amylose absorbs at  $\lambda_{\max}$  of 620 nm (Juliano, 1971). The respective apparent and absolute blue values for the starches were Rongai white (0.543, 0.362), Rongai brown (0.499, 0.490) and Highworth black (0.549, 0.472), and were significantly different at  $p < 0.05$ . Rongai brown had the highest absolute amylose content (23.45%) while Rongai white had the least (17.53%). The absolute amylose was lower than the apparent amylose for each starch variety and the two were significantly different ( $p < 0.05$ ). Several workers (Spence & Jane, 1999; Stevenson et al., 2006; Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda, 2003) have reported differences between the apparent and absolute amylose for other starches. El Tinay et al. (1983) have reported an amylose content of 31.0% for *D. lablab* starch while Lai and Varriano-Marston (1979) reported an amylose content of 38% for black bean starch. Wickramasinghe and Noda (2008) observed that amylose content was a major factor controlling most physicochemical properties of rice starch and showed a negative correlation to gelatinization temperature, swelling power, viscosity peak and breakdown, and enzyme digestibility.

### 3.5. Gelatinization properties

Starch gelatinization is an order-disorder phase transition of starch granules in the presence of water and heat. This transition occurs at a critical temperature which depends on starch concentration, granule structure and composition, and the presence of electrolytes (Ahmad & Williams, 1999). The DSC thermograms and the gelatinization parameters of the Lablab starches are presented in Fig. 6 and Table 4, respectively. The onset temperature ( $T_0$ ) for Rongai white was 73.8 °C, Rongai brown 73.5 °C and High-

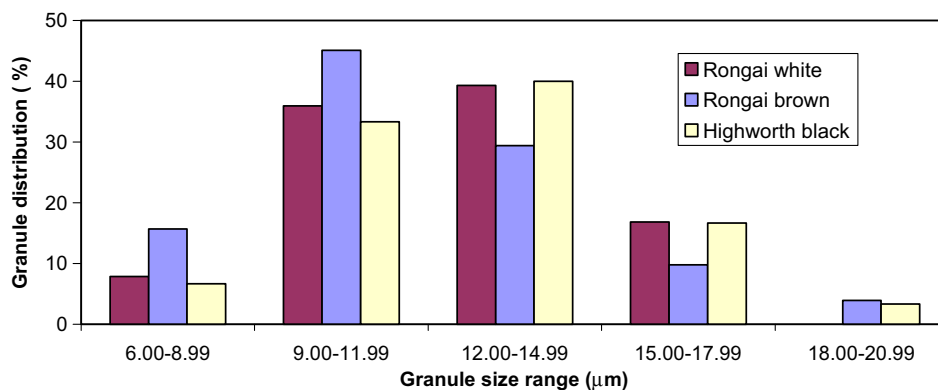


Fig. 3. Granule size distribution of *Lablab purpureus* starches.

Table 2

Granule size analysis of *Lablab purpureus* starches.

Particle characteristics	Rongai white	Highworth black	Rongai brown
Particle number	89	68	58
Maximum diameter (μm)	17.77	19.76	20.56
Minimum diameter (μm)	6.44	8.18	7.57
Average granule size (μm)	12.51	13.18	12.59
Length/diameter, L/D	1.29	1.26	1.28
Roundness	0.67	0.71	0.66

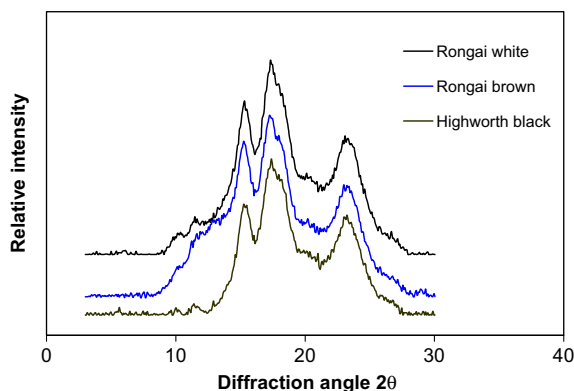


Fig. 4. X-ray diffraction patterns of *Lablab purpureus* starches.

worth black 75.7 °C. The gelatinization temperature was highest in Highworth black and least in Rongai brown. The gelatinization ranges ( $T_c-T_o$ , 12.9–14.7 °C) of the starches were wide indicating a high heterogeneity of the granule crystallites. The order of varia-

Table 3

Starch-iodine absorption characteristics of *Lablab purpureus* starches.

Starch source	Rongai white	Rongai brown	Highworth black
Apparent $\lambda_{max}$ (nm)	594	589	601
Absolute $\lambda_{max}$ (nm)	604–605	604	603–605
Apparent blue value	0.5431 <sup>a,(a)</sup>	0.4986 <sup>b,(a)</sup>	0.5458 <sup>a,(a)</sup>
Absolute blue value	0.3624 <sup>a,(b)</sup>	0.4902 <sup>b,(b)</sup>	0.4720 <sup>c,(b)</sup>
Apparent amylose (%)	25.515 <sup>a,(a)</sup>	23.049 <sup>a,(a)</sup>	25.981 <sup>a,(a)</sup>
Absolute amylose (%)	17.527 <sup>a,(b)</sup>	23.45 <sup>b,(b)</sup>	22.638 <sup>c,(b)</sup>

Values with superscripts are mean of two determinations. Values in a row with different superscripts are significantly different ( $p < 0.05$ ). Values of related parameters in brackets in a column with different superscripts are significantly different ( $p < 0.05$ ).

tion of  $T_p$ ,  $T_c-T_o$  and  $\Delta H$  is: Highworth black > Rongai white > Rongai brown. The gelatinization parameters ( $T_p$ ,  $T_c-T_o$  and  $\Delta H$ ) showed positive correlation with apparent amylose content, however, Rongai brown with the highest absolute amylose content had the lowest gelatinization temperature. Difference in transition temperatures between different starches could be attributed to difference in the degree of crystallinity (Abdel-Aal, Hucl, Chibbar, Han, & Demeke, 2002; Qi et al., 2004; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003). High transition temperatures have been reported to result from a high degree of crystallinity which provides structural stability and makes the granule more resistant toward gelatinization (Barichello, Yada, Coffin, & Stanley, 1990;

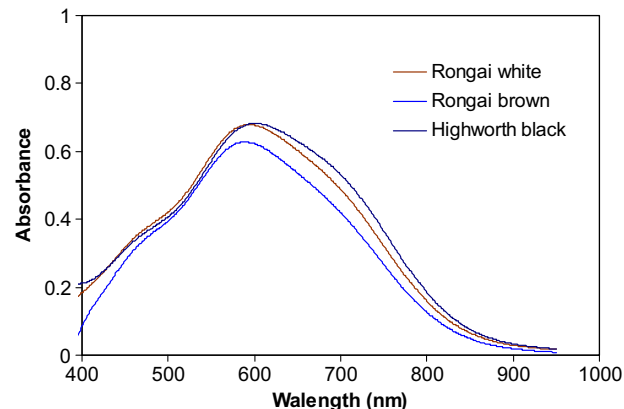


Fig. 5. (a) Starch-iodine absorption spectra of *Lablab purpureus* starches.

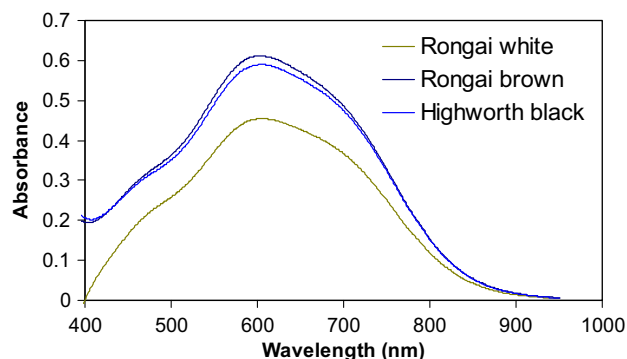


Fig. 5. (b) Starch-iodine spectra of defatted *Lablab purpureus* starches.

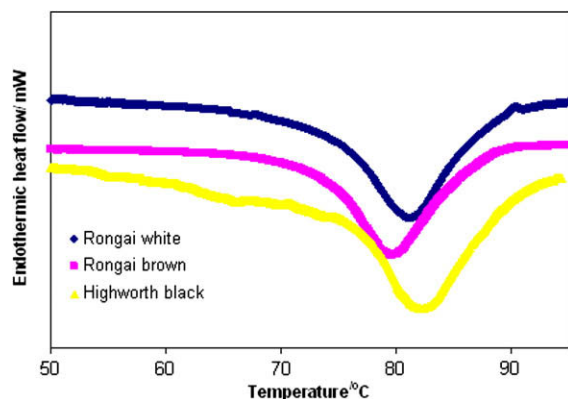


Fig. 6. DSC thermograms of *Lablab purpureus* starches.

**Table 4**  
Gelatinization properties of *Lablab purpureus* starches.

Gelatinization parameters	Rongai white	Rongai brown	Highworth black
$T_o$ (°C)	73.8280	73.5226	75.6967
$T_p$ (°C)	81.1472	79.5782	82.0957
$T_c$ (°C)	87.5850	86.4057	89.4351
$T_c - T_o$ (°C)	13.757	12.8831	14.7384
Enthalpy (J/g)	14.564	12.262	18.786

Krueger, Knutson, Inglett, & Walker, 1987). The high gelatinization temperatures of Lablab starches could be attributed to their high crystallinity values; however, Highworth black with the highest gelatinization temperature had the lowest crystallinity. The gelatinization temperatures of Rongai white, Rongai brown and Highworth black starches fall within the range (78–80 °C; El Tinay et al., 1983) and (65–76 °C; Rosenthal et al., 1971) reported for other Lablab starches. Other legume starches have been reported to gelatinize in the range: 67–76 °C (chickpea), 71–78 °C (pigeon pea) (El Tinay et al., 1983), 56–57.9 °C ( $T_p$ ) (Soybean) (Stevenson et al., 2006). The cooking quality of starch has been associated with the gelatinization temperature (Waters, Henry, Reinke, & Fitzgerald, 2005); starches with low gelatinization temperature have good cooking quality.

### 3.6. Swelling power and amylose leaching

Figs. 7 and 8 show the swelling and amylose leaching patterns of the Lablab starches. No significant swelling and solubilization occurred at temperatures up to 70 °C; thereafter, both the swelling power and amylose leaching increased rapidly. The starch varieties did not show any wide difference in swelling power but showed remarkable difference in amylose leaching. Rongai brown starch showed evidence of granule relaxation before the other varieties as indicated by its slightly initial higher swelling power and amylose leaching at 65 and 70 °C. The amount of amylose leached as a function of temperature was lowest for Highworth black when compared with the other varieties. The higher swelling power observed for Rongai brown at lower temperatures could be attributed to its higher population of smaller granules and the higher specific surface area (Tang et al., 2002). Fig. 9 shows that for the low swelling power range, the greatest amount of amylose leached was observed in Rongai brown, in the region of medium swelling range, both Rongai white and brown starches released about the same amount of amylose while Rongai brown was highest at high swelling range. In the regions of low swelling power (SP < 15 g/g), the amylose leaching was low ( $\leq 2$  mg/100 mg starch), thereafter both increased rapidly. This is because the process of swelling loosens

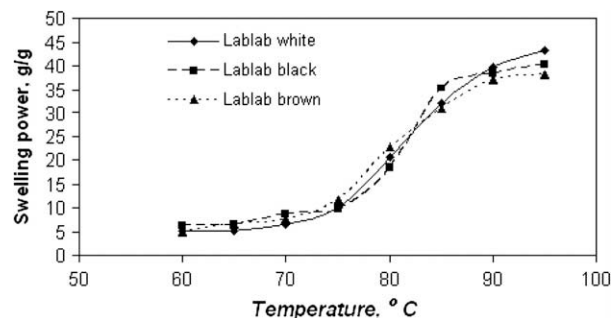


Fig. 7. Swelling patterns of *Lablab purpureus* starches.

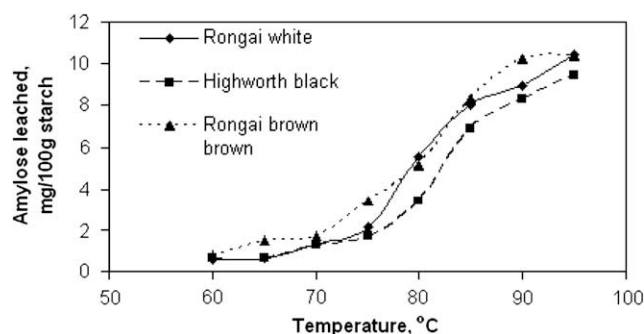


Fig. 8. Amylose leaching patterns of *Lablab purpureus* starches.

the granules permitting water uptake and diffusion of the amylose from the granule interior into the surrounding medium. Solubilized amylose has been reported to contribute greatly to both rheological and textural properties of cooked starch (Iturriaga, de Mishima, & Anon, 2006). The respective swelling power and percentage amylose leached from the starches at 95 °C were: Rongai white (43.2, 10.5), Rongai brown (38.2, 10.4) and Highworth black (40.3, 9.4). The swelling powers of the Lablab starches are in the range of values reported for Lima and Mung bean starches (Schoch & Maywald, 1968). Low swelling powers was reported for Bonavist bean (Lablab), Chickpea, pigeon pea (El Tinay et al., 1983) and Black bean starches (Lai & Varriano-Marston, 1979). Swelling power and amylose leaching are affected by starch composition, granule structure and the presence of amylose–lipid complexes (Lindeboom, Chang, & Tyler, 2004).

### 3.7. Paste clarity and freeze–thaw stability

Fig. 10 shows the light transmittance of the *L. purpureus* starch pastes. The native samples exhibited very low clarity. The order of

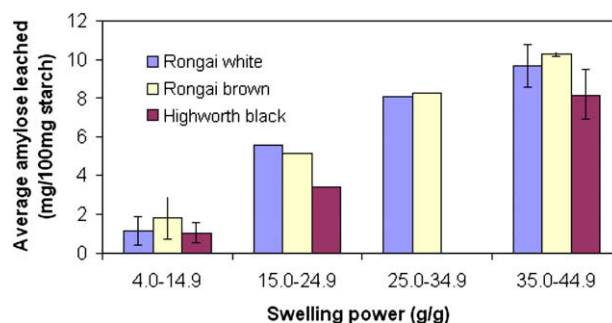


Fig. 9. Amylose leached as a function of swelling power.

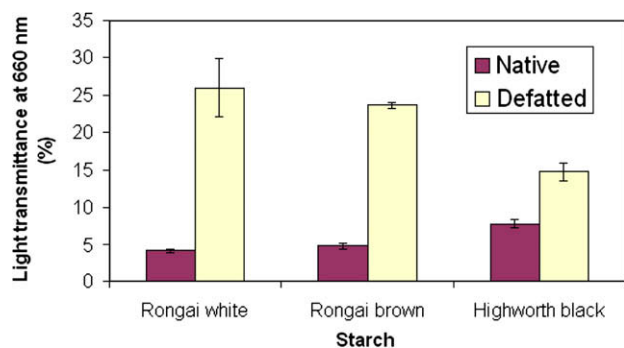


Fig. 10. Paste clarity of *Lablab purpureus* starch paste at 1.0% (w/v) concentration.

clarity was Highworth black > Rongai brown > Rongai white. The defatted samples showed higher paste clarity than the native samples, Rongai white with lowest absolute amylose gave the highest paste clarity. The lower clarity of the undefatted starch is due to formation of amylose–lipid complexes which are difficult to disperse and therefore reduce the light transmittance (Lindeboom et al., 2004; Wang & White, 1994). Previous workers have reported negative correlation between paste clarity and amylose content (Visser, Suurs, Bruinenberg, Bleeker, & Jacobsen, 1997; Wang & White, 1994). Fig. 11 shows the freeze–thaw stability curves of the starches. The three starches exhibited high instability to freeze–thaw cycles. At the first cycle, Rongai brown gave the highest amount of exudates while Highworth black gave the least. After 6 freeze–thaw cycles, the level of syneresis was Rongai brown (57%), Rongai white (46.6%) and Highworth black (44.7%). This indicates a high retrogradation tendency of the starch pastes. There was a positive correlation between amount of leached amylose and syneresis. Highworth black with the lowest amount of leached amylose was the most freeze–thaw stable. Apart from amylose content, retrogradation is known to be affected by other factors such as granule size, amylose and amylopectin chain lengths, and amylose–lipid complexes (Mua & Jackson, 1998; Philpot, Martin, Butardo, Willoughby, & Fitzgerald, 2006; Yao, Zhang, & Ding, 2002). Starch retrogradation is an important factor when starch is used in food processing because it affects the quality of the food texture at the end of the production and distribution chain. Native starch generally presents unstable pastes as a result of re-association of dispersed amylose molecules. Only a limited number of native starches have been reported to show improved freeze–thaw stability, these are mostly the waxy starches (Kuntz, 1995) and some others like *Amaranthus paniculatus* (Singhal & Kulkarni,

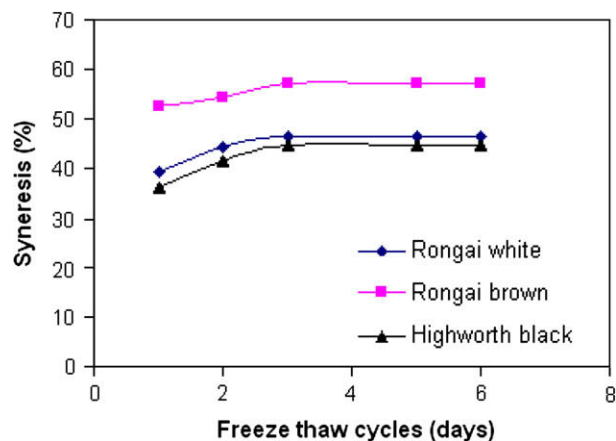


Fig. 11. Freeze–thaw stability of *Lablab purpureus* starches.

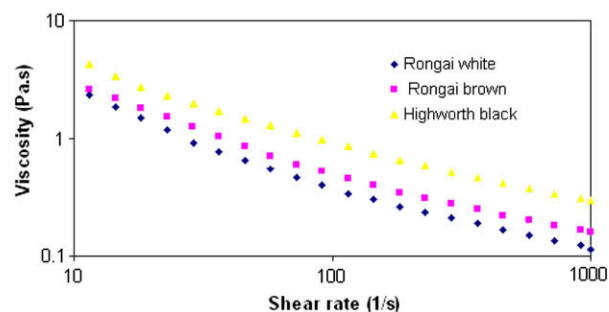


Fig. 12. Viscosity–shear rate profiles of 50% pastes of *Lablab purpureus* starches.

Table 5

Flow properties for 6% gels of *Lablab purpureus* starch pastes at 25 °C fitted with the Sisko model.

	Rongai white	Rongai brown	Highworth black
$\eta_{\infty}$ (Pa s)	0.07426	0.1042	0.1495
$\kappa$ (s)	19.24	15.26	24.80
$n$	0.1164	0.2053	0.2557
S.E.	11.40	18.79	18.25

$\eta_{\infty}$ , infinite shear viscosity;  $\kappa$ , consistency;  $n$ , rate index; S.E., standard error.

1990), sweetsop (Nwokocha & Williams, 2009) and Peruvian carrot (Takeiti, Fakhouri, Ormenese, Steel, & Collares, 2007). Hence most starches for food application are employed in modified form (Duxbury, 1993).

### 3.8. Rheological properties

In Fig. 12 the flow curves of the three varieties of *L. purpureus* starch pastes were compared in the high shear rate region where the curves were distinct from each other. They were fitted with the Sisko model (Eq. (1)); the best fitting viscosity–shear rate model, the flow properties are presented in Table 5.

$$\eta = \eta_{\infty} + \kappa * (\dot{\gamma})^{n-1} \quad (1)$$

where  $\eta$  = viscosity (Pa s),  $\eta_{\infty}$  = infinite shear viscosity (Pa s),  $\dot{\gamma}$  = shear rate (1/s),  $\kappa$  = consistency (s),  $n$  = flow behaviour index (dimensionless).

The starches showed a non-Newtonian behaviour with the viscosity decreasing with increase in shear rate. Rongai white starch paste showed the greatest shear thinning characteristics and the

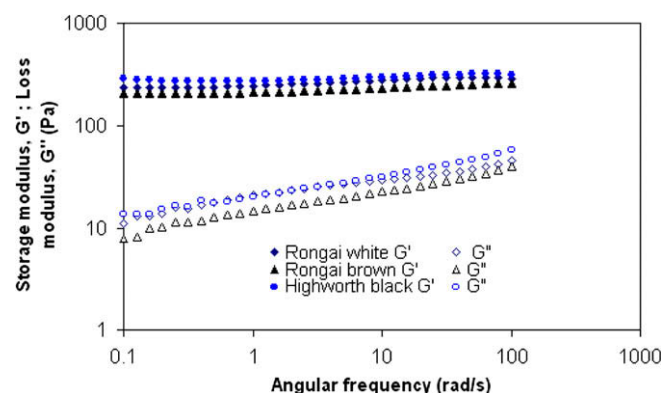


Fig. 13. Frequency sweep showing  $G'$  and  $G''$  for 6% gels of *Lablab purpureus* starches.



lowest value of  $\eta_{\infty}$ . Highworth black had the highest value of  $\kappa$  while Rongai white had the least. All the starch pastes had very low values of  $n$  ( $n < 1$ ) indicating a high degree of shear thinning. The value of  $n$  was highest for Highworth black and least for Rongai white. Similar shear thinning characteristics have been reported for other polysaccharides (Nurul, Mohd. Azemi, & Manan, 1999; Singhal & Kulkarni, 1990; Tan, Tan, Gao, & Gu, 2007). Fig. 13 shows the mechanical spectra of 6% starch gels. The storage modulus,  $G'$ , is significantly greater than the loss modulus,  $G''$ , for the three varieties and both moduli exhibit some frequency dependence indicating weak gel characteristics. The starches had similar gel characteristics as shown by the close  $G'$  values.

#### 4. Conclusion

Starch isolated from three varieties of *L. purpureus* ranged from 13.2% to 15.8% of the seeds. The granule size ranged from 12.51 to 20.56  $\mu\text{m}$ , with Rongai brown having the highest proportion of small granules. The starches exhibited a C-type diffraction pattern with degree of crystallinity ranging from 37.0% to 46.3%. The apparent and absolute amylose contents were 23.1–26.0% and 17.5–23.6%, respectively. The starches had high gelatinization onset temperatures ( $T_o = 73.5\text{--}75.7^\circ\text{C}$ ) and single stage swelling and amylose leaching patterns. The starch pastes exhibited significant shear thinning, low clarity and poor freeze–thaw stability.

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