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Dynamic rheological and physicochemical properties of annealed starches from two cultivars of cassava

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ABSTRACT

Starch from tubers of "sweet" (low cyanide) and "bitter" (high cyanide) cassava (*Manihot esculentum* Crantz) crops was isolated and subjected to annealing treatment at 50 °C for 24,96,144 and 192 h in 1:5 starch to water ratio. Annealing generally reduced the swelling power and solubility of the starches. Storage modulus (G') and loss modulus (G'') increased with decrease in complex viscosity (Eta*) as a function of angular frequency (ω). The magnitudes of G' reported were greater than those of G'' revealing frequency dependency except sweet starch cultivar annealed at 192 h. The tan δ (ratio of G''/G') of annealed starches were within the ranges of 0.31–0.39 and 0.23–0.45 for sweet and bitter cultivars, respectively. The tan δ values of the starches were lower than one, indicating that the samples are elastic in nature than viscous. The X-ray patterns of the A-type starches were not altered on annealing denoting that the double helices in the amorphous region were not disrupted. Annealing as revealed by scanning electron micrographs changed the structure of the sweet cultivar, while there was no change in the size and shape of the bitter cultivar.

1. Introduction

Cassava (*Manihot esculentum* Crantz) is one of the world's cheapest and most popular staple crops in many countries including Nigeria, Brazil and Thailand. World production of cassava in the year 2000 amounted to 165×10^6 t with Africa producing over 91 million metric tonnes of cassava annually and Nigeria has been reported to be the highest world producer of cassava with 33.9 million metric tonnes per annum (Sriroth, Piyachomwan, Wanlapatit, & Oates, 2000). In 2004, the annual output was over 44 million metric tonnes (CBN, 2004). Traditionally, products from cassava have served as staple foods for millions of people throughout the hot and humid region of the world (Cooke, 1978). However, there is the need to source value-added products such as sweeteners, dextrin, amino acids and starch from cassava for economic reasons rather than subsistence (Sriroth et al., 2002).

In plants, starch is deposited in the form of partially crystalline granules whose morphology, chemical composition and super molecular structure are characteristics of plant species. Starch is a homoglycan consisting of anhydrous glucose units linked primarily through alpha-D-(1, 4) glucosidic bond, which owes majority of its functionality to two high molecular weight components: amylose and amylopectin, in addition to the physical organization of these macromolecules into granular structure. Great variation exists in properties of starches from different botanical sources and even among cultivars (Ellis et al., 1998). Although the use of native starch in the food system is comparatively limited, expansion of the range of application in the food industry is by modification. The properties of starches can be modified by hydrothermal treatments. Heat-moisture treatments of different roots and tuber starches have been carried out with different ratio of starch/water, temperature and time of treatment (Gunaratne & Hoover, 2002).

The annealing treatment is carried out in excess (>60%, w/w) or at intermediate (40–55%, w/w) water content for a long period, below the gelatinization temperature (T_o) and above the glass transition temperature (T_g) of starches (Jacobs & Delcour, 1998). The annealing treatment provokes a reorganization of starch molecules (Hoover and Vasanthan, 1994) which modifies the physicochemical properties of starches. The principal changes are: decrease in the swelling power and solubility (Tester, Debon, & Sommerville, 2000), increase in enzyme susceptibility (Wang, Powell, & Oates, 1997) and decrease in peak viscosity and retrogradation tendency (Jacobs & Delcour, 1998).

The heat-moisture treatment (HMT) is similar to annealing. It modifies the physicochemical properties of starch, without destroying the granular structure. Heat-moisture treatment is how-

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ever, carried out in low moisture level (<35%, w/w), normally for 16 h at 100 °C (Jacobs & Delcour, 1998). Kawabata et al. (1994) reported a change in X-ray pattern of potato starch from B to A due to heat-moisture treatment. Annealing at 50 °C for different periods of time (0, 48, 96, 120,144 and 148 h) has been reported to show an increase in intensity of the peaks at 15°, 18° and 23° in fermented cassava starch which is an indication of increase in crystallinity (Mendes da Silva, Sasaki, Freitas e Silva, Jorge, & Romeu, 1997). Most researchers have studied the rheological properties of annealed starches using the Brabender Amylograph or Rapid Visco Analyser which do not give information on the structural properties of starch pastes because of shear deformation during heating. However, pasting properties like peak viscosity, breakdown, setback and pasting temperature are evaluated. The dynamic rheological tests for small-deformation oscillatory measurements in general have generated valuable information on the viscoelastic properties of starch pastes without breaking the structural element.

The objectives of this study are to verify the changes in properties of annealed starches from two popular cultivars of cassava, provide information on dynamic rheological properties and crystallinity of the starches which may be useful when considering application of starch in the food industry.

2. Materials and methods

2.1. Materials

The cassava tubers, TMS 30572 (Sweet) and TMS 50395 (Bitter) cultivars were collected from the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria. In the following text only the abbreviation SCS and BCS will be used, respectively.

2.2. Isolation of starch

The starches were extracted using established procedures (Desrosier, 1977). Briefly, peeled tubers were washed thoroughly and grated. The grated pulp was mixed with sufficient amount of water to form slurry with subsequent filtration. The resulting starch was allowed to sediment and the supernatant decanted. The starch was spread in a thin layer on a tray and dried in air oven at temperature of 35 ± 2 °C, the dried starch was pulverized and screened through standard 100 mesh opening (Retsch Analysensieb DIN 4188). Analytical sieve shaker (Model 03-502, Fritsch GmbH & Co., Haan, Germany) was used.

2.3. Annealing of starches

The starch samples were subjected to steeping at 50 °C in aqueous suspensions at a starch to water ratio 1:5 ratio for 24, 96, 144 and 192 h. After the incubation period the samples were filtered and dried in a circulating air oven.

2.4. Determination of swelling power and solubility

Method of Schoch (1964) as described with minor modifications (Osundahunsi, Fagbemi, Kesselman, & Shimoni, 2003) was used. Suspension 1% (w/v) of starch was weighed into graduated centrifuge tube with distilled water. These tubes were immersed in water bath at a temperature range from 45 to 95 °C at 10 °C interval for 30 min, with shaking every 5 min during heating. The tubes were removed, cooled to room temperature and centrifuged (Megafuge 1.0R Heraeus Thermo Scientific, USA) at $G = 221$ for 30 min after which the supernatant was carefully sucked into already weighed Petri dishes and dried in the air oven at 110 °C for 4 h. The weight of the pastes was determined and used to calculate the swelling power as gram of sediment paste per gram starch. The difference in weight of the Petri dish after drying the supernatant was taken as the weight of soluble starch percentage; solubility was calculated as gram of soluble starch per gram starch. All measurements were done in triplicate.

2.5. Preparation of starch pastes for rheological measurements

Dispersions (5%, w/v) of annealed CS with different periods of treatment (0, 24, 96, 144 and 192 h) were prepared by mixing starch with distilled water. The starch dispersions were moderately stirred for 30 min at room temperature and then heated at 85 °C in a water bath for 30 min with constant mild agitation to prevent sedimentation and agglomeration. At the end of the heating period, the hot paste was immediately transferred to the rheometer plate for the measurement of rheological properties.

2.6. Rheological measurements

Dynamic rheological measurements were conducted with a rheometer (ARES RDA III, TA Instruments, New Castle, DE, USA), using a parallel plate system (40 mm dia.) at a gap of 500 μm. Dynamic shear data were obtained from frequency sweeps over the range of 0.63–78.9 rad/s at 2% strain. The 2% strain was in the linear viscoelastic region. Frequency sweep tests were also performed at 25 °C. TA-Orchestrator Data Analysis software was used to obtain the experimental data and to calculate the storage modulus (G', a measure of elastic response), loss modulus (G'', a measure of viscous response), complex viscosity (Eta*, a measure of overall resistance to flow) and loss tangent ($\tan\delta = G''/G'$). In order to relax the samples before the dynamic shear rheological measurements, all samples were allowed to rest at the initial temperature for 5 min. Average of three recorded measurements were reported.

2.7. X-ray powder diffraction

The X-ray diffraction pattern (XRD) of native and annealed starch samples were recorded with a Brucker axs X-ray diffractometer (Germany) using a CuK2 radiation detector. The motor was D8 Discover detection system. The starch powders were tightly packed in a sample holder and scanned over the range of 10–40° at Bragg angle (2θ). 2θ in step of 0.02° 2θ per s. The crystalline nature of the granules was determined by the position of the XRD peaks.

2.8. Scanning electron microscopy

A starch powder in thin layer was mounted on an aluminium specimen holder by a double-sided sticky tape. The specimen holders were loaded for critical point drying (Bal-tec AG, model SCD 020, Balzers) and sputtered with gold (Bal-tec AG, model SCD 040, Balzers). Samples were examined with scanning electron microscope (LEO Gemini 1530, LEO Inc., Oberkochen, Germany).

2.9. Statistical analysis

All results are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed using Statistical Software (SPSS version 15). Differences in means were determined using Duncan's multiple range tests.

3. Results and discussion

Swelling power of native and annealed sweet and bitter cultivars of cassava is presented in Figs. 1 and 2, respectively. Swelling power of native starch increased from 2.3 to 24.3 while those of annealed samples ranged from 2.3 to 28.4. The bitter cultivar had valued ranging from 2.3 to 29.3 (Fig. 2). There was reduction in the swelling

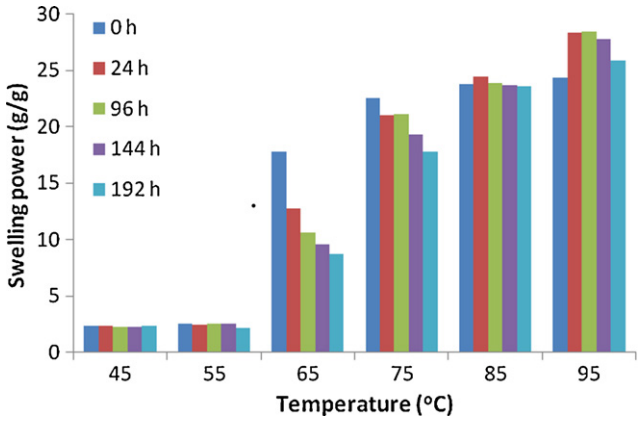

Fig. 1. Swelling power of native and annealed sweet cassava starch.

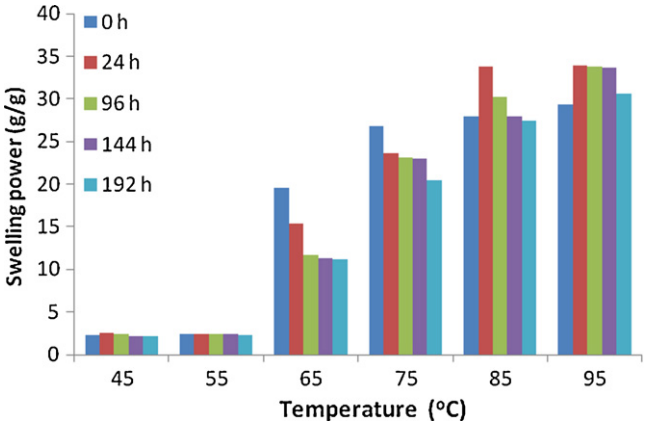

Fig. 2. Swelling power of native and annealed bitter cassava starch.

power of the starches as the length of time of annealing increased. Moreover, swelling power increased substantially from the temperature range 65–95 °C for both cultivars. The reduction in the swelling power was less pronounced at higher temperatures. This observation is in agreement with the lowering of swelling power of some roots and tuber starches after heat-moisture conditioning (Gunaratne & Hoover, 2002). Also, swelling power of all the samples increased as a function of temperature. There was reduction in the solubility of the samples as the period of annealing increased (Figs. 3 and 4). There was strengthening of bond between starch molecules as confirmed by the solubility of the samples. It could be

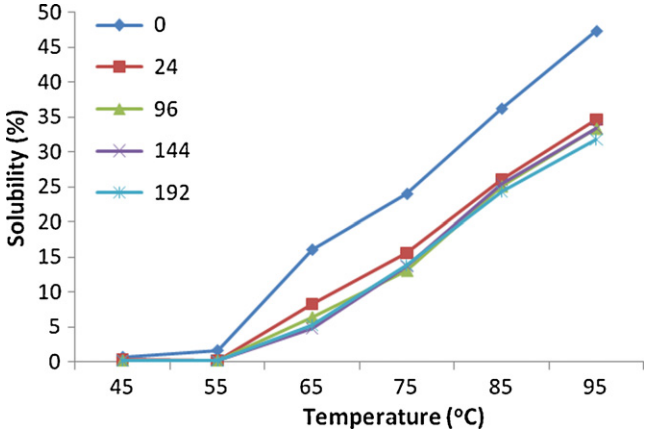

Fig. 3. Solubility of native and annealed sweet cassava starch.

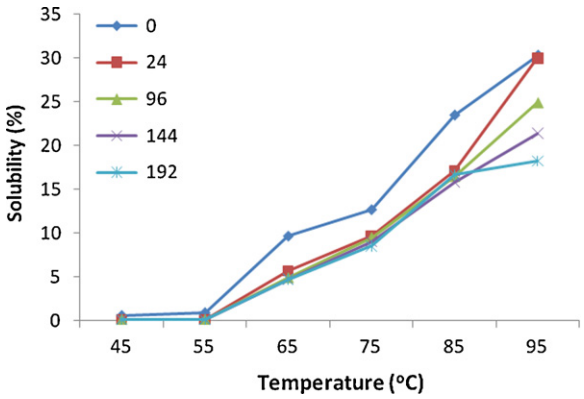

Fig. 4. Solubility of native and annealed bitter cassava starch.

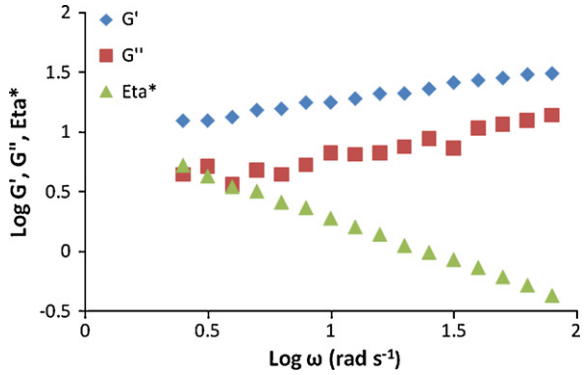

Fig. 5. Plot of log G', G'', Eta* versus log ω for annealed sweet cultivar paste (5%) at 25 °C: (◊) G', (□) G'', and (△) Eta*.

that the temperature was insufficient to disrupt the bond and leach amylose. It has been suggested that the decrease observed in heat-moisture treatment and/or annealing may be due to interaction between amylose–amylose or amylose–amylopectin chain. Tester and Morrison (1990) reported that swelling is primarily a property of amylopectin and that amylose is a diluent. Furthermore, such changes have been attributed to internal rearrangement of starch granule. The double helical amylopectin side chains are more organized and tend to increase crystallinity. Cooke and Gidley (1992) opined that the forces holding granules are mainly at the double helical level and that the starch crystallinity enhances dense packing not just as a primary provider of structure. Lowering of swelling power in annealed samples could also be due to increased starch crystallinity. Increased starch crystallinity may be responsible for

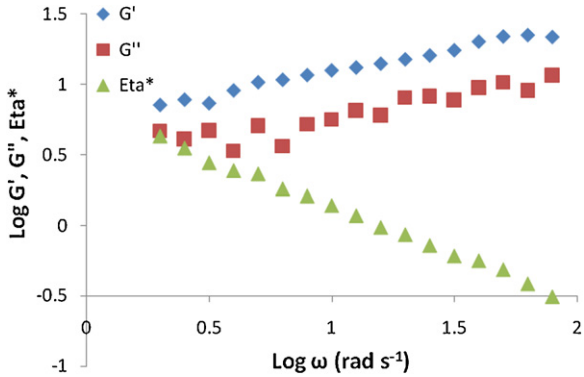

Fig. 6. Plot of log G', G'', Eta* versus log ω for annealed bitter cultivar paste (5%) at 25 °C: (◊) G', (□) G'', and (△) Eta*.

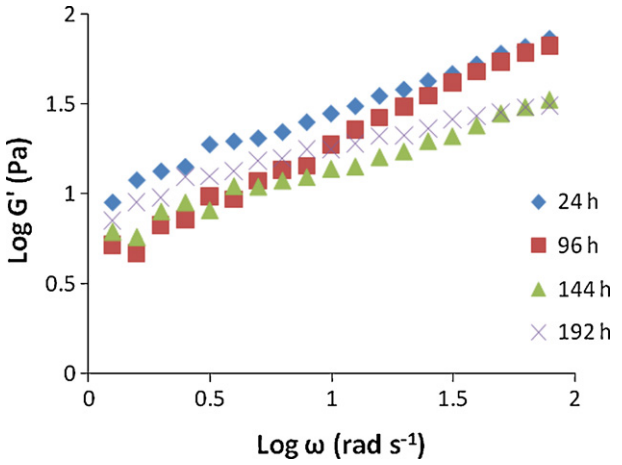

Fig. 7. Plot of log G' versus log ω for annealed sweet cultivar pastes at 25 °C: (◊) 24 h, (□) 96 h, (△) 144 h, and (×) 192 h.

the reduction in swelling power since the crystalline granules limit starch swelling (Tester et al., 2000).

Figs. 5 and 6 show the changes in storage modulus (G'), loss modulus (G'') and complex viscosity (Eta*) as a function of angular frequency (ω) for sweet and bitter annealed cassava starch pastes at 25 °C, respectively. The magnitudes of G' reported were greater than those of G'' revealing frequency dependency. Both G' and G'' increased with decrease in Eta* also. The starches from the two cultivars exhibited rheological behavior similar to weak gels. This tendency is in agreement with the report on sweet potato starch (Lee & Yoo, 2009). For the SCS, the G' and G'' values (Figs. 7 and 8) of the annealed starch reduced but not dependent on the length of time for annealing. Starch annealed at 192 h had higher G' values than one or two of the other samples (Fig. 7). Whereas, the reduction in magnitude of G' and G'' values (Figs. 9 and 10) for BCS was with respect to the length of period for annealing. Table 1 shows G', G'', Eta* and tan δ values at 7.89 rad/s of both native and annealed sweet and bitter cassava starch pastes at 25 °C. The dynamic moduli values (G' and G'') of annealed pastes except the storage modulus of sweet cultivar show a decrease with increase in the length of hour for annealing. This type of reduction in dynamic moduli may be attributed to decrease in swelling power of starch granules caused by annealing treatment (Figs. 1 and 2). The variation

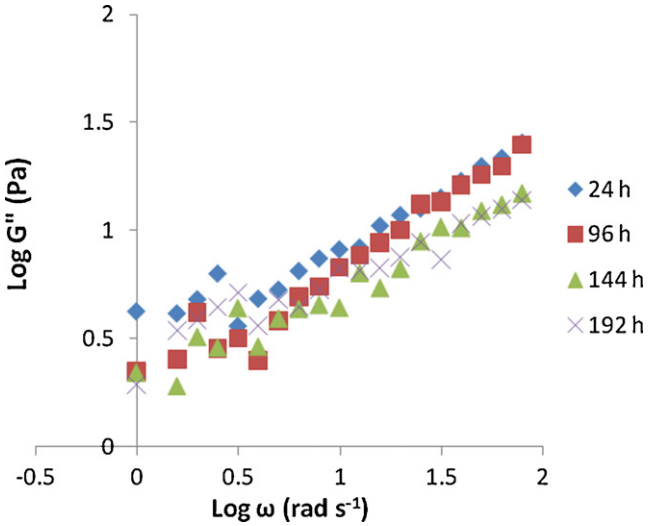

Fig. 8. Plot of log G'' versus log ω for annealed sweet cultivar pastes at 25 °C: (◊) 24 h, (□) 96 h, (△) 144 h, and (×) 192 h.

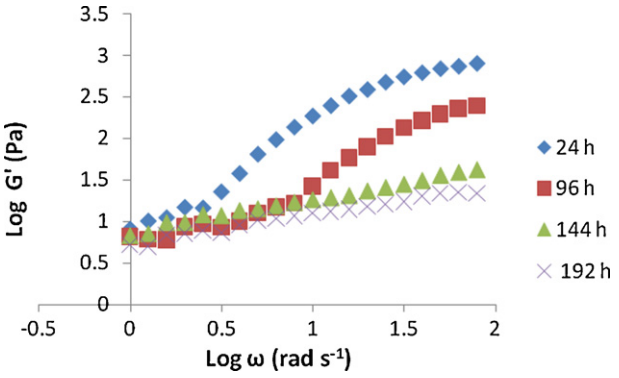

Fig. 9. Plot of log G' versus log ω for annealed bitter cultivar pastes at 25 °C: (◊) 24 h, (□) 96 h, (△) 144 h, and (×) 192 h.

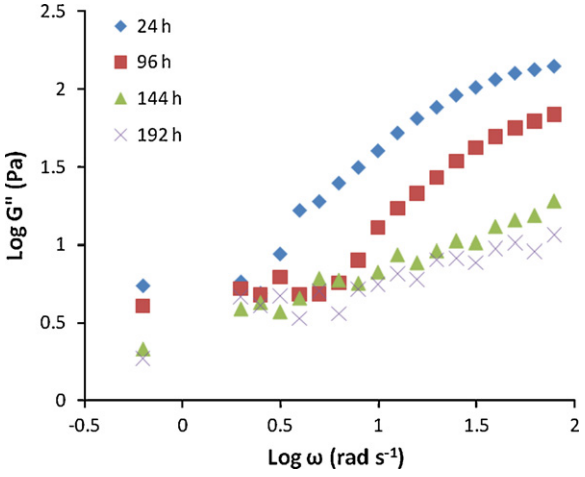

Fig. 10. Plot of log G'' versus log ω for annealed bitter cultivar pastes at 25 °C: (◊) 24 h, (□) 96 h, (△) 144 h, and (×) 192 h.

recorded in storage modulus of annealed sweet cultivar may be caused by an intrinsic factor. The two starch cultivars were treated under similar conditions in the laboratory. It has been reported that swelling power and temperatures govern the viscoelastic properties of gelatinised starch dispersion (Shon & Yoo, 2006). The loss factor (tan δ), stating directly the G''/G' ratio of annealed SCS and BCS were within the ranges of 0.31–0.39 and 0.23–0.45 for sweet and bitter cultivars, respectively (Table 1). The tan δ values of the starches were lower than one, indicating that the samples are elastic in nature. There was reduction in the magnitude of G' and G'' values of the annealed samples except SCS annealed at 192 h. This

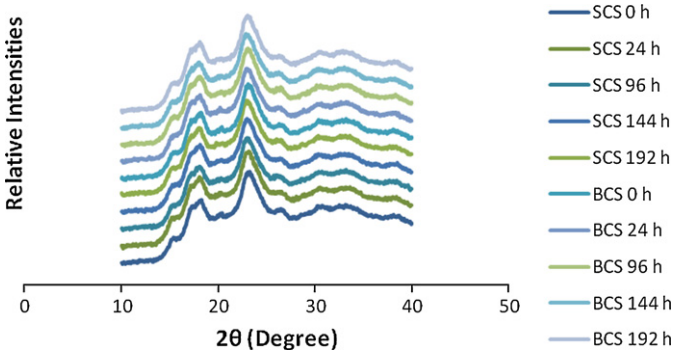

Fig. 11. SEM of cassava starches (A: NATIVE sweet, B: annealed sweet, C: Native bitter, and D: anealed bitter).

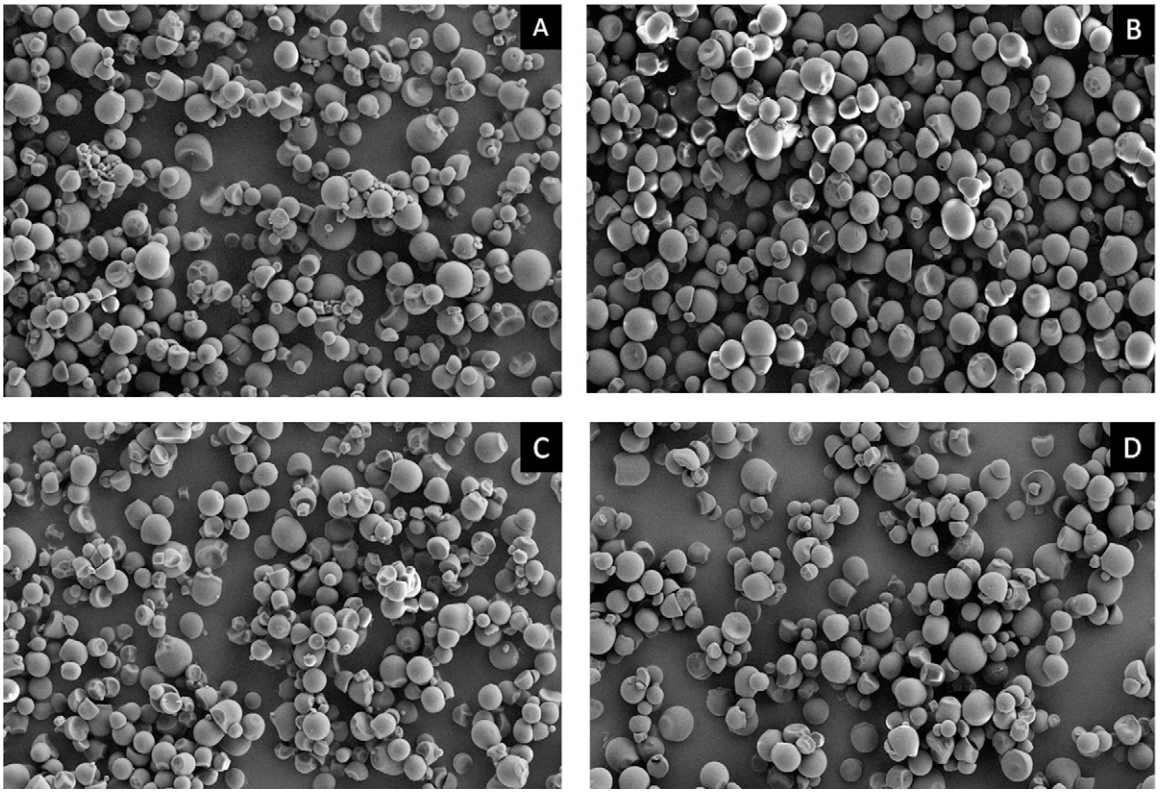

Fig. 12. X-ray pattern of native and annealed bitter (BSC) and sweet (SCS) cassava cultivar.

observation may be due to the increased stability of the granular integrity caused by strengthening of bonds in the swollen granules of sweet cassava paste due to annealing for a longer period of time. The tan δ values of the annealed SCS were all lower than that of native starch (0.51) while the values for annealed BCS were generally higher than the native (Table 1). As discussed earlier, the elastic properties can be explained by the swelling power of the granules. Singh, Kaur, and Singh (2004) indicated that G' and G'' values of acetylated corn and potato starches were primarily governed by the volume fraction of the swollen granules. Therefore, the decrease in values with increasing length of period for annealing may be due to stronger strengthening of bond of the starch. In conclusion, the starches were more elastic in nature than viscous.

X-ray diffraction patterns of native SCS, BCS and annealed counterparts at different time of annealing are depicted in Fig. 11. The patterns show the typical feature of A-type starch with peaks at (2θ) about 15°, 17° and 23°. The A-type starches are based on parallel standard double helices, in which the helices are more closely packed in the A-type than B-type. In addition, the starches also differ in content of intra-helical water (B > A) (Gunaratne & Hoover, 2002). Annealing did not change the X-ray pattern of the two cultivars of starches evaluated. This finding agrees with the report of Tukomane, Leerapongnun, Shobsngob, and Varanit (2007) in which X-ray diffraction remained unchanged after annealing of Tapioca starch. The results are contrary to the report on annealed *Polvilho doce* cassava starch from a C_A-type pattern (intermediate between A- and B-type) being converted to A-type. Gunaratne and Hoover (2002) reported that A- and B-type starches are packed in a pseudo-hexagonal array. However, in A-type, the lattices contain the helices at the centre rather than a column of water as in B-type. As such, dehydration (vapourisation) of the water molecule usually cause a change in X-ray pattern of B-type due to the movement of a pair of double helices into the centre which was originally occupied by the vapourised water molecule. This may explain the attribute of A-type remaining unchanged since the helices were at the centre rather than water molecule.

The photomicrographs showed that the shape of the starch granules varied considerably. The main difference being that the sweet cultivar (Fig. 12A and B) is more compact than the bitter cultivar. The annealed sweet sample has even more compact granules (Fig. 12B) than the native counterpart. Both native and annealed bitter granules remained scattered without visible changes (Fig. 12C and D). The starch granules had round, convex–biconcave polyhedral round and truncated shapes. The granule surface appeared to

Table 1
Storage modulus (G'), loss modulus (G''), complex viscosity (Eta*) and tan δ values at 7.89 rad/s of native and annealed starches from sweet and bitter cultivars of cassava at 25 °C[a].

Time [h]	G'[Pa]		G'' [Pa]		Eta* [Pa s]		tan δ	
	SCS	BCS	SCS	BCS	SCS	BCS	SCS	BCS
0 (Native)	5.44 ± 0.17e	111.79 ± 0.02a	2.74 ± 0.01e	27.54 ± 0.06b	0.39 ± 0.02e	14.55 ± 0.05b	0.51 ± 0.01a	0.27 ± 0.01d
24	25.05 ± 0.04a	117.29 ± 2.15a	7.39 ± 0.01a	31.68 ± 0.19a	3.32 ± 0.02a	16.10 ± 1.33a	0.31 ± 0.01d	0.23 ± 0.01e
96	14.42 ± 0.13c	16.68 ± 0.03b	5.51 ± 0.01b	8.12 ± 0.19c	1.95 ± 0.02c	2.33 ± 0.01c	0.39 ± 0.01b	0.48 ± 0.01a
144	12.41 ± 0.03d	16.89 ± 0.01b	4.54 ± 0.02d	5.73 ± 0.02d	1.70 ± 0.05d	2.24 ± 0.01c	0.37 ± 0.00c	0.34 ± 0.00c
192	17.52 ± 0.15b	11.64 ± 0.03b	5.31 ± 0.02c	5.23 ± 0.02e	2.33 ± 0.01b	1.61 ± 0.02c	0.31 ± 0.01d	0.45 ± 0.01b

[a] Mean values in the same column with different letters are significantly different ($p < 0.05$).

be smooth and showed no evidence of pin holes under scanning electron microscope.

Cassava starch granules of different varieties have been reported to be mostly round with a flat surface on one side (Richard, Asaoka, & Blanshard, 1991). Although, earlier studies on cassava varieties did not report noticeable variation in sizes and shapes, Tukomane et al. (2007) reported observable fusion possibly by agglomeration of granules ascribed to partial gelatinization at the granule surface due to spray drying at 60 °C. Annealing did not alter the size or shape of the starch granules of bitter cultivar of the starch (Fig. 12C and D). Similar observation was made on HMT cassava starch (Gunaratne & Hoover, 2002).

4. Conclusions

The production of cassava tuber in some developing countries has been reported to be on the increase. This is a great challenge for scientists in such countries to exploit the properties of the roots and tuber starches by heat-moisture/annealing treatment to produce modified starches that will be applied in the industry to the transformation of rural African economies especially and improve livelihood. The wider utilisation of cassava starch will encourage farmers to produce the crop for economic reasons rather than subsistence. This will in turn enhance these countries to compete more favourably for both food and non-food sectors of the market.

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