

Isolation and characterization of starches from two cowpea (*Vigna unguiculata*) cultivars

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Abstract

This study was conducted to evaluate and compare the gross chemical compositions and functional properties of starches derived from two cultivars of cowpea. White cowpea starch (WCS) and brown cowpea starch (BCS) were isolated from their respective seeds. The starch yield (40.00%) with its residual protein content (0.09%) from white cowpea seeds was higher than that obtained from brown cowpea seeds. The apparent amylose (AAM) content of WCS was lower than in BCS. Moisture, fats and ash contents for the WCS and BCS were 11.54 and 10.18%, 0.05 and 0.07%, 0.03 and 0.05%, respectively, and their pH was 6.98 and 6.93, respectively. Bulk density of WCS was lower than in BCS; in contrast the percentage dispersibility of WCS was higher than in BCS. The shapes of the starch granules obtained from SEM were round to elliptical with many granules occurring in clusters. WCS granules were clustered while BCS granules were singled. Fissures were also observed on the surfaces of BCS granules. When heated from 55 to 95°C at 10°C intervals, the swelling power (SP) and water solubility index (WSI) of both starches were evaluated. SP of WCS and BCS increased with increased temperature, but were more pronounced in BCS. The WSI of WCS increased progressively with increased temperature until a decrease occurred after 85°C, for BCS the WSI values were inconsistent with increase in temperature. Pasting parameters (PP) were evaluated using RVA. Significant differences were observed in the PP of the two cowpea starches, especially in PV, TV, FV and SV. The results revealed that cultivar difference has an effect on pasting properties of cowpea starch.

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Introduction

Legumes are an excellent source of carbohydrates and provide an inexpensive source of protein. Cowpea (*Vigna unguiculata*) is a legume that originated in Africa (Sasanam *et al.*, 2011). There are many local names for cowpea around the world, these include, “niebe”, “wake”, and “ewa” for most of West Africa and “caupi” in Brazil. Other names to describe cowpeas are, “southernpeas”, “blackeyed peas”, “pinkeyes” and “crowders”. It is widely distributed in tropical and temperate climates and differs in shape, size and colour of seed coat. Cowpea is important in the human diet because it is nutritious and a typical cultivar contains 11% moisture, 24% protein, 1.3% fat, 56.8% carbohydrate, 3.9% fiber, and 3.6% ash (Deshpande and Damodaran, 1990). Generally, in West Africa, cowpea seeds are consumed as boiled seeds alone or in combination with other foods (e.g., plantain, maize and rice) (Henshaw, 2008). The paste of cowpea can also be fried (Akara) or steamed (Moinmoin).

Cowpea seeds can be processed into value

added products like protein concentrate and food-grade starch. Starch is a natural, cheap, available, renewable, and biodegradable polymer produced by many plants as a source of stored energy. It is the second most abundant biomass material in nature. Starch owes much of its functionality to the proportion of its two major constituents, amylose (AM) and amylopectin (AP), though the contributions of minor components (lipids and proteins) cannot be rule out. AM is a mixture of branched and linear molecules with a degree of polymerization (DP_n) of 1100-1700 and 700-900 glucose units, respectively (Hizukuri *et al.*, 1989). AP is a branched polymer with one of the higher molecular weights (MWs) known among naturally occurring polymers (Karim *et al.*, 2000). Its MW range from 10⁷ to 10⁹ (Lineback and Rasper, 1988). It has been reported that starch is the most abundant polysaccharide in the legume seed (22-45%) (Hoover and Sosulski, 1991). The versatility of starch is manifested in its wide field of applications. Legume starch pastes have been widely reported to be comparatively more viscous than that from cereal starches, indicating their higher resistance to swelling

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and rupture of starch granules.

Cowpeas are likely the most significant starch-protein grain legume seeds in the West Africa sub-region, offering wider pattern of utilization than any other legume (Atuobi *et al.*, 2011). It has been previously indicated that the isolation of pure starch from some legumes was made difficult due the presence of insoluble protein and highly hydrated fine fiber fraction (Schoch and Maywald, 1968). The fine fibers are probably derived from the cell walls covering the starch granules.

Various workers (McEwen *et al.*, 1974; Rockland *et al.*, 1974) have studied the size and shape of legume starch granules. The individuality of starches is best seen in the differences of the morphology of their granules. The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant (Bodenhuizen, 1969). Generally, legume starches have variable granule diameters, normally ranged between 4 and 80 μm . Granule shape may be oval, spherical, elliptical, or irregular depending on the botanical source. Pasting encompasses the changes that occur after gelatinization upon further heating and these include further swelling of granules, leaching of molecular components from the granules and eventual disruption of granules especially with the application of shear forces (Tester and Morrison, 1990). Atuobi *et al.* (2011) studied starches from four cowpea cultivars and concluded that there are differences in their pasting properties, indicating discrepancies in cooking time. According to Henshaw and Adebawale (2004), swelling power (SP) increased progressively with increasing temperature for all starches of cowpea cultivars evaluated by them. Literature review reveals plenty of information on cowpea flours (Kerr *et al.*, 2000; Henshaw *et al.*, 2002). There is limited information in the literature on cowpea starches, especially in the areas of pasting, dispersibility, pH and bulk density. Therefore, the objective of this work was to investigate and compare the functional properties of starches from two cowpea cultivars (white and brown seed coats). The results would help determine specific end user applications and form the basis for further investigations on physical and chemical modifications to improve the functionality of cowpea starches.

Materials and Methods

Materials

White cowpea and brown cowpea seeds were purchased from the local market in Akungba, Ondo state, Nigeria. All other chemicals were of analytical

reagent grade.

Isolation of cowpea starch

400 g of the cowpea seeds were steeped in distilled water for 2 hr. The seed coats were manually removed and the inner endosperm blended for 5 min at slow rotation using a laboratory blender. The slurry was diluted with distilled water and allowed to stand for 1 hr. The supernatant was decanted and distilled water added to the starch residue. Repeated dilution and decantation continues until the pH is neutral. The prime starch residue was collected and dried in a vacuum oven (N505F, YOGOII, Genlab Widnes, England) at 40°C for 48 hr.

Gross chemical composition of isolated cowpea starches

Apparent amylose content (%) was determined by colorimetric iodine assay index method, according to Juliano (1985). The moisture, protein, lipid, and ash content in cowpea starch were determined using procedure of AACC method (2000).

Morphology of cowpea starch granules

The morphology of cowpea starch granules was evaluated by scanning electron microscope (SEM) (QUANTA FEG 250 ESEM). Starch samples were suspended in 95% ethanol and mounted on circular aluminum stubs with double-sided sticky tape. The starch granules were evenly distributed on the surface of the tape, and the ethanol was allowed to evaporate. The samples were then coated with 12 nm gold, examined and photographed at an accelerating voltage of 5kV with a magnification of x500.

Functional properties

Swelling power and solubility

Swelling power (SP) and water solubility index (WSI) determinations were carried out in the temperature range 55-95°C at 10°C intervals using the method of Leach *et al.* (1959) and Holm *et al.* (1985), respectively.

Bulk density

This was determined by the method of Wang and Kinsella (1976) with slight modification. 10 mL capacity graduated cylinder was filled with the starch powdery sample. This was done by gently tapping the bottom of the cylinder on the laboratory bench several times until there is no further diminution of the sample level after filling to the 10 mL mark.

$$\text{Bulk density (g/mL)} = \frac{\text{Weight of samples (g)}}{\text{Volume of sample (mL)}}$$

Dispersibility

This was determined by the method described by Kulkarni *et al.* (1991) as recently modified by Akanbi *et al.* (2009).

pH

Cowpea starch samples (5 g) were weighed in triplicate into a beaker, mixed with 20 ml of distilled water. The resulting suspension stirred for 5 min and left to settle for 10 min. The pH of the water phase was measured using a calibrated pH meter (Benesi, 2005).

Pasting properties of starches

The pasting properties of the cowpea starches were evaluated by using a Rapid Visco Analyzer (Newport Scientific, RVA Super 3, Switzerland). Starch suspensions (9%, w/w; dry starch basis, 28 g total weight) were equilibrated at 30°C for 1 min, heated at 95°C for 5.5 mins, at a rate of 6°C/min, held at 95°C for 5.5 min, cooled down to 50°C at a rate of 6°C/min and finally held at 50°C for 2 mins. It was a programmed heating and cooling cycle. Parameters recorded were pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), or trough viscosity (TV), final viscosity (FV), and peak time (PTime). Breakdown viscosity (BV) was calculated as the difference between PV minus MV, while total setback viscosity (SV) was determined as the FV minus MV. All determinations were performed in triplicate.

Statistical analysis

Experimental data were analysed statistically using Microsoft Excel and SPSS V. 12.0

Results and Discussion

Yield and gross chemical composition of cowpea starches

The yield and gross chemical composition of the two isolated cowpea starches i.e., white cowpea starch (WCS) and brown cowpea starch (BCS) are shown in Table 1. The yield of 40% and 38% for WCS and BCS falls within the range reported in the literature for most legume starches (Hoover and Sosulski, 1991). The yield of isolated cowpea starches was low when compared to other legume starches such as black gram (45%), red bean (46%) (Hoover and Sosulski, 1991) but high when compared to some other legume starches such as beach pea (12.3%), grass pea (26%), green pea (30%) (Chavan *et al.*, 1999) and adzuki bean (21.5%) (Naivikul and D'Appolonia, 1979). The rather low yield of isolated cowpea starches could be attributed to the presence of highly hydrated fine fiber

Table 1. Yield and Gross chemical composition of Cowpea Starches

Starch samples	Yield (%)	Moisture (%)	Protein (%)	Fats (%)	Ash (%)	AM (%)
WCS	40.00 ^a ±1.1	11.54 ^a ±0.03	0.09±0.01	0.05±0.00	0.03±0.01	22.06 ^a ±0.03
BCS	38.00 ^b ±1.9	10.18 ^b ±0.42	0.07±0.02	0.07±0.01	0.05±0.01	26.53 ^b ±0.05

Uncommon superscripts along columns indicate statistically significant difference ($p < 0.05$).

fraction (Vose, 1977) which is derived from the cell wall enclosing the starch granules. Additional reasons might be due to presence of some insoluble proteins and compact association of cowpea starch granules with other biomolecules that could be present.

The difference in moisture content between the two cowpea starches (Table 1) might be attributed to differences in cultivar (Chein *et al.*, 2003). The values of moisture content also concur with the established goal necessary to reach a stable shell life (less than 14% moisture content; Juliano and Villareal, 1993). There are no significant differences in the values of residual protein, fats and ash contents between the two cowpea starches and these are in accordance with literature values for other legume starches such as adzuki bean and lima bean (Tjahjadi and Breene, 1984; Betancur-Ancona *et al.*, 2003). Apparent amylose (AAM) concentration differ significantly between the two cowpea starches (Table 1) but falls within the range stipulated in previous literature for cowpea starches (Akinyele *et al.*, 1986; Aremu, 1991). According to the latter investigators, the AAM concentration of starches from cowpea cultivars ranged from 6.92% to 39.30%, averaging at 17.73%. Typically for legume starches these values (22.06% and 26.53%) for WCS and BCS are very low and the consequences for retrogradation and syneresis are pretty obvious. The differences in AAM concentration between WCS and BCS could be attributed to variations in climatic conditions and soil type during growth (Morrison and Azudin, 1987; Asaoka *et al.*, 1985). It is absolutely necessary to point out the difficulties involved in an attempt to compare lipid (fats) values in legume starches, because different lipid extractants were utilized by different researchers (Goshima *et al.*, 1985; Kawano *et al.*, 1989). These different lipid extractants differ in their ability to extract firmly bound lipids (Vasanthan and Hoover, 1992) and become obviously difficult to compare results from published data.

Morphological properties of isolated starches

The granules of WCS and BCS are shown in Figure 1. Microscopic examination showed that WCS and BCS granules had irregular shapes which varied from round to elliptical as reported for other legume starch granules (Singh *et al.*, 2004; Hoover and Sosulski, 1991). Both cowpea starch granules

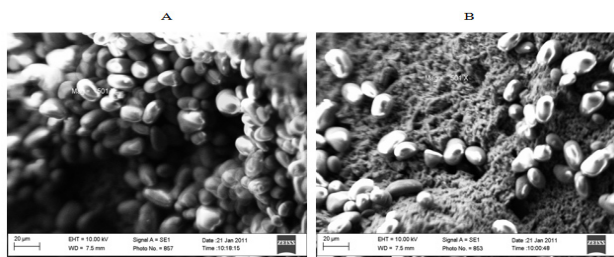


Figure 1. SEM micrographs of the isolated cowpea starches of magnification (Mag.) X500; A (WCS granules), B (BCS granules)

had diameter of 10 to 20 μm and this fall within the range indicated by Jane *et al.* (1994) for other cowpea starch granules and also concurs with value generally reported for legume starch granules (Sathe *et al.*, 1982).

Granule clustering was evident in WCS, similar to that reported by Chavan *et al.* (1999) for beach pea, green pea and grass pea. In contrast, there was no clustering tendency in BCS and fissures appeared on their surfaces. The cracks on the surfaces of BCS granules were more pronounced than that in WCS granules. These clustering of granules or the formation of compound granules in WCS had been previously reported for rice starch granules (Ashogbon and Akintayo, 2011, 2012). It is probably due to the presence of residual protein (Cardoso *et al.*, 2006, 2007) or could be attributed to the drying conditions that produce slight gelatinization on the surface of granules and cause the granules to adhere together to form aggregates (Newman *et al.*, 2007).

Functional properties of cowpea starches

The values of bulk density, dispersibility, and pH are presented in Table 2. The bulk density is a measure of the degree of coarseness of the sample, it is lower for WCS (0.58 g/ml) and higher for BCS (0.60 g/ml). The higher the bulk density, the coarser the particles of the sample. It has been previously observed by other researchers (Bhattacharya *et al.*, 1972) that bulk density is related to the kernel shape (length/breadth ratio), the more round the kernel, the higher the bulk density.

Dispersibility is a measure of reconstitution of starch flour in water, the higher the dispersibility the better the flour reconstitutes in water (Kulkarni *et al.*, 1991). The percentage dispersibility varies significantly between the two cowpea starches (Table 2). It was observed that the settling rate of the particles of the BCS was faster than that of WCS, but the latter occupied smaller surface area in the measuring cylinder than the former (BCS). It could be rationalized that the particles of the BCS were larger and heavier when compared to WCS. Therefore the

Table 2. Bulk density, Dispersibility and pH of Cowpea Starches

Cowpea cultivar	Bulk density (g/ml)	Dispersibility (%)	pH
WCS	0.58±0.01	74.20±0.26 ^a	6.98±0.03
BCS	0.60±0.02	72.10±0.24 ^b	6.93±0.02

Uncommon superscripts along columns indicate statistically significant difference ($P < 0.05$).

Table 3. Temperature ($^{\circ}\text{C}$) effects on SP (g/g) of Cowpea Starches

Cowpea cultivar	Swelling Power with different Temperature				
	55 $^{\circ}\text{C}$	65 $^{\circ}\text{C}$	75 $^{\circ}\text{C}$	85 $^{\circ}\text{C}$	95 $^{\circ}\text{C}$
WCS	1.85±0.13 ^a	2.07±0.16	4.88±0.17 ^a	5.22±0.25 ^a	6.48±0.23 ^a
BCS	1.66±0.11 ^b	2.07±0.12	5.86±0.12 ^b	6.59±0.24 ^b	7.85±0.22 ^b

Uncommon superscripts along columns indicate statistically significant difference ($P < 0.05$).

Table 4. Temperature ($^{\circ}\text{C}$) effects on WSI (%) of Cowpea Starches

Cowpea cultivar	Water solubility index at different temperatures				
	55 $^{\circ}\text{C}$	65 $^{\circ}\text{C}$	75 $^{\circ}\text{C}$	85 $^{\circ}\text{C}$	95 $^{\circ}\text{C}$
WCS	0.29±0.16 ^a	0.51±0.12 ^a	1.09±0.23 ^a	4.28±0.11 ^a	1.60±0.13 ^a
BCS	0.53±0.17 ^b	0.28±0.13 ^b	1.16±0.24 ^b	1.58±0.13 ^b	2.75±0.14 ^b

Uncommon superscripts along columns indicate statistically significant difference ($P < 0.05$).

BCS particles due to the influence of gravity settles down faster and the WCS particles settle slower on account of their smaller size and agglutination might have contributed to its occupation of smaller surface area. Since the higher the dispersibility the better the starch flour reconstitutes, the values obtained for these cowpea starches (72.10-74.20%) are better than the 40.67% obtained by Akanbi *et al.* (2009) for breadfruit starch, but not as good as 75.10-82.12% obtained for rice starches (Ashogbon and Akintayo, 2012).

pH is an important property in starch industrial applications, being used generally to indicate the acidic or alkaline properties of liquid media. pH values for the cowpea starches were 6.98 and 6.93, this shows that the cowpea starches are slightly acidic. But Ahmed *et al.* (2007) had reported a pH values of 3.71-3.90 for some rice starch media.

The values for swelling power (SP) (g/ml) and water solubility index (WSI) (%) for the cowpea starch samples heated from 55 $^{\circ}\text{C}$ to 95 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}$ intervals are summarized in Table 3 and 4. The SP for WCS and BCS increased progressively with increasing temperature, similar trend were reported by Henshaw and Adebawale (2004) for starch from cowpea varieties, by Ratnayake *et al.* (2001) for starch from field pea cultivars and by Chavan *et al.* (1999) for beach pea starch. SP for BCS was more pronounced than for WCS. In contrast, the values for WSI were inconsistent, for WCS it increases from 55 $^{\circ}\text{C}$ to 85 $^{\circ}\text{C}$ and then decreases to 95 $^{\circ}\text{C}$. The WSI for BCS was more inconsistent. The difference in SP among starches from different cowpea cultivars

Table 5. Pasting properties of cowpea starches

Rice Cultivars	PV (RVU)	TV (RVU)	BV (RVU)	FV (RVU)	SV (RVU)	Peak Time (Min)	PT (°C)
WCS	451.67±0.1 ^a	236.58±0.1 ^a	215.08±0.1 ^a	313.75±0.1 ^a	77.17±0.1 ^a	4.43±0.1 ^a	50.30±0.1 ^a
BCS	474.83±0.2 ^b	266.75±0.1 ^b	208.08±0.2 ^b	358.33±0.1 ^b	91.58±0.2 ^b	4.50±0.1 ^b	50.20±0.2 ^b

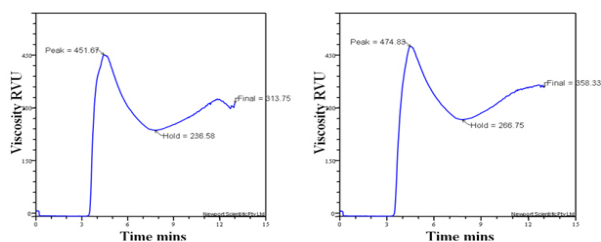


Figure 2. Pasting curves showing different pasting properties of isolated starches: A. WCS; B. BCS

indicate variation in the strength of associative bonding forces within the granules (Leach *et al.*, 1959). The higher SP shown by BCS might be indicative of weak bonding forces within its granules and the fact that it is less compact when compared to WCS granules. SP of starch is also affected by the presence of lipids (Swinkels, 1985). These may have no effect on SP in our study, as the cowpea starches contain only traces of lipids.

Pasting properties of cowpea starch

Pasting is an episode following gelatinization in the solubilization of starches. It involves granular swelling, exudation of the granular molecular components, and lastly complete disruption of the granules (Atwell *et al.*, 1988). Changes in the viscosity of starch suspensions as temperature changes were measured with RVA. The pasting characteristics of the two cowpea starches are shown in Table 5. PT (temperature at the onset of rise in viscosity) of the two cowpea starches; white cowpea starch (WCS) and brown cowpea starch (BCS) are significantly different (Table 5). Pasting temperatures range of 50.20 to 52.50°C for black gram, chickpea, field pea, lentil, mung bean, and pigeon pea starches studied by Sandhu and Lim (2008) concurs with our study. Higher pasting temperatures were previously reported by some investigators: identical values of 79.50°C for four cultivars of field pea starches (Ratnayake *et al.*, 2001); and a range of 75.80-80.30°C for some Indian black gram starches (Singh *et al.*, 2004). The lower PT for cowpea starches may be attributed to their lower resistance towards swelling.

PV is the maximum viscosity attained by gelatinized starch during heating in water and corresponds to the point when the numbers of swollen, but still intact starch granules are maximal. It also indicates the water binding capacity of the starch granules (Shimelis *et al.*, 2006) and it is frequently correlated with final product quality. PV differed

significantly between the two cowpea starches; it was higher for BCS (Table 5). The PV of these cowpea starches is in accordance with the work of Singh *et al.* (2004). PV is accompanied immediately by a reduction in viscosity to a minimum (TV), due to granule rupture and leaching of the lower molecular weight glucan polymers, e.g. AM, as a result of exposure to higher temperature and shear.

The BV (measure of the vulnerability of cooked starch to disintegration) was higher for WCS (215.08 RVU) when compared to BCS (208.08 RVU). The higher the breakdown in viscosity, the lower the ability of the starch sample to withstand heating and shear stress during cooking (Adebowale *et al.*, 2005). Therefore, BCS might be able to withstand more heating and shear stress when compared to WCS because of its lower breakdown value. These cowpea starches possess less ability to resist heating and shear stress when compared to the starches from 13 improved Indian black gram cultivars of Singh *et al.* (2004) due to their higher BV values.

Both the FV (indicates the ability of the starch to form a viscous paste) and SV (measure of re-crystallization of gelatinized starch when cooled) are higher for BCS when compared to WCS. The higher FV for BCS indicated that the paste could be more resistant to shearing, and could form a more rigid gel (Zhang *et al.*, 2005). SV is usually correlated with the texture of various end products and related to the AAM content of the starch sample. High AM starches re-associate more readily than high AP starches. These indicated that BCS were more susceptible to retrograde than WCS during the cooling of the cooking processes due to its higher concentration of AAM. This is in absolute agreement with works in the literature (Gudmundsson, 1994) that constantly link high AAM concentration with the tendencies of syneresis and retrogradation especially in legume starches (Adebowale and Lawal, 2003; Ashogbon and Akintayo, 2011; Ashogbon *et al.*, 2011). The differences in setback among different starches may also be due to the amount and the molecular weight of AM leached out from the granules and the ghost of the gelatinized starch granules (Loh, 1992). In Table 5, it can be seen that the FV was higher for the starch with higher AAM concentration (Table 1). This is in accordance with Miles *et al.* (1985) that previously reported an increase in FV might probably be due to the re-association of AAM molecules. According to Juliano *et al.* (1987), varietal differences in pasting characteristics of starch can be attributed to differences in AP molecular structure rather than AAM. It is also possible that the differences in the degree of randomly limited branching in AAM

concentration might have contributed to varietal differences (Ashogbon and Akintayo, 2011). Other reasons for varietal differences may be inherent differences in the structure of starch or maybe due to different degree of interactions between starch and its associated compounds during pasting (Zhang and Hamaker, 2008).

As seen in Figure 2, the pasting curves of the different starches were significantly different. This is clearly obvious in the values of PV, TV and FV, despite the insignificant differences between the residual proteins, fats and ash contents of the two cowpea starches.

Conclusions

This study showed that differences in starch properties could occur among the cultivars of the same species, even under identical experimental conditions. The isolated cowpea starches showed ash, protein, fats and AAM contents ranging between 0.03-0.05%, 0.07-0.09%, 0.05-0.07%, and 22.06-26.53%, respectively. Clustering of granules was observed for WCS and fissures appeared on the surfaces of BCS granules. The shapes of the granules were round to elliptical. The significant differences in the functional properties of the two cowpea starches especially their pasting properties indicated that these observed differences could be used in the selection of the best cultivars for specific food processing applications.

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