

Physicochemical characterization of sprouted cowpea starch by varieties

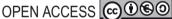
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Abstract

This current work explored the physicochemical characteristics of sprouted cowpea starch across different varieties. Specifically, cowpea varieties (IR48B, IT89KD-288, IT82D-716W, and TV32-36WS) were sprouted (steeping = 36 h; germinating = 72 h), then milled into a slurry, followed by starch extraction. Physicochemical characterization involved measurements of starch yield, moisture, protein, pH, amylose, water absorption capacity, gelation, solubility index, bulk density, swelling power, and pasting attributes. Results showed that sprouting significantly enhanced the protein, water absorption capacity, total titratable acidity, swelling power, solubility index, and emulsion capacity of cowpea starch. However, sprouting significantly reduced starch yield, pH, bulk density, gelation capacity, and amylose content. Comparative analysis revealed that sprouted cowpea starch had superior pasting properties, including higher peak viscosity and setback viscosity, especially when compared to other starch sources. A direct correlation between amylose content and setback viscosity appeared evident, although the quality of fit for sprouted cowpea starch suggested that additional factors might influence the pasting behavior. Sprouted cowpea starch seems to be a nutritional and versatile alternative in food formulations, functionally positioned particularly for health-conscious consumers.

Keywords: cowpea starch; functional ingredient; pasting properties; physicochemical properties; sprouting

Introduction

Pulses are dicotyledonous seeds of the global Leguminosae plant family, with about 60 domesticated species (Hedey, 2001). Among different pulses, cowpea (Vigna unguiculata L. Walp) remains the most consumed source of protein (Hedey, 2001), with seeds constituting about 25% protein and 64% carbohydrate, while starch makes up about 52% of the total carbohydrate content (Kerr et al., 2000; Ihediohanma et al., 2014). Besides comprising a well-balanced amino acid content, cowpea provides several B-complex diet vitamins (Hedey, 2001), which, when utilized, can directly help combat malnutrition in developing countries. Believed to have originated from Africa before spreading into Asia and Europe, cowpea appeared long before Phaseolus beans were introduced from the Americas (Sasanam et al., 2011). Given its distribution in both tropical and temperate climates, cowpea can have different seed coat shapes, sizes, and colors (Afoakwa *et al.*, 2006; FAO, 2000). In Nigeria, cowpea is widely consumed as boiled seeds alone or in combination with other foods (e.g., plantain, maize, and rice) (Henshaw, 2008). Cowpea paste can also be fried (Akara) or steamed (moi moi) (Nwosu *et al.*, 2014). Compared to other legumes, cowpea remains one of the most significant starch-protein grains with potential industrial applications in the West African sub-region (Atuobi *et al.*, 2011; Oyeyinka *et al.*, 2021).

In recent times, food and non-food industries have increased their attention to utilizing cowpea components as functional ingredients. Processing cowpea seeds into value-added products, such as protein concentrate and/ or food-grade starch, has become useful for extenders, gelling agents, stabilizers, texture modifiers, and thickeners in food formulations (Thomas and Atwell, 1997). However, isolating pure starch from some legumes, which are largely occupied with insoluble protein and highly hydrated fine fiber fractions, remains challenging (Schoch and Maywald, 1968; Ashogbon and Akintayo, 2013). The fine fibers likely reflect the cell walls that cover the starch granules. Comparatively, legume starches appear more viscous than those of cereals, indicative of higher granule resistance to swelling and rupture. Additionally, legume starches serve as better substrates than those of cereals or tubers (Hoover and Zhou, 2003), probably due to the absence of pores on the granule surface (Hoover and Sosulski, 1985), the presence of only trace quantities of bound lipids (Hoover and Sosulski, 1991), wide variations in 'B' type crystallite quantities (Cairns et al., 1997; Ratnayake et al., 2001), uniform granule size (Hoover and Sosulski, 1991), and variations in starch chain interactions within the amorphous and crystalline domains (Hoover and Sosulski, 1985). Understanding the susceptibility of cowpea starches, whether within the same or different biotypes, may help identify the structural factors that limit amylolysis and provide greater insights into the legume's lower glycemic index (GI).

Native starches can easily undergo syneresis, making them unsuitable for certain types of processing (Din et al., 2015), due to their poor shear and thermal stability, as well as a high degree of retrogradation (Jayakody and Hoover, 2008). To enhance starch utilization in various food applications, inherent drawbacks such as excessive paste turbidity, retrogradability, low resistance to shear and temperature, and limited structure formability must be addressed (Atienza & Rubiales, 2017). However, starch restructuring through various modifications is necessary, which can increase their stability against excessive heat, acid, shear, time, cooling, or freezing, as well as either increase or decrease their viscosity, shorten or lengthen the gelatinization period, change their texture, and/or improve their visco-stability (Zeeman et al., 2010).

Various modification processes, such as physical, chemical (acid-thinning, oxidation, etc.), and enzymatic techniques, have been employed to improve the functionality and digestibility of legume starch (Rostamabadi *et al.*, 2024; Bangar *et al.*, 2022; Ashogbon *et al.*, 2020). While concerns about chemical starch modifications are increasing, legume starches treated without chemicals are gaining popularity (BeMiller, 2018).

Bioprocessing methods such as solid-state fermentation, dry fractionation, air classification, and sprouting have been used to modify legume starch (Di Stefano et al., 2019; Xing, 2020; Xing et al., 2020). Specifically, sprouting stands out because it modifies the starch through induced enzyme activity on the legume's carbohydrates, significantly enhancing the nutritional profile and improving digestibility (Ofoedu et al., 2020; Eke-Ejiofor et al., 2021). Additionally, sprouting reduces the levels of anti-nutritional components, such as phytic acid and tannins, which would otherwise inhibit the absorption of vital nutrients. Also considered a "clean-label" method, sprouting is regarded as a natural and simple process, yet it is cost-effective, energy-efficient, and requires minimal external additives or chemicals (Di Stefano et al., 2019). Despite previous studies on the effects of cross-linking and acetylation in modifying the starch properties of cowpea seeds (Haungb et al., 2007; Mwasaru and Ishibashi, 2006), there is a lack of relevant information regarding the starch characteristics across different varieties modified by sprouting, particularly in relation to emergent functional and pasting properties. Understanding the functionality and rheological behavior of cowpea starch within the food system (post-modification) can help enhance variety selection for high-quality output. As a functional ingredient, therefore, the utilization of cowpea starch with hope of reducing the over-dependence on more familiar sources should be a promising food application strategy. To extend the body of knowledge, this current work explored the physicochemical characterization of sprouted cowpea starch across different varieties.

Materials and Methods

Schematic overview of the experimental program

The schematic overview of the experimental program, as shown in Figure 1, presents the crucial processing and treatment stages of cowpea seeds, from sprouting through the extraction of cowpea starch to the quality evaluation of the starch. For emphasis, this current work was specifically designed to characterize the starch produced from different cowpea varieties after the sprouting treatment. Starch analysis was conducted through duplicate determinations of aliquots collected from the sample population (sprouted and unsprouted cowpea starches) across the cowpea varieties.

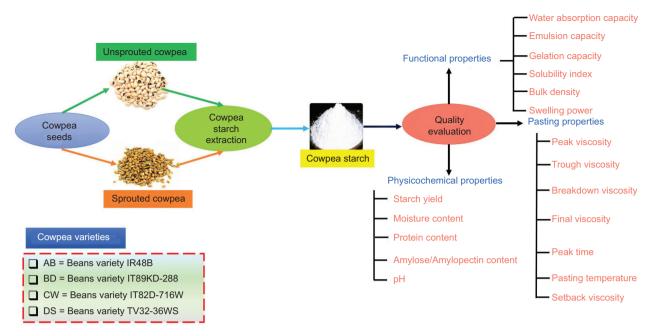


Figure 1. Schematic representation of the experimental program.

Procurement of materials

Four commercially available cowpea varieties, namely: Beans Variety IR48B (denoted herein as AB), Beans Variety IT89KD-288 (denoted herein as BD), Beans Variety IT82D-716W (denoted herein as CW), and Beans Variety TV32-36WS (denoted herein as DS), were sourced from the National Root Crop Research Institute (NRCRI) Umudike, Abia State, Nigeria. Importantly, these cowpea varieties were grown and stored under standard field agronomic practices. Additionally, all chemicals and reagents used in this current work were of analytical grade and procured from certified sources.

Sprouting of cowpea samples

Before the sprouting activity, the cowpea seeds of different varieties were manually cleaned by sorting to remove extraneous materials and damaged seeds, followed by winnowing to remove dust, before being subjected to further processing through sprouting and starch extraction. The sprouting activity followed the barley malting protocol (Kunze, 2005; Osuji *et al.*, 2019) with slight modifications. Cowpea samples were steeped in water at a temperature of 20–25°C for 36 h. Further, the steep cycle alternated between a wet-steep cycle of 12 h and an air-rest period of 45 min. Once the steeping operation was completed, the cowpea seeds were placed on (dry heat) sterilized jute bags, allowed to sprout at a temperature range of 25–30°C, and removed after 72 h.

Starch extraction from cowpea seeds

Starch extraction from cowpea seeds (sprouted and unsprouted) followed the method described by Osuji and Anih (2011) with slight modifications. The cowpea seeds were washed, steeped in water, and their coats manually removed. This was followed by wet milling of the cotyledons into a slurry, then stirring and allowing it to settle (~6 h) until a heterogeneous mixture was observed. The top portion formed a transparent liquid, whereas the bottom part formed a thick deposit. The supernatant was decanted. The starch sediment was re-dissolved in 0.05 M NaOH and allowed to stand for 2 h, after which it was neutralized with 1 M NaNO3 to pH 6. The starch sediment was rinsed with distilled water and allowed to settle until a firm and dense deposit was seen at the bottom, facilitating the gradual recovery of the sediment. This was followed by gently drying (~60°C) in a hot air oven (Genlab, England, Model M 30 C, S/N 92B060) for about 6 h. Thereafter, the resultant starch was ground using an electric blender (Blendtec FIT Model, Blendtec Inc., USA) to achieve a powder, then sieved and stored in a sealed container until required for further analysis.

Physicochemical characterization of cowpea starch

Determination of yield

The cowpea starch yield (sprouted and unsprouted) was determined using the method described by

Adebowale *et al.* (2010), and was expressed as a percentage on a dry matter basis using Eqn 1 below:

Starch Yield

$$= \frac{\text{Weight of Cowpea Starch after extraction}}{\text{Weight of Cowpea seeds before treatment}} \times 100^{-(1)}$$

Determination of moisture content

The moisture content of cowpea starch (sprouted and unsprouted) was determined using the extraction oven method (AOAC, 2004), expressed as a percentage (%) and calculated from Eqn 2 below:

Moisture content =
$$\frac{W_2 - W_3}{W_2 - W_1} \times 100$$
 (2)

where W_1 = initial weight of the empty dish W_2 = weight of the dish + undried sample W_3 = weight of the dish + dried

Determination of protein content

The protein content of cowpea starch (sprouted and unsprouted) was determined using the Kjeldahl method (AOAC, 2004), expressed as a percentage (%) and calculated from Eqn 3 and 4 below:

% Nitrogen =
$$\frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of Sample} \times V}$$
 (3)

% Crude protein =
$$6.25 \times \%N$$
 (4)

where S = Sample titration reading 0.014 = Milli equivalent weight of Nitrogen

N = Normality of HCl

V = Volume taken for distillation

B = Blank titration reading

D = Dilution of sample after digestion

Determination of pH

The pH of cowpea starch (sprouted and unsprouted) was determined using a pH meter electrode (probe) (AOAC, 2004).

Determination of amylose content

Amylose content of cowpea starch (sprouted and unsprouted) was determined using the method described by Udachan *et al.* (2012) with slight modifications. Standard amylose (70%) and about 0.1 g of the cowpea starch samples were weighed into different test tubes. Then, 9 ml of 1 M NaOH and 95% ethanol were added and mixed in a vortex mixer. A boiling water bath was used to heat and gelatinize the starch in the test tubes, which was then allowed to cool (it should contain 10 ml of extract). An aliquot (1 ml) was taken from each extract into another test tube and made up to 10 ml with distilled

water (9 ml). Subsequently, 0.5 ml was taken from the 10 ml diluent into another test tube, then 0.2 ml of iodine solution and 0.1 ml of acetic acid solution were added, and the entire volume was made up to 10 ml with 9.2 ml of distilled water. The mixture was allowed to stand for 20 minutes to facilitate color development (dark blue complex). The test tubes were vortexed, and the absorbance was read on the spectrophotometer (Spectrumlab 22pc) at 620 nm. The % amylose content was calculated using Eqn 5 below:

% Amylose content

 $= \frac{\% Absorbance of standard \times Absorbance of sample}{Absorbance of standard} (5)$

Determination of water absorption capacity

The water absorption capacity of cowpea starch (sprouted and unsprouted) was determined using the method described by Onwuka (2005). The water absorption capacity, expressed as g/g, was calculated using Eqn 6 below:

Water absorption capacity
$$(g/g) = \frac{\text{Weight of sample after centrifuging}}{\text{Weight of sample before centrifuging}}$$
 (6)

Determination of emulsion capacity

The emulsion capacity of cowpea starch (sprouted and unsprouted) was determined using the method described by Onwuka (2005). The emulsion capacity, expressed as %, was read from the centrifuge tubes and calculated using Eqn 7 below:

Emulsion Capacity (%) = $\frac{\text{Height of emulsified layer}}{\text{Height of whole solution in the centrifuge tube}} \times 100$ (7)

Determination of gelation capacity

The gelation capacity of cowpea starch (sprouted and unsprouted) was determined using the method described by Onwuka (2005) with slight modifications. Suspensions of 4, 8, 12, 14, 16, 18, and 20% (w/v) in 5 ml of distilled water were prepared in test tubes. These were immersed in a boiling water bath and heated for 1 h, followed by rapid cooling under running cold tap water. The lowest gelation concentration (LGC) was identified when the sample did not slide when the tube was inverted.

Determination of solubility index

The solubility index of cowpea starch (sprouted and unsprouted) was determined using the method described by Adebowale *et al.* (2009) with slight modifications. The starch sample (5 g) was weighed into a pre-weighed centrifuge tube, and 20 ml of distilled water was added

and thoroughly shaken on a vortex. The sample was then heated to temperatures of 50°C, 60°C, 70°C, and 80°C for 30 min in a water bath (HH-4, Techmel and Techmel, USA). The samples were centrifuged at 3000 rpm for 20 min. The supernatant was carefully decanted and dried to a constant weight at 110°C in a hot air oven (TT 9053A, Techmel and Techmel, USA). The residue obtained after drying the supernatant represented the amount of starch solubilized in water. The solubility index was calculated using Eqn 8 below:

Solubility index (%) =
$$\frac{\text{Weight of soluble}}{\text{Weight of sample}} \times 100$$
 (8)

Determination of bulk density and swelling power

The swelling power and bulk density of cowpea starch (sprouted and unsprouted) were determined using the method described by Onwuka (2005). The percentage swelling power and bulk density, expressed as g/mL, were calculated using Eqns 9 and 10 below:

Bulk density (g/ml) =
$$\frac{\text{Weight of sample (g)}}{\text{Volume of sample (ml)}}$$
 (9)

Swelling power (%) =
$$\frac{\text{Final volume}}{\text{Initial volume}} \times 100$$
 (10)

Determinations of pasting attributes

The pasting attributes of cowpea starch (sprouted and unsprouted) were determined using the Rapid Visco Analyzer (Model: RVA-4, Newport Scientific Pty. Ltd., Sydney, Australia, 1995), which operated with Thermocline for Windows software. The starch sample (2.5 g) was weighed into a previously dried canister, and 25 ml of distilled water was dispensed and added. The suspension was then well mixed and fitted into the Rapid Visco Analyzer (RVA), which followed the standard profile 1: 1 min of mixing, stirring, and warming up to 50°C; 3 min and 42 sec of

heating at 12°C/min up to 95°C; 2.5 min of holding at 95°C; 3 min and 48 sec of cooling down to 50°C at the same rate as the heating (12°C/min); and 2 min of holding at 50°C, with the process ending after 13 minutes (Deffenbaugh and Walker, 1989). Using the starch gelatinization (pasting) curves, the viscosity was determined, expressed in terms of Rapid Visco Units (RVU), which is equivalent to 10 centipoises. The viscogram profile/pasting curves show the relationship between time, viscosity, and temperature during the cooking processes. The pasting attributes determined included: (a) peak viscosity, (b) peaking time (pasting time) breakdown, (c) trough viscosity, (d) setback, (e) final viscosity, and (f) pasting temperature.

Statistical analysis

Data obtained from two determinations were subjected to analysis of variance (ANOVA) in a 4 (Cowpea Variety) x 2 (Treatments) approach, and results were expressed as mean \pm standard deviation (SD). Mean differences were resolved using the LSD test. Correlation analysis was applied to determine if any relationship existed between amylopectin content of sprouted and unsprouted cowpea starch and pasting attributes (setback). The significance level was set at p < 0.05. SPSS Software Package version 16 was used to perform the data analysis.

Results and Discussion

Variations in yield, moisture and protein content of cowpea starch

The variations in yield, moisture, and protein content of starches from sprouted and unsprouted cowpea of different varieties are shown in Table 1. While the starch yield and moisture content were similar (p > 0.05), the protein content significantly differed (p < 0.05) due to sprouting

Table 1. Variations in starch yield, moisture content, and protein content of starches from sprouted and unsprouted cowpea varieties.

Cowpea variety	ea variety Starch yield (%)		Moisture c	ontent (%)	Protein content (%)	
	Unsprouted	Sprouted	Unsprouted	Sprouted	Unsprouted	Sprouted
AB	*38.02b±0.05	26.61b±0.21	11.00°±0.49	10.8 ^{0a} ±0.00	4.92d±0.01	*6.73b±0.01
BD	*37.79°±0.21	26.45b±0.22	11.45°±0.71	10.00°±0.00	5.42°±0.01	*6.97°±0.01
CW	*39.27a±0.67	27.49°±0.03	11.20a±0.42	10.95°±0.42	5.06°±0.01	*6.47° ±0.01
DS	*36.75d±0.81	25.73°±0.56	11.02a±0.06	10.01a±0.37	5.26 ^{ab} ±0.01	*6.66bc±0.01
LSD	1.04	0.86	NS	NS	0.25	0.24

Values are the means of duplicate determinations.

a,b indicates that values with the same superscript in a column for each treatment are not significantly different (p > 0.05).

An asterisk (*) within a row indicates that the values for swelling power, total titratable acidity, water binding capacity, and total solids are significantly different (p < 0.05).

AB = Beans Variety IR48B; BD = Beans Variety IT89KD-288; CW = Beans Variety IT82D-716W; DS = Beans Variety TV32-36WS.

treatments and varietal differences. Starch yield peaked at CW (unsprouted and sprouted) (39.27% and 27.49%) but was lowest at DS (unsprouted and sprouted) (36.75% and 25.73%), which is consistent with other cowpea data (38-40%) (Ashogbon and Akintayo, 2013) but appears higher than that of Indonesian cowpea (17.78-22.93%) (Ratnaningsih et al., 2016). The significantly lower starch yield of sprouted cowpea (p < 0.05) compared to unsprouted cowpea seems to reflect amylose/amylopectin degradation, physiological state, genotype variations, and differing starch isolation methods. The moisture content of sprouted and unsprouted cowpea starch is lower than the <14% recommended for flours by NDSU (2018). Increased moisture content in flour naturally fosters microbial growth and the production of off-odors and flavors. Lowering the initial moisture content would enhance storage stability (Fellows, 2000; Akubor and Badifu, 2004).

The protein content of unsprouted cowpea starch peaked at BD (5.42%) but was lowest at AB (4.92%), whereas in the sprouted samples, the protein content peaked at BD (6.97%) and was lowest at CW (6.47%). The protein content range (sprouted and unsprouted) was higher than the values (0.14–0.49% for cowpea starch) reported by Ratnaningsih et al. (2016) but lower than those (23.74-27.01% for cowpea flour) reported by Ihediohanma et al. (2014). The sprouting process may increase the protein content in cowpea starch through net enzymatic synthesis (Masood et al., 2014; Erba et al., 2018). Sprouting initiates the de novo synthesis of starch-degrading enzymes (α-amylase/glucosidase) within the scutellum/aleurone cells (Duke, 2009; Saman et al., 2008). Higher proteolytic activity during germination might also contribute to the protein content in sprouted cowpea starch, due to a shift in protein distribution from high (less soluble) to low (more soluble) molecular constituents (Owuamanam et al., 2013; Lemmens et al., 2018; Nwosu et al., 2019). Leaching of water-soluble peptides in the steeping water (Afify et al., 2012; Elmaki et al., 1999) may not significantly decrease the protein content of legumes after sprouting (Lemmens *et al.*, 2018), despite carbohydrate loss via respiration (Mbithi-Mwikya *et al.*, 2000; Tizazu *et al.*, 2010). The presence of proteins, either as co-extractives or impurities, might have a functional influence on cowpea starch. For instance, proteins could enhance the water absorption capacity of cowpea starch, improving hydration and swelling during cooking, which would provide textural benefits in food products (Scott & Awika, 2023). Additionally, proteins could strengthen the emulsion capacity of cowpea starch, improving oil and water mixture stability. Protein interactions with starch during cooking could influence gelation properties, shaping the product's consistency and mouthfeel (Scott, G., & Awika, J. M. (2023).

Variations in pH, amylose, and amylopectin content of cowpea starch

The variations in pH, amylose, and amylopectin content of starches from sprouted and unsprouted cowpea of different varieties are shown in Table 2. The pH, amylose, and amylopectin content significantly differed (p<0.05) due to sprouting treatments (unsprouted and sprouted) and varietal differences. For unsprouted cowpeas, pH peaked at CW (6.30) and was lowest at BD (5.70), whereas in sprouted cowpeas, pH peaked at DS (4.70) and was lowest at BD (4.10). For unsprouted samples, amylose and amylopectin content peaked at BD (amylose = 36.66%) and AB (amylopectin = 67.11%), respectively, and were lowest at AB (amylose = 32.89%) and BD (amylopectin = 63.34%). For sprouted samples, both amylose and amylopectin contents peaked at BD (amylose = 28.80%) and AB (amylopectin = 75.40%), respectively, and were lowest at AB (amylose = 24.60%) and BD (amylopectin = 71.20%). The pH of cowpea starch (sprouted and unsprouted) ranged between 4.10 and 6.30, which aligns well with legume data (pH = 5.10-6.40) reported by Benitez et al., (2013).

Table 2. Variations in pH, amylose content, and amylopectin content of starches from sprouted and unsprouted cowpea varieties.

Cowpea variety	рН		Amylo	Amylose (%)		Amylopectin (%)	
	Unsprouted	Sprouted	Unsprouted	Sprouted	Unsprouted	Sprouted	
AB	*5.80 ^{ab} ±0.01	4.40 ^b ±0.01	*32.89°±0.20	24.60°±0.13	67.11a±0.22	*75.40°±0.02	
BD	*5.70b±0.01	4.10°±0.01	*36.66a±0.14	28.80°±0.55	63.34 ^d ±0.32	*71.20d±0.21	
CW	*6.30°±0.69	4.60°±0.01	*34.58b±0.07	26.50b±0.32	65.42°±0.56	*73.50°±0.11	
DS	*5.80b±0.03	4.70°±0.01	*33.61 ^d ±0.11	25.40 ^d ±0.15	66.39b±0.26	*74.60b±0.34	
LSD	0.49	0.49	0.52	0.40	0.52	0.40	

Values are the means of duplicate determinations.

Values with the same superscript (a, b) within a column for each treatment are not significantly different (p > 0.05).

Values with an asterisk (*) within a row indicate significant differences (p < 0.05) for starch yield, pH, moisture content, and protein content.

AB = Beans Variety IR48B; BD = Beans Variety IT89KD-288; CW = Beans Variety IT82D-716W; DS = Beans Variety TV32-36WS

Besides controlling the stability of bioactive compounds in food products (Sanchez-Moreno et al., 2006), the pH in flour-water suspension influences both emulsion and foaming properties (Benitez et al., 2013). High amylose content can affect the pasting, gelatinization, retrogradation, and swelling behavior of starch (Blazek and Copeland, 2008). In this study, sprouting affected the amylose content in cowpea starch, where the unsprouted samples had significantly higher amylose content (p<0.05) than the sprouted ones. This difference is potentially attributable to hydrolysis of the legume starch (Benincasa et al., 2019; Morad et al., 1980; Otutu et al., 2014). Moreover, cowpea naturally contains resistant starch and dietary fiber, which contribute to its low glycemic index (GI) properties. Sprouting further improves this by altering the starch structure, increasing fiber, and lowering the glucose absorption rate (Jayathilake et al., 2018; Abebe and Alemayehu, 2022). For health-conscious consumers and those managing diabetes, sprouted cowpea starch would be a valuable ingredient option, given its ability to lower the glycemic index and contribute to better overall health and well-being (Devi et al., 2015; Sunitha et al., 2023).

Variations in emulsion, gelation and water absorption capacities of cowpea starch

The variations in water absorption capacity (WAC), emulsion capacity, and gelation capacity of starches from sprouted and unsprouted cowpea of different varieties are shown in Table 3. The emulsion capacity significantly differed (p<0.05) across sprouted and unsprouted cowpea varieties, but there were no significant differences for gelation capacity and WAC. Specifically, for unsprouted cowpeas, the emulsion capacity peaked at CW (36.96%)

and was lowest at DS (30.73%). For sprouted cowpeas, the emulsion capacity peaked at CW (44.21%) and was lowest at DS (34.06%). For unsprouted cowpeas, the gelation capacity was highest at AB, BD, and DS (8.00%) and lowest at CW (6.00%). For sprouted cowpeas, the gelation capacity peaked at AB, BD, and DS (6.00%) and was lowest at CW (4.00%). For unsprouted cowpeas, the WAC peaked at DS (1.68 g/g) and was lowest at BD (1.52 g/g). For sprouted cowpeas, the WAC peaked at DS (1.75 g/g) and was lowest at BD (1.57 g/g). The emulsion capacity of sprouted and unsprouted cowpea starch remained lower than the 52% reported for sprouted cowpea flour (Owuamanam *et al.*, 2013). The emulsion capacity of sprouted cowpea starch was noticeably higher (p<0.05) than that of unsprouted starch.

The WAC of cowpea starch from sprouted varieties was significantly higher (p<0.05) than that of unsprouted varieties, which could be due to the increased protein content (refer to Table 3) (Otutu et al., 2014), given the insoluble dietary fiber and available starches that may differ across cowpea varieties (Benitez et al., 2013). This indicates that sprouted cowpea starch can absorb water more effectively, which is important for achieving the desired texture in food products. Polysaccharides could influence the WAC due to their affinity with water molecules, which are largely available in starch/polar amino acid residues (Ghavidel and Prakash, 2006). The lower WAC of unsprouted cowpea starch is likely associated with hydroxyl groups, forming hydrogen and covalent bonds between starch chains and water (Nawab et al., 2014). The crux of WAC lies in controlling the hydration process, ensuring that a food system achieves the right consistency. This property can influence the formulation of gluten-free products, where water absorption is critical for mimicking the texture of traditional wheat-based

Table 3. Variations in water absorption, emulsion capacity, and gelation capacity of starches from sprouted and unsprouted cowpea varieties.

Cowpea Variety	ty Water Absorption capacity (g/g) Emulsion capacity (%)		capacity (%)	Gelation capacity (%)		
	Unsprouted	Sprouted	Unsprouted	Sprouted	Unsprouted	Sprouted
AB	1.53b±0.01	*1.72b±0.03	32.42b±0.01	*34.07b±0.75	*8.00b±0.00	6.00a±0.00
BD	1.52b±0.01	1.57b±0.27	36.81b±0.01	*42.12a±0.01	*8.00b±0.00	6.00a±0.00
CW	1.61b±0.01	*1.71b±0.01	36.96b±0.01	*44.21a±0.01	*6.00°±0.00	4.00b±0.00
DS	1.68b±0.01	*1.75b±0.01	30.73b±0.01	*34.06b±0.70	*8.00b±0.00	6.00°±0.00
LSD	0.19	0.19	7.02	7.02	0.80	0.80

Values are the means of duplicate determinations.

Values with the same superscript (a, b) along a column for each treatment indicate no significant difference (p > 0.05).

Values with an asterisk (*) within a row indicate significant differences (p < 0.05) for water absorption, emulsion capacity, gelation capacity, solubility index, and bulk density.

AB = Beans Variety IR48B; BD = Beans Variety IT89KD-288; CW = Beans Variety IT82D-716W; DS = Beans Variety TV32-36WS; NS = Not Significant.

products. In addition to sprouting improving protein solubility and emulsification capacity (Owuamanam et al., 2013), it also enhances the formation of adsorption films around the globules, which is crucial for lowering the interfacial tension at the oil-water interface (Zayas, 1997). While emulsifying activity depends on the properties of proteins, the conditions of emulsification vary by the protein source, protein concentration, pH, ionic strength (salt type and concentration), and the viscosity of the food system (Zayas, 1997). However, the gelation capacity of sprouted cowpea starch was significantly lower than that of unsprouted cowpea starch (consistent across the varieties) (Table 3). This might reflect the relative amounts of protein, lipids, and carbohydrates (Benitez et al., 2013; Ihediohanma et al., 2014). High protein and starch content in pulse/legume flours might influence the gelation capacity (Kaushal et al., 2012). During sprouting, enhanced amylase activity, which reduces the amylose chain lengths, might have limited the gelation capacity (Phattanakulkaewmorie et al., 2011; Wichamanee and Teerarat, 2012; Xu et al., 2012; Onyeka and Dibia, 2002).

Variations in bulk density, swelling power, solubility index of cowpea starch

The variations in solubility index, bulk density, and swelling power of starches from sprouted and unsprouted cowpea of different varieties are shown in Table 4. The swelling power of cowpea starch (unsprouted and sprouted) significantly differed (p<0.05) across different varieties, but not for bulk densities and the solubility index (p>0.05). Specifically, for unsprouted starch, the bulk density peaked at BD (0.82 g/ml) but was lowest at both DS and CW (0.73 g/ml). In comparison, sprouted starch peaked at AB (0.73 g/ml) and was lowest at both DS and CW (0.67 g/ml). For unsprouted starch, the swelling power peaked at CW (4.84%) but was lowest at DS (4.14%), whereas for sprouted starch, the swelling power

peaked at BD (6.47%) and was lowest at DS (4.46%). For unsprouted starch, the solubility index peaked at CW (1.65%) but was lowest at AB (1.61%), whereas for sprouted starch, it peaked at AB (1.77%) but was lowest at DS (1.73%). Sprouting significantly decreased the bulk density of cowpea starch, which is consistent with sorghum starch data reported by Otutu *et al.* (2014). Moreover, the sprouting process can influence the particle size distribution of cowpea starch (Elkhalifa and Bernhardt, 2010), which is important for determining packaging requirements, material handling, and wet processing applications (Adebowale *et al.*, 2005a).

The swelling power of sprouted cowpea starches was significantly higher (p<0.05) than that of unsprouted starches, which probably resulted from the combined action of lower molecular proteins and proteolytic enzymes during sprouting, increasing the bioavailability of amino acids. The protein-starch matrix, more loosely bound by sprouting, increases water absorption and influences the swelling of legume flour (Henshaw and Adebowale, 2004; Phattanakulkaewmorie et al., 2011; Ihediohanma et al., 2014), which could improve the nutritional and product quality (Enujiugha et al., 2003). The lower swelling power of unsprouted cowpea starches might have inhibited the leaching of amylose chains, thereby preventing the starch granules from opening up (Chaisawang and Suphantharika, 2006). The solubility index of sprouted cowpea starches, being significantly higher (p<0.05), might suggest weaker bonding forces within the granules, as well as reduced compactness of internal starch molecules and increased water uptake (Chaisawang and Suphantharika, 2006). Additionally, sprouting initiates grain softening as the protein matrix interacts with the decreased starch endosperm granules, facilitated by peptidase and amylase action (Dziki et al., 2015). The degree of interaction between starch chains in the amorphous/crystalline regions is influenced by the amylose-to-amylopectin ratio and specific characteristics,

Table 4. Variations in solubility index, bulk density, and swelling power of starches from sprouted and unsprouted cowpea varieties.

Cowpea variety	Solubility index (%)		Bulk density (g/ml)		Swelling power (%)	
	Unsprouted	Sprouted	Unsprouted	Sprouted	Unsprouted	Sprouted
AB	1.61a±0.01	*1.77°±0.01	*0.81a±0.01	0.73a±0.01	4.19b±0.01	*4.79b±0.69
BD	1.59°±0.01	*1.75°±0.01	*0.82a±0.01	0.72°±0.01	4.37 ^{ab} ±0.01	*6.47°±0.01
CW	1.65°±0.01	*1.77°±0.01	*0.73°±0.08	0.67°±0.01	4.84°±0.01	*6.43°±0.01
DS	1.62°±0.00	*1.73°±0.01	*0.73°±0.08	0.67°±0.01	4.14b±0.01	4.46b±0.02
LSD	NS	NS	0.10	0.10	0.49	0.49

Values are the means of duplicate determinations.

Values with the same superscript (a, b...) within a column for each treatment indicate no significant difference (p > 0.05).

Values with an asterisk (*) within a row indicate significant differences (p < 0.05) for swelling power, total titratable acidity, water binding capacity, and total solids.

AB = Beans Variety IR48B; BD = Beans Variety IT89KD-288; CW = Beans Variety IT82D-716W; DS = Beans Variety TV32-36WS.

such as molecular weight/distribution, degree and length of branching, and conformation (Hoover, 2001).

Variations in pasting attributes of cowpea starches

Pasting occurs in starches upon further heating after gelatinization, which may include further granule swelling and starch leaching, as well as increased viscosity due to the application of shear forces (Hoover et al., 2010). There were significant differences (p<0.05) in the pasting properties of cowpea starch from different varieties (refer to Tables 5 and 6). For peak viscosity, the unsprouted CW showed the highest (6250 cp), while sprouted EW showed the lowest (2232 cp). Peak viscosity suggests the water-binding capacity of starch, which freely swelled before physical breakdown. The relatively high viscosity in unsprouted cowpea starch and the low viscosity in sprouted cowpea starch suggest that the latter's ability to decrease granule swelling resistance when forming a stable gel is significant. Thus, the unsprouted cowpea starch sample might be suitable for products requiring high gel elasticity/strength (Ikegwu et al., 2010). The breakdown viscosity peaked at unsprouted CW (1648 cp) but was lowest at sprouted BD (906 cp). High breakdown viscosity suggests that the flour is unable to withstand heating and shear stress during cooking (Adebowale et al., 2005b). Unsprouted CW obtained the peak trough viscosity (4620 cp), while sprouted EW had the least (1319 cp). Unsprouted CW also obtained the peak final viscosity (7861 cp), while sprouted EW had the least (2662 cp). The final viscosity, which is the change in viscosity after holding the cooked cowpea starch at 50°C, differed significantly (p<0.05) between sprouted and unsprouted samples. Final viscosity indicates the ability of the starch to form a stable and viscous paste or gel after cooking and cooling (Maziya-Dixon et al., 2007).

Furthermore, unsprouted EW recorded the highest peak time (6.20 min), while sprouted CW recorded the least peak time (4.90 min). The unsprouted BD showed the highest pasting temperature (87.35°C), whereas sprouted EW showed the least pasting temperature (71°C). Pasting temperature represents the minimum temperature required for a sample to cook and gel. A reduced pasting temperature translates to lower energy costs and better stability of other components. Unsprouted CW obtained the highest setback viscosity (3240 cp), whereas sprouted EW obtained the least (1343 cp). The low setback in sprouted cowpea starch samples may be associated with high resistance to cooked paste retrogradation (Sanni et al., 2001). Setback viscosity depicts the interaction between the leached amylose chains during the cooling cycle, despite the presence of intact and/or fragmented granules being embedded in the amylose network (Ambigaipalan et al., 2011). The lower

Mean values of peak, trough, breakdown, and final viscosity of different varieties of sprouted and unsprouted cowpea Table 5.

Cowpea variety	Peak viscosity (cp)	sity (cp)	Trough viscosity (cp)	cosity (cp)	Breakdown viscosity (cp)	iscosity (cp)	Final viscosity (cp)	sity (cp)
	Unsprouted	Sprouted	Unsprouted	Sprouted	Unsprouted	Sprouted	Unsprouted	Sprouted
AB	*4191.00°±60.81	4097.50°±37.48	*4030.00 ^b ±34.35	3996.506 ±26.16	*161.00°±7.35	101.00⁴±11.31	*5791.00 ⁴ ±23.04	5395.00°±41.01
BD	*5177.50°±80.20	5148.00b±94.45	4220.00b±57.98	4271.50a±63.34	*928.00b±62.23	906.00 ^b ±31.11	7257.50b±20.12	7237.50b±40.60
CW	*6250.00°±89.10	6125.50a±74.24	*4620.50°±50.20	4477.50°±21.21	*1648.00°±38.89	1629.50°±76.36	7861.00°±76.77	7719.00°±90.91
DS	*4923.00°±47.08	4917.00°±55.15	*4290.50 ^b ±70.11	4192.00 ^b ±46.37	*725.00°±23.04	632.50°±91.22	*7249.50b±71.52	6613.00°±77.07
LSD	217.73	217.73	268.94	268.94	142.98	142.98	404.46	404.46

Values are the means of duplicate determinations.

Values with the same superscript (a, b, etc.) within a column for each treatment are not significantly different (p > 0.05).

Values with an asterisk (*) within a row indicate significant differences (p < 0.05) for peak, trough, breakdown, and final viscosity.

AB = Beans Variety IR48B: BD = Beans Variety IT89KD-288: CW = Beans Variety IT82D-716W: DS = Beans Variety IT832-36W

Table 6. Mean values of peak time, pasting temperature, and setback of different varieties of sprouted and unsprouted cowpea.

Cowpea variety Peak tim		(min) Pasting temperature (°C)			Setback viscosity (cp)		
	Unsprouted	Sprouted	Unsprouted	Sprouted	Unsprouted	Sprouted	
AB	*6.17a±0.05	5.97b±0.06	*87.28a±0.04	86.40b±0.00	*1761.00°±11.31	1398.50°±14.85	
BD	5.27°±0.00	5.27c±0.09	87.35a±0.00	87.25°±0.07	*3037.50°±60.10	2966.00b±43.24	
CW	4.93d±0.00	4.90d±0.04	84.85°±0.07	84.78°±0.11	3240.50a±26.57	3241.50°±93.04	
DS	*5.50b±0.14	5.33c±0.00	86.43b±0.07	86.35b±0.10	*2959.00b±11.41	2421.00b±69.29	
LSD	0.14	0.14	0.20	0.20	245.32	245.32	

Values are the means of duplicate determinations.

Values with the same superscript (a, b, etc.) along a column for each treatment are not significantly different (p > 0.05).

setback viscosity in sprouted cowpea starch reveals, to a great extent, the magnitude of starch granule disruption during the heating cycle, largely due to the sprouting process. The pasting properties of the cowpea starches significantly decreased with sprouting, reflecting enzymatic (starch) degradation. Indeed, sprouting reduces the average molecular weights of β -glucans in the cowpea starches, thereby reducing their ability to form a viscous fluid (Juhasz *et al.*, 2005 and Xu *et al.*, 2012).

Comparisons and correlations involving cowpea starches

The comparison of some physicochemical properties (protein, amylose, swelling index, solubility power, water absorption capacity, pasting temperature, peak viscosity, and setback viscosity) of sprouted and unsprouted cowpea starch with cereal (corn) and tuber (cassava) starch from previous literature is shown in Table 7. Cowpea starch exhibits higher peak viscosity, setback viscosity, pasting temperature, amylose, and protein content compared to corn and cassava starch. On the other hand, cowpea starch has a lower water absorption capacity (WAC) compared to cassava starch, but higher

than corn starch. The swelling index of cowpea starch is lower compared to corn starch but higher than cassava starch. These variations could be attributed to differences in crop type and composition. The quality and quantity of protein and starch content significantly influence the functionality of food systems. Protein and starch might account for legume flour swelling at low temperatures (Henshaw and Adebowale, 2004; Ihediohanma et al., 2014). Cowpea starch could offer greater added-value potential, being more viscous than equivalent cereal or tuber starches, suggesting that it can form thicker gels and maintain stability during heating and cooling cycles. For food manufacturers, these properties are crucial when formulating products that require thickening or gelling agents. Higher viscosity can enhance the texture of sauces, soups, and gravies, making them more appealing to consumers. Additionally, the stability of cowpea starch during processing can lead to improved product consistency, which is essential for maintaining quality in mass-produced food items.

Amylose content and setback viscosity were directly correlated in both sprouted and unsprouted cowpea starch (refer to Table 8). This suggests that, during cooling, an

Table 7. Comparison of some physicochemical properties of beans (sprouted and unsprouted) starch with cereal and tuber starch.

Parameters	Cowpea starch		Cassava starch	Corn starch	Reference(s)	
	Sprouted	Unsprouted				
Protein (%)	6.66 – 6.97	4.92 – 5.42	0.51 – 1.26	0.31 – 0.55	Ojo et al. (2017); Ali et al. (2016)	
Amylose (%)	24.60 - 28.80	32.89 - 36.66	16.27 – 20.52	24.74 - 29.44	Chisenga et al. (2019); Onitilo et al. (2007)	
Swelling power (g/g)	4.46 - 6.47	4.14 – 4.84	2.22 - 3.01	11.34 – 13.55	Oyeyinka et al. (2019); Mishra and Rai (2006)	
Solubility index (%)	1.73 – 1.77	1.59 - 1.65	1.62 – 4.18	1.01 – 3.89	Onitilo et al. (2007); Mishra and Rai (2006)	
Water absorption capacity (g/g)	1.57 – 1.75	1.52 - 1.68	2.79 - 3.53	1.01 – 1.15	Ali et al. (2016); Ojo et al. (2017)	
Pasting temperature (°C)	84.78 - 87.25	84.85 - 87.35	80.2 - 83.2	78.3 – 65.22	Mishra and Rai (2006); Oyeyinka et al. (2019)	
Peak viscosity (cp)	4097 – 6125	4191 – 6250	1769 – 1921	3096 - 4867	Ojo et al. (2017); Chisenga et al. (2019)	
Setback viscosity	1148 – 3241	1761 – 3240	660 – 859	1444 – 2193	Oyeyinka et al. (2019); Ali et al. (2016)	

^{*}Values with an asterisk (*) within a row indicate significant differences (p < 0.05) for peak time, pasting temperature, setback, and power.

AB = Beans Variety IR48B; BD = Beans Variety IT89KD-288; CW = Beans Variety IT82D-716W; DS = Beans Variety TV32-36WS.

Table 8. Correlation analysis between amylose content and setback viscosity of sprouted and unsprouted cowpea starch.

Unsprouted	cowpea starch	Sprouted cowpea starch		
Parameters	Quantity	Parameters	Quantity	
Multiple R	0.628	Multiple R	0.731	
R Squared (R ²)	0.395	R Squared (R ²)	0.534	
Intercept (a)	–6101.537 cp	Intercept (a)	-6076.123 ср	
Variable (b)	257.0361 cp/%	Variable (b)	326.0350 cp/%	

increase in amylose content could stabilize the starch gel. Additionally, the R2 value for sprouted cowpea starch indicates a quality of fit at 0.5345 (less than 0.75), which is not satisfactory. This implies that 53.45% of the variations in the pasting property (setback viscosity) could be attributed to amylose content, while the remaining 46.55% is due to circumstantial or unaccounted factors. The variable coefficient (b) for sprouted cowpea starch indicates that about 326.0350 cp of setback viscosity is produced for every percentage increase in amylose content. Similarly, the R² value for unsprouted cowpea starch shows a quality of fit at 0.395 (less than 0.75), which is also not satisfactory. This implies that 39.5% of the variations in pasting property (setback viscosity) are attributed to amylose content, while the remaining 60.51% is due to other factors. The variable coefficient (b) for unsprouted cowpea starch indicates that about 257.04 cp of setback viscosity is produced for every percentage increase in amylose. These differences suggest that cowpea varieties, treatments, and variations in amylose content are responsible for the changes and variations in pasting properties (setback viscosity). Since the development of products such as puddings and custards require a stable gel structure, understanding this relationship is important. It allows for the optimization of starch blends to achieve the desired textural properties. For consumers, products made with sprouted cowpea starch may offer better mouthfeel and texture, enhancing the overall eating experience.

Conclusion

The physicochemical properties of sprouted cowpea starch across various varieties were investigated. The cowpea starch from different varieties exhibited distinct differences in yield, moisture, and protein content, indicating that the selection of specific cultivars can optimize starch functionality for targeted applications. Overall, the study demonstrated that sprouting enhances the protein content, water absorption capacity, swelling power, solubility, and emulsion capacity of cowpea starch, while also improving its pasting properties. The elevated protein levels in sprouted cowpea starch contribute not only to improved functional attributes but also to its nutritional

value, positioning it as a versatile ingredient for use in plant-based yogurt, protein gels, and as a stabilizer in various food systems.

Moreover, the positive influence of sprouting on the pasting characteristics of cowpea starch highlights its potential for use in food products requiring specific textural properties, such as sauces, soups, and baked goods. Given its unique properties, sprouted cowpea starch presents opportunities for applications in the production of gluten-free foods, thickening agents, and emulsions. However, the research does have certain limitations. The investigation was limited to a small number of cowpea varieties, which may not fully capture the genetic diversity within the species. Additionally, the study did not examine the long-term storage stability of sprouted cowpea starch, which is an important consideration, given the tendency of starches to retrograde over time. While the study identified unsatisfactory but positive correlation between amylose content and pasting properties, it did not delve into the mechanistic aspects of how the structural interactions between starch and proteins influence functionality during processing. Further studies are needed to elucidate these interactions and transformations, especially during the process of sprouting and cooking.

Future research should also focus on exploring diverse processing techniques, including both thermal and non-thermal methods (such as ultrasound, microwave, pulsed electric fields, high-pressure processing, and radio frequency), to optimize the functionality of cowpea starch for specific industrial applications. Additionally, it is important to investigate how sprouted cowpea starch behaves in different food matrices, as this could impact the overall glycemic response when consumed. Further studies are needed to examine the effect of sprouting on in vitro starch digestibility, other functional properties (such as freeze-thaw stability, paste clarity, etc.), structural characteristics, molecular interactions, and thermal profiles.

Author Contributions

C.U.M., U.E.O., C.E.O.: conceptualization, methodology, investigation, formal analysis, resources, supervision,

writing – original draft; C.O.R.O.: formal analysis, visualization, funding acquisition, writing – review & editing, project administration.

Conflict of Interest

Charles Odilichukwu R. Okpala who is an Editorial Board Member of Quality Assurance and Safety of Crops and Foods was neither involved in the decision-making nor review process of this manuscript. The other authors declare no conflict of interest.

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