

Evaluation of Different Modification Techniques and Their Impact on Red Sorghum Starch Properties

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ABSTRACT

Background: Red sorghum is an important cereal crop with starch that has limited functional properties for food processing applications. This study aimed to evaluate the effects of pre-gelatinization, annealing, and citric acid modification on the physicochemical, structural, and functional properties of red sorghum starch. **Materials and Methods:** Native red sorghum starch was extracted and modified by Pre-gelatinization (PGRSS), annealing (ARSS), and citric acid treatment (CMRSS). The starch samples were characterized for flow properties (Hausner ratio), Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), apparent amylose content, amylose/amylopectin ratio, swelling power, solubility, crystallinity (XRD), functional groups (FTIR), and Surface Morphology (SEM). **Results:** PGRSS and ARSS exhibited good flowability based on the Hausner ratio. Water absorption capacity increased from $88.66 \pm 5.15\%$ (native starch) to $126.35 \pm 0.81\%$ (PGRSS), $91.70 \pm 1.43\%$ (ARSS), and $93.35 \pm 0.72\%$ (CMRSS). PGRSS showed the highest oil absorption capacity ($52.61 \pm 1.08\%$) and swelling power (12.59 ± 0.49 g/g). CMRSS exhibited the highest apparent amylose content ($15.66 \pm 0.22\%$) and amylose/amylopectin ratio (0.18 ± 0.003). Solubility increased to $15.40 \pm 0.65\%$ in PGRSS and $15.06 \pm 0.65\%$ in CMRSS compared with native starch ($14.18 \pm 0.59\%$). XRD analysis showed that native starch exhibited A-type crystallinity, PGRSS showed C-type crystallinity, and ARSS and CMRSS exhibited B-type crystallinity. FTIR confirmed structural modifications, while SEM revealed round, irregular, and polygonal starch granules with altered surface morphology following modification. **Conclusion:** pre-gelatinization was the most effective treatment for improving water absorption, swelling power, and oil absorption, whereas citric acid modification produced the highest amylose content and amylose/amylopectin ratio. These modifications improve the potential of red sorghum starch for food processing and other industrial applications.

Keywords: Annealing, Citric Acid, Functional Properties, Physicochemical Properties, Pre-gelatinization, Red Sorghum Starch, Structural Properties.

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INTRODUCTION

Sorghum is the primary crop consumed by more than 750 million people in semi-arid tropical parts of Africa, Asia, and Latin America (Khady *et al.*, 2010). Grain is a staple food and feed in many semi-arid Asian and African nations, including Senegal. People, particularly farmers, who frequently lack the resources to sustain themselves with other food sources high in protein, vitamins, and minerals use sorghum grains (Evers and Millar, 2002).

Grain impairment is frequently identified by the presence of a Testa, seed coat, and brownish coloring. While the Testa is brightly colored in some sorghum genotypes, it can occasionally be partial, invisible, or even absent. In sorghum grain, phenolic compounds-including flavonoids, tannins, and phenolic acids-are the most prevalent and widely distributed secondary metabolites (Dicko *et al.*, 2005).

Starches are composed of 70-90% amylopectin and 10-30% amylose. The linear polymer amylose contains only D-glucose units joined by α -1,4-glycosidic bonds (Lindeboom, Chang and Tyler, 2004). Amylose has been shown to be coiled like a spring, with six glucose monomers per turn, rather than being a straight chain of glucose units. The branching activity of amylopectin is attributed to its branched-chain structure, which is composed of glucose units joined primarily by α -1,4-glycosidic bonds but also occasionally by α -1,6-glycosidic links. An amylopectin molecule



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is composed of thousands of glucose units, with branch points appearing approximately every 25 to 30 units (Waigh *et al.*, 1998).

Annealing occurs when starch is treated at temperatures lower than the onset temperature of gelatinization, either with surplus water (<65% w/w) or with intermediate water concentrations (40-50% w/w). By getting closer to the glass transition temperature, which increases molecular mobility without causing gelatinization, annealing serves a physical purpose. Additionally, it has been demonstrated that annealing lentil, smooth pea, and wrinkled pea starch increases gelatinization temperatures, thermal stability, and susceptibility to α -amylase while reducing granular swelling and amylose leaching (Perry and Donald, 2001).

Pre-gelatinized starch is starch that has been cooked through to full gelatinization and then dried to a consistent consistency (Buckow, Heinz and Knorr, 2007). The main characteristics of PGS include an increase in swelling properties, depression in cold water, and solubility (Marechal, 1996). The PGS functioning is a result of the combined effects of drying, starch supply, and cooking conditions (Perrier-Cornet, Gervais and Coquille, 1998). According to physically modified starches, PGS is mostly used as a thickening agent in a variety of immediate products, such as baby food, soups, and desserts, due to its ability to dissolve in cold water and produce pastes. PGS is preferred in sensible foods since it does not require heating to make a paste (Blaszczak *et al.*, 2005).

This process involves the acid-catalyzed hydrolysis of the α -1,4 and α -1,6 glycosidic linkages in the starch molecule. Acid hydrolysis primarily affects the amorphous regions of the starch granule, while the crystalline regions remain protected. As a result, starch with a more crystalline structure is generated. The resultant starch retains its granular structure but exhibits reduced viscosity, swelling capacity, and increased solubility and gel strength (Gohel *et al.*, 2011).

The process of acid hydrolysis typically involves adding hydrochloric or sulfuric acid to a starch slurry under agitation at temperatures ranging from 25°C to just below the typical starch gelatinization temperature until the desired degree of hydrolysis is achieved. Afterward, the acid is neutralized, and the converted starch granules are recovered through filtration, washing, and drying (Okunlola and Akingbala, 2013). When used as a filler-binder, this modified starch demonstrates better flow and compressibility properties than microcrystalline cellulose in formulations. The compacts made from the modified starch exhibit significantly higher crushing strength and lower friability, indicating its effective capabilities as a directly compressible excipient (Khalid *et al.*, 2016).

MATERIALS AND METHODS

Materials

The sample of red sorghum (*Sorghum bicolor*) was taken from a local market in Hyderabad. The equipment used in this study includes a 100-mesh sieve, microwave oven for drying, laboratory grinding machine, hot air oven, beakers, magnetic stirrer, muslin cloth, spectrophotometry, distill water.

Isolation of Red sorghum starch

Isolation of red sorghum starch was carried out following the method described by (Singh and Sodhi, 2009). For 24 hr at 5°C, 100 g of sorghum grain was steeped in 200cc of 0.25% w/v Noah. Following steeping, the grains were rinsed and blended for three min with an equivalent volume of water. To filter the slurry, a 200-mesh screen was used. To rinse the residue from the sieve, water was utilized. This substance underwent screening and grinding three times. Cleaning and disposal of the residue were done. An hr was spent letting the filtrate stand. The filtrate underwent a 10-min centrifugation at 6000 rpm. The top layer was scraped off with a spatula; it was rich in protein and grey. Additionally, centrifugation was performed. for a further five min to resuspend the sample with more water. Until it turned white, the top layer of starch underwent numerous washings and centrifugations at 40°C, the starch was dried for 24 hr. A sample weighing 100 g was used to calculate the recovery percentage. Characteristics of Sorghum Starch.

Preparation of pre-gelatinization modified red sorghum starch

Preparation of pre-gelatinization modified red sorghum starch was done by the method of (Onimawo and Akubor, 2019). After suspending 300 g of starch in 1 L of distilled water, the mixture was gradually stirred and heated for 15 min to 80°C. Spread out the pregelatinized starch (1 cm thick) on a stainless-steel tray and dry it in a convection oven at 40°C for 48 hr. Following grinding, it was stored at room temperature (30±2°C) in airtight glass jars until it was required.

Preparation of annealed modified red sorghum starch

Annealed modified red sorghum starch was prepared following the method described by (Ikegwu *et al.*, 2011). In a sealed container in a water bath set at 50°C, 10 g of native starch and 20 mL of distilled water were combined and cooked for 24 hr After incubating the mixture for 24 hr, the suspension was After passing through Whatman No. 1 filter paper, it was hot air oven-dried for 72 hr at 40°C. In order to store the dried samples at room temperature before usage, they were first sealed in polyethylene bags.

Preparation of citric acid modified red sorghum starch

Citric acid-modified red sorghum starch was prepared according to the method described by (Zambrano and Camargo, 2002). starch slurry was prepared by dispersing 40 g (dry basis) of in a 3% citric acid solution, which was then maintained in a water bath at 45°C for 4 hr with constant stirring. Following hydrolysis, the pH of the solution was adjusted to 5.5±0.2 by gradually adding an aqueous sodium hydroxide solution (5 g/100 mL). The modified starch was washed three times with double the volume of deionized water, filtered, and dried in a convection oven at 45°C for 48 hr. The resulting dried starch was ground into a fine powder and stored in airtight containers for further use.

Physicochemical properties of native and modified red sorghum starch

Starch yield

The starch yield obtained from red sorghum starch was calculated by the following formula:

$$\text{Starch yield (\%)} = \frac{\text{isolated starch}}{\text{Total amount of raw red sorghum}} \times 100$$

Recovery yield

The recovery yield obtained from red sorghum starch was calculated by the following formula

$$\text{recovery yield (\%)} = \frac{\text{Dry weight of starch after hydrolysis}}{\text{Dry weight of starch before hydrolysis}} \times 100$$

Apparent amylose

Apparent amylose content determination was carried out using a colorimetric iodine affinity procedure briefly a mixture of 0.1 g of starch sample, 1 mL of ethanol and 9 mL 1 N sodium hydroxide boiled for 10 min in a boiling water bath and allowed to cool. To a portion (5 mL) of the mixture, 1 mL of 1 N acetic acid and 2 mL of iodine solution will add, and Absorbance (A) was read using a Spectrophotometer at 620 nm. The apparent amylose content was calculated as follows (Williams *et al.*, 1958).

$$\text{Apparent amylose content (\%)} = 3.06 \times A \times 20$$

Where A = absorbance value.

Determination of amylopectin content and amylose/amylopectin ratio

Amylopectin content and amylose/amylopectin ratio were estimated by the method given by (Iegwe *et al.*, 2018). Amylopectin content and amylose/amylopectin ratios were calculated by the following formulas:

$$\text{Amylopectin} = 100 - \text{amylose}$$

$$\text{Amylose/Amylopectin ratio} = \frac{\text{amylose}}{\text{amylopectin}}$$

Bulk Properties of native and modified red sorghum starches

Bulk density

(Eleazu *et al.*, 2014) reported that the bulk density was determined by adding 50 g of wheat sample to a graduated cylinder and measuring the volume.

$$\text{Loose bulk density} \left(\frac{\text{g}}{\text{mL}} \right) = \frac{\text{bulk mass of sample}}{\text{bulk volume}}$$

Tapped density

The flour samples were carefully put into the 10 mL graduated plastic cylinders. On a foam-covered lab bench, the cylinder's bottom was gently tapped repeatedly until the sample level stopped dropping. According to (shuma, 2023) the bulk density was calculated by dividing the sample weight by the sample volume.

$$\text{Tapped density} \left(\frac{\text{g}}{\text{mL}} \right) = \frac{\text{mass of tapped sample}}{\text{tapped volume}}$$

Carr's Index (CI)

The compressibility index was calculated using the formula provided by the method of (Schwartz, 1975).

$$\text{C.I} = \frac{\text{TD} - \text{BD}}{\text{TD}} \times 100$$

Where, C.I = Compressibility Index, TD = Tapped Density, BD = Bulk Density

Hausner's Ratio

This was calculated as the ratio of tapped bulk density to poured bulk density, as described by (Apeji *et al.*, 2011).

$$\text{HR} = \frac{\text{TD}}{\text{BD}}$$

Functional properties of native and modified red sorghum starch

Water binding capacity

The method described by (Yamazaki, 1953) was used to determine the water binding capacity of native and modified red sorghum starch. Starch (5 g, dry basis) was added to 75 mL distilled water in a tared 100 mL centrifuge bottle. The bottle was stoppered and agitated on a wrist-action shaker for one hr. It was then centrifuged for 10 min at 2,200 x g, the water was decanted, and the bottle was tipped up and allowed to drain for 10 min. more. The bottle was weighed and the amount of water held by the starch was determined. The water binding capacity was calculated from the formula below:

$$\% \text{ water absorption capacity} = \frac{\text{grams of bound water} - \text{Dry weight of the starch}}{\text{Dry weight of the starch}} \times 100$$

Oil absorption capacity

The oil absorption capacity of the samples was determined using the method of with slight modifications (Jackson *et al.*, 2018). The samples (0.5 g) were mix with 5 mL of corn oil (commercial) in reweighed centrifuge tubes. "A glass rod was used to agitate the contents for one min in order to distribute the sample throughout the oil. After being stored for 30 min, the tubes were centrifuged for 25 min at 3000×g. The separated oil layer was then removed with a pipette and the tubes were inverted for 25 min to drain any remaining oil prior to reweighing. The oil absorption capacity was expressed as gram of oil bound per gram of the sample on a dry weight basis.

Swelling power and solubility

The swelling power and solubility of the starch samples were determined using the method described by (Gani *et al.*, 2010). The flask was heated to 50, 60, 70, 80, and 90°C for 30 min in order to prepare five suspensions of starches (1% w/v). After cooling to room temperature and centrifuging for 15 min at 3000×g, shake every 5 min. After decanting the supernatant, the volume that remained was calculated. At 130°C, the solid portion was dried for 2 hr.

Color analysis of native and modified red sorghum starches

The color of the different starches was examined using a hand-held Colorimeter (CR-10, Minolta, Osaka, Japan). The CIE lab system was used to express the results (L^* , a^* , b^*). Each analysis was performed in five duplicates under the same illumination conditions and at room temperature (Syed, 2023).

Structural properties of native and modified red sorghum starch

X-ray Diffraction (XRD)

The X-ray diffraction patterns of the starch samples were determined using the method described by (Surendra *et al.*, 2014) with slight modifications. X-ray diffraction patterns of starch samples were obtained using a Powder Ray Diffractometer (Rigaku Mini Hex-II, Japan). Before obtaining the X-ray diffraction pattern, samples were conditioned to 75% relative humidity, which had an impact on the X-ray intensity of the starch. The software Powder (Chinese Academy of Sciences, Beijing) was used to smooth the graphs after they were plotted between 2θ angles of 10 and 60. Using Origin version 6.0 software (Microcal Software, Inc., Northampton, MA 01060 USA) and the % crystallinity of the starches was determined by the method of (Nara and Komiya, 1983).

FT-IR spectroscopy

The FTIR spectra of the starch samples were determined using the method described by (Surendra *et al.*, 2019) with slight

modifications. Each starch sample's infrared spectrum was obtained using an FT-IR spectrometer (Brucker, Tensor-27). The apparatus has a resolution of 2.0 cm^{-1} and a scanning range of 4000-370 cm^{-1} . Before the sample was collected, it was mixed with KBr (1:100, w/w) to acquire the background value from pure KBr.

Scanning Electron Microscope (SEM)

The SEM analysis of the starch samples was carried out using the method described by (Surendra *et al.*, 2014). with slight modifications. The starch granules were examined using a Scanning Electron Microscope (SEM) (JEOL-Model 6390, Japan). The samples were secured using a 20 nm-thick layer of coal and conductive copper double adhesive tape. It was inserted into a JEOL electron microscope using an evaporator and vacuum. In the metal ionizer JEOL, it was subsequently covered with a 50 nm-thick layer of gold. Under a microscope, this was observed and captured on camera. Film segments were positioned on aluminum stubs using double-sided tape and overlaid with a layer of gold (40-50 nm) to view the surface and cross-section. An accelerating voltage of 5Kv was used to evaluate each sample.

Statistical analysis

The samples were examined in three duplicates. The mean score and standard deviation of the three replicates for each test (for all samples) were calculated using Microsoft Excel 2023. All of the gathered data will be subjected to a One-Way Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS). The means were compared using LSD at $p < 0.05$.

RESULTS

Physicochemical properties of native and modified red sorghum starches

The Physicochemical properties of native and modified starches were presented in Table 1. The apparent amylose of native starch, PGRSS, ARSS and CMRSS was 9.71 ± 0.44 , 10.36 ± 1.46 , 8.03 ± 0.62 , and 15.66 ± 0.22 respectively. PGRSS showed a higher apparent amylose content (10.36 ± 1.46) than native starch (9.71 ± 0.44). Similar results were reported by (Sobini *et al.*, 2022) where pre-gelatinized palmyrah starch had more amylose (21.69 ± 0.34) than native palmyrah starch (18.77 ± 0.06). Likewise (Ulfa *et al.*, 2021), found that pre-gelatinized sweet potato starch had a higher amylose content (43.08 ± 1.15) compared to native starch (41.40 ± 1.62). ARSS exhibited a lower apparent amylose content (8.03 ± 0.62) compared to native starch (9.71 ± 0.44). A similar trend was reported by (Kolawole and Oluwatoyin, 2017) who found that annealed white yam starch (31.43 ± 0.74) and yellow yam starch (36.45 ± 0.22) had reduced amylose content compared to their native forms, which showed higher values of 33.54 ± 1.13 and 38.92 ± 0.17 respectively. CMRSS had a greater apparent amylose content (15.66 ± 0.22) than native starch (9.71 ± 0.44). According

to (Sobini *et al.*, 2022) citric acid palmyrah starch has a greater amylose concentration (20.42 ± 0.42) compared to native palmyrah starch (18.77 ± 0.06). The Amylopectin of native starch, PGRSS, ARSS and CMRSS was 90.28 ± 0.44 , 89.63 ± 1.46 , 91.96 ± 0.62 and 84.33 ± 0.22 respectively. PGRSS had a lower amylopectin content (89.63 ± 1.46) than native starch (90.28 ± 0.44). Compared to native starch (90.28 ± 0.44), the amylopectin of CMRSS (84.33 ± 0.22) was lower. The citric acid Palmyrah starch has a lower amylopectin (79.58 ± 0.42) than native Palmyrah starch (81.23 ± 0.06), according to (Sobini *et al.*, 2022).

Bulk properties of native and modified red sorghum starches

The Bulk properties of native and modified starches were presented in Table 2. The Bulk properties of native starch, PGRSS, ARSS and CMRSS was 0.51 ± 0.00 , 0.77 ± 0.01 , 0.50 ± 0.00 and 0.48 ± 0.00 respectively. This result is confirmed with (Verma, 2022) who stated that the bulk density of native cassava flour was $0.421 \pm 0.002\%$. The bulk density depends on the initial moisture content and particle size of the sample. Bulk density is an essential quality factor in food industries for making food products. The tapped density of native starch, PGRSS, ARSS and CMRSS was 0.64 ± 0.01 , 0.85 ± 0.01 , 0.62 ± 0.02 and 0.66 ± 0.01 respectively. This result is confirmed with (Shuma, 2023) who stated that the tapped density of native cassava flour was 0.68%. The tapped density of a powder represents its random dense packing. The low tapped density indicates the non-cohesive properties of the material. Higher tapped density is suitable to the packaging and the greater amount of material is packed with in constant unit volume.

The Carr's index of native starch, PGRSS, ARSS and CMRSS was 20.17 ± 2.36 , 9.07 ± 1.50 , 19.68 ± 3.21 and 27.97 ± 2.29 respectively. The Carr's index of CMRSS (27.97 ± 2.29) was higher than that of NRSS (20.17 ± 2.36), ARSS (19.68 ± 3.21) and PGRSS (9.07 ± 1.50).

Hausner ratio of native starch, PGRSS, ARSS, CMRSS was 1.25 ± 0.03 , 1.09 ± 0.01 , 1.24 ± 0.04 and 1.39 ± 0.04 respectively. The Hausner Ratio of CMRSS (1.39 ± 0.04) was higher than that of NRSS (1.25 ± 0.03), PGRSS (1.09 ± 0.01) and ARSS (1.24 ± 0.04).

Functional properties of native and modified red sorghum starches

The functional properties of native and modified starches were presented in Table 3. The water absorption capacity of native starch, PGRSS, ARSS and CMRSS was 88.66 ± 5.15 , 126.35 ± 0.81 , 91.7 ± 1.43 and 93.35 ± 0.72 respectively.

PGRSS exhibited a higher water absorption capacity (126.35 ± 0.81) compared to native starch (88.66 ± 5.15). This finding is consistent with (Herlina, 2017) who reported that pre-gelatinized sweet potato starch has greater water absorption capacity (5.42) than native sweet potato starch (2.32). ARSS exhibited a higher water absorption capacity (91.7 ± 1.43) compared to native starch (88.66 ± 5.15). This finding is consistent with (Kolawole and Oluwatoyin, 2015) who reported that annealed white yam starch has greater water absorption capacity (0.723 ± 0.012) than native white yam starch (0.630 ± 0.010).

CMRSS showed a higher water absorption capacity (93.35 ± 1.43) compared to native starch (88.66 ± 5.15). This conclusion is in arrangement with (Kolawole and Oluwatoyin, 2015) who reported that citric acid-modified white, water, and yellow yam starches exhibit greater water absorption capacities (0.803 ± 0.006 , 0.853 ± 0.021 , and 0.823 ± 0.021 , respectively) than their corresponding native starches (0.630 ± 0.010 , 0.823 ± 0.021 , and 0.810 ± 0.020). The oil absorption capacity of native starch, PGRSS, ARSS and CMRSS was 49.59 ± 3.85 , 52.61 ± 1.08 , 50.36 ± 4.61 and 45.5 ± 3.35 respectively. Compared to native starch (49.59 ± 3.85), PGRSS has a greater oil absorption capacity (52.61 ± 1.08). According to (Peter and Theresa, 2019) pre-gelatinized unripe banana fruit starch has a better oil absorption capacity (88.52 ± 0.04) than native unripe banana fruit starch (84.15 ± 0.21), which supports this result. Compared to native starch (49.59 ± 3.85), CMRSS has a poorer oil absorption ability (45.5 ± 3.35) (Kolawole and Oluwatoyin, 2015). That native white, water, and bitter yam starch had a higher oil absorption capacity (0.530 ± 0.044 , 0.617 ± 0.006 , 1.003 ± 0.040) than citric acid white, water, and bitter yam starch (0.437 ± 0.134 , 0.600 ± 0.030 , 1.000 ± 0.017). The oil absorption capacity of native starch (49.59 ± 3.85) was lower than that of ARSS (50.36 ± 4.61) (Kolawole and Oluwatoyin, 2015). Who reported that annealed bitter yam starch has a

Table 1: Physicochemical Properties of native and modified red sorghum starches.

Physicochemical Properties	NRSS	PGRSS	ARSS	CMRSS
Apparent Amylose (%)	9.71 ± 0.44^a	10.36 ± 1.46^{ac}	8.03 ± 0.62^{ad}	15.66 ± 0.22^b
Amylopectin (%)	90.28 ± 0.44^a	89.63 ± 1.46^{ac}	91.96 ± 0.62^{ad}	84.33 ± 0.22^b
Amylose/Amylopectin Ratio	0.10 ± 0.00^a	0.11 ± 0.01^{ac}	0.08 ± 0.00^{ad}	0.18 ± 0.003^b
Ash (%)	0.43 ± 0.22^a	0.37 ± 0.17^a	0.61 ± 0.28^a	0.34 ± 0.01^a
Moisture (%)	8.61 ± 0.52^a	8.42 ± 2.30^a	10.30 ± 0.20^a	10.15 ± 0.20^a
Dry matter (%)	91.38 ± 0.52^a	91.57 ± 2.30^a	89.69 ± 0.20^a	89.84 ± 0.20^a

Mean value followed by the different letters with subscript within the row are significantly different ($p < 0.05$). NRSS: Native red sorghum starch, PGRSS: Pre-gelatinization red sorghum starch, ARSS: Annealing red sorghum starch, CMRSS: Citric acid modified red sorghum starch.

Table 2: Bulk properties of native and modified red sorghum starches.

Bulk Properties	NRSS	PGRSS	ARSS	CMRSS
Bulk density(g/cm ³)	0.51±0.00 ^a	0.77±0.01 ^b	0.50±0.00 ^a	0.48±0.00 ^c
Tapped density(g/cm ³)	0.64±0.01 ^a	0.85±0.01 ^b	0.62±0.02 ^a	0.66±0.01 ^a
Carr's index (%)	20.17±2.36 ^a	9.07±1.50 ^b	19.68±3.21 ^a	27.97±2.29 ^c
Hausner Ratio (HR)	1.25±0.03 ^a	1.09±0.01 ^b	1.24±0.04 ^a	1.39±0.04 ^c

Mean value followed by the different letters with subscript within the row are significantly different ($p<0.05$). **BD:** Bulk density, **TD:** Tapped density, **HR:** Hausner Ratio, **CI:** Carr's index.

Table 3: Functional Properties of native and modified red sorghum starches.

Functional properties	NRSS	PGRSS	ARSS	CMRSS
WAC (%)	88.66±5.15 ^a	126.35±0.81 ^b	91.7±1.43 ^a	93.35±0.72 ^a
OAC (%)	49.59±3.85 ^a	52.61±1.08 ^a	50.36±4.61 ^a	45.5±3.35 ^a
Swelling power (g/g)	8.73±0.67 ^a	12.59±0.49 ^{bc}	10.43±0.95 ^{bde}	9.27±0.14 ^{ae}
Solubility (%)	14.18±0.59 ^a	15.40±0.65 ^a	12.48±0.73 ^b	15.06±0.65 ^a

Mean value followed by the different letters with subscript within the row are significantly different ($p<0.05$). **WAC:** Water absorption capacity, **OAC:** Oil absorption capacity.

Table 4: Color analysis of native and modified red sorghum starches.

Color analysis	NRSS	PGRSS	ARSS	CMRSS
L*	84.64±1.07 ^a	69.66±1.50 ^b	82.72±1.61 ^a	83.04±0.82 ^a
a*	2.63±0.25 ^a	4.32±1.23 ^{bc}	2.10±0.16 ^a	3.50±0.01 ^{ac}
b*	6.94±0.39 ^a	9.64±1.71 ^{bc}	6.10±0.29 ^a	7.61±0.08 ^{ac}

Mean value followed by the different letters with subscript within the row are significantly different ($p<0.05$). Lightness (L* Value), redness (a* value) and yellowness (b* value) of starches.

greater oil absorption capacity (1.053±0.047) than native bitter yam starch (1.003±0.040), corroborate this finding. The swelling power of native starch, PGRSS, ARSS and CMRSS was 8.73±0.67, 12.59±0.49, 10.43±0.95 and 9.27±0.14 respectively. Compared to native starch (8.73±0.67), ARSS had a greater swelling power (10.43±0.95). According to (Sandhiya *et al.*, 2025). annealed proso millet starch has a larger swelling power (6.85±0.23) than native proso millet starch (3.27±0.62), which supports this result. The solubility of native starch, PGRSS, ARSS and CMRSS was 14.18±0.59, 15.40±0.65, 12.48±0.73 and 15.06±0.65 respectively. Compared to native starch (14.18±0.59), ARSS (12.48±0.73%) was less soluble. According to (Ali and Hasnain, 2016) annealed white sorghum exhibits a lesser solubility (5.6±0.06) compared to native white sorghum starch (6.4±0.04), which supports this finding.

Color analysis of native and modified red sorghum starches

The color analysis of native and modified starches was presented in Table 4. The color analysis of native starch, PGRSS, ARSS and CMRSS was respectively.

Lightness (L* Value) of the starch increased after the conversion of NRSS into modified starch Whereas, redness (a* value) and yellowness (b* value) of starches significantly increased after the starch modification.

The Lightness (L* Value) of native starch (84.64±1.07) was higher than that of PGRSS, ARSS and CMRSS. The redness (a* value) and yellowness (b* value) of PGRSS (4.32±1.23) and (9.64±1.71) and CMRSS (3.50±0.01) and (7.61±0.08) was higher than that of NRSS (2.63±0.25) and (6.94±0.39) whereas lower than that of ARSS (2.10±0.16) and (6.10±0.29).

Structural Properties of native and modified red sorghum starches

The Structural properties of native and modified starches were presented in Table 5 and Figure 1. The X-ray diffraction of native starch, PGRSS, ARSS and CMRSS was respectively.

The X-ray pattern of the NRSS showed 15.02°, 17.26°, 20.23° peaks which indicates A type crystallinity. The X-ray pattern of the PGRSS showed 5.59°, 7.59°, 12.75°, 15.10°, 17.20°, 18.03°, 20.03°, 23.19° peaks which indicates C type crystallinity.

The X-ray pattern of the ARSS showed 10.05°, 15.22°, 17.20°, 18.15°, 20.14°, 23.19° peaks which indicates B type crystallinity. The X-ray pattern of the CMRSS 4H showed 11.34°, 15.10°, 17.09°, 18.15°, 20.14°, 22.84° peaks which indicates B type crystallinity.

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transforms infrared spectroscopy of native starch, PGRSS, ARSS and CMRSS was respectively (Figure 2). The FTIR

spectra are used to examine how modifications affect the unique properties of functional groups in starch molecules.

The FTIR of NRSS distinct peaks were identified in the region of 3269.87cm^{-1} represents the O-H stretch, free hydroxyl bonding of starches and it indicates the functional groups of alcohols and phenols, it denotes the functional group of nitro compounds and is 1337.07cm^{-1} , representing the N-O asymmetric stretch. The C-N stretch of a bond is represented by the values 1149.29cm^{-1} and 1076.56cm^{-1} , which denote the aliphatic amines of the functional group, 994.92cm^{-1} represents the =C-H bend the bond and it indicates the functional group of alkenes, The C-H stretch of the bond, which denotes the alkanes' functional group, is 2926.69cm^{-1} . 1643.77cm^{-1} represents the -C=C- stretch and N-H bend bond and it indicates the functional group of primary amines and alkenes, 860.59cm^{-1} represents the C-H "group" and N-H wag bonds and it indicates the functional group of aromatics, primary and secondary amines, $505.88\text{--}521.63\text{cm}^{-1}$ represents the C-Br stretch bond and it indicates the alkyl halides group.

The PGRSS of FTIR characteristics peak at 3286.95cm^{-1} denotes the alkynes and -C=C-H: C-H stretching of bond, 2925.71cm^{-1} represents the C-H stretch of bond and it indicates the alkanes group, 1643.46cm^{-1} represents the -C=C- stretch and N-H bend and it indicates the functional group of primary amines and alkenes, 1362.43cm^{-1} peak denotes the alkanes and C-H rock bond, 1148.54cm^{-1} it represents the C-N stretch and it indicates the aliphatic amines of functional group, 1077.10cm^{-1} represents the C=O stretch of bond and it shows the α , β - unsaturated, aldehydes and ketone functional groups, 993.40cm^{-1} , 930.83cm^{-1} , 858.52cm^{-1} , 760.43cm^{-1} , 705.95cm^{-1} these wavenumbers are

denotes the=C-H bend of bond and alkenes group, 521.34cm^{-1} - 570.69cm^{-1} these peaks represents the C-Br stretch and alkyl halides functional group.

The ARSS of FTIR structural characteristics peak at 3271.47cm^{-1} it represents the O-H stretch, H-bonded and O-H bond and it indicates the phenols, alcohols and primary, secondary amines and amides of functional groups, 2927.01cm^{-1} peak denotes the C-H stretch, O-H stretch and it indicates the alkanes and carboxylic acids, 1643.27cm^{-1} it denotes the -C=C- stretch and alkenes group, 1337.34cm^{-1} represents the N-O symmetric stretch and functional group of nitro compounds, 1149.25cm^{-1} it represents the stretch C-N stretch and aliphatic amines group, 994.67cm^{-1} , 860.52cm^{-1} , 763.78cm^{-1} , 707.21cm^{-1} it represents the =C-H bend of bond and alkenes group, and the peaks at 519.75cm^{-1} to 571.53cm^{-1} indicate the C-Br stretch and the functional group of alkyl halides.

The FTIR of CMRSS 4H distinct peaks were identified in the region of 3269.00cm^{-1} represents the O-H stretch; it indicates the functional group alcohols and phenols. The sharp band at 2925.5cm^{-1} represents the C-H stretch, it indicates the functional group hydrogen ring and 1643.1cm^{-1} represents the -C=C- stretch, it indicates the functional group of alkenes. The sharp band at 1336.45cm^{-1} represents the N-O symmetric stretch, it indicates the functional group of nitro compounds and 1149.29cm^{-1} represents the C-N stretch, it indicates the functional group of aliphatic amines. The sharp band at 1076.30cm^{-1} and 995.30cm^{-1} represents the =C-H bend, it indicates the alkenes and C-N stretch, it indicates the aliphatic amines. The sharp band at 860.51cm^{-1} represents the C-Cl stretch, it indicates the functional group

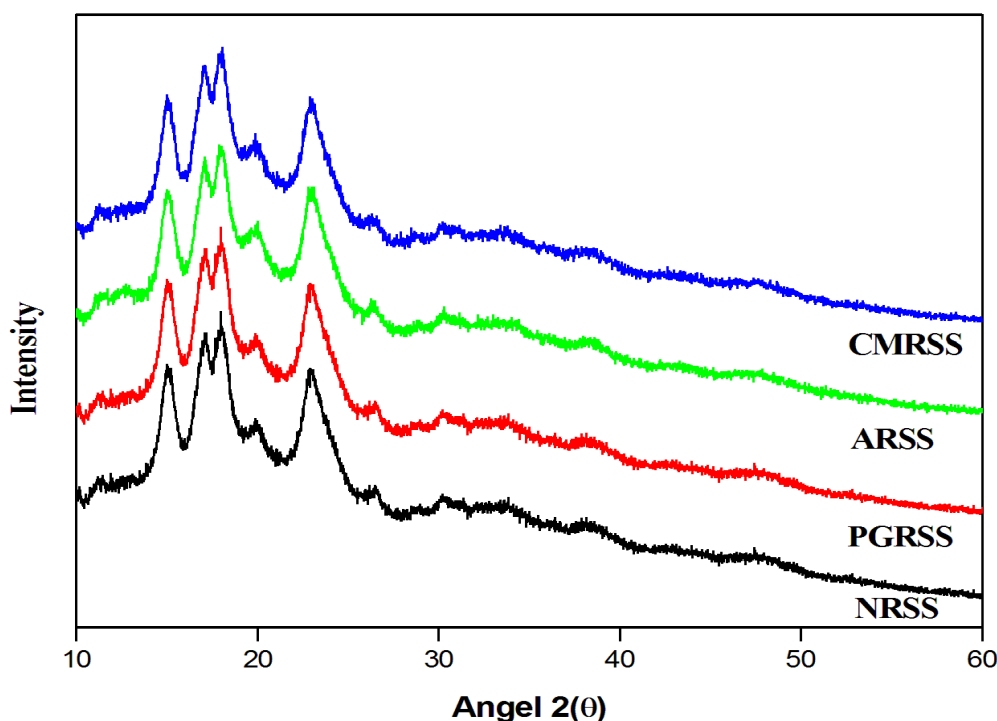


Figure 1: The XRD Spectra of native and modified red sorghum starches.

of alkyl halides. The sharp band appeared at $570.23\text{ cm}^{-1}(\text{m})$ C-Br stretch it indicates the functional group of alkyl halides groups.

Scanning Electron Microscope (SEM)

The scanning electron microscope of native starch, PGRSS, ARSS and CMRSS was respectively (Figure 3). The scanning electron microscope of native and modified starches shows the spherical, polygonal, irregular shapes, shrinking in granule size, disrupted the hydrogen bonds between starch molecules, smooth, non-porous granule surface with an oval and elongated shape.

DISCUSSION

Pre-gelatinized starch showed higher levels of amylose, total starch, and whiteness index. These changes may be due to the breakdown of starch granules. Similarly (Adedokun and Itiola, 2010), reported that pre-gelatinized rice and yam starch also have higher amylose content. Variations in the amylose-to-amylopectin ratio of native starches may result from factors such as enzyme activity during starch synthesis, genetic differences, climate conditions, cultivation practices, and the botanical source, as reported by (Odeku *et al.*, 2007). These changes may be due to partial hydrolysis and the leaching of some amylose caused by heat treatment (Adebowale *et al.*, 2009). Also reported that tempering can lead to partial hydrolysis and physical reorganization of starch without altering the granular structure.

Acid attacks the amorphous regions of dextrinized starch and breakdown its glycosidic linkages (Akin-Ajani *et al.*, 2014). According to (Sobini *et al.*, 2022) pre-gelatinization palmyrah starch has a lower amylopectin content (78.32 ± 0.34) compared to native palmyrah starch (81.23 ± 0.06), which supports this conclusion. According to (María and Alvarez, 2017) the three main processes that occur during dextrinization are glycosidic bond cleavage, glycosidic bond creation, and repolymerization.

The bulk density depends on the initial moisture content and particle size of the sample. Bulk density is an essential quality

Table 5: Structural properties of native and modified red sorghum starches.

Samples	Diffraction peaks	Crystallinity type
NRSS	A	15.02° , 17.26° , 20.23°
PGRSS	C	5.59° , 7.59° , 12.75° , 15.10° , 17.20° , 18.03° , 20.03° , 23.19°
ARSS	B	10.05° , 15.22° , 17.20° , 18.15° , 20.14° , 23.19°
CMRSS	B	11.34° , 15.10° , 17.09° , 18.15° , 20.14° , 22.84°

NRSS: Native red sorghum starch, **PGRSS:** Pre-gelatinization red sorghum starch, **ARSS:** Annealing red sorghum starch, **CMRSS:** Citric acid modified red sorghum starch.

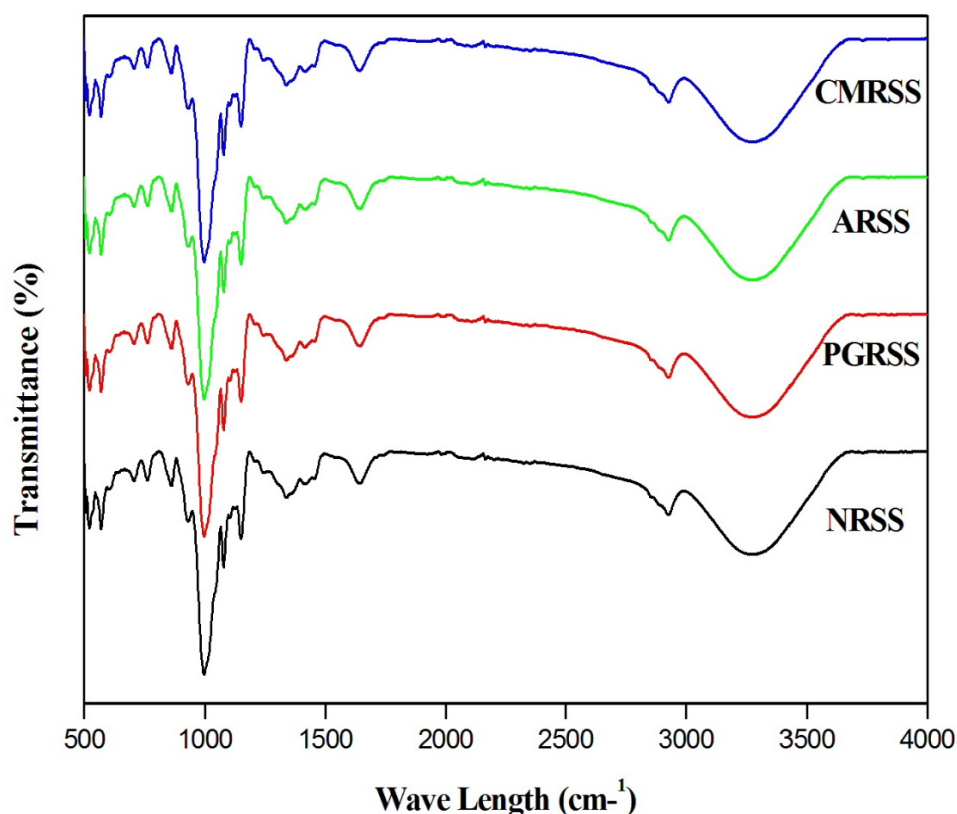


Figure 2: The FTIR Spectra of native and modified red sorghum starches.

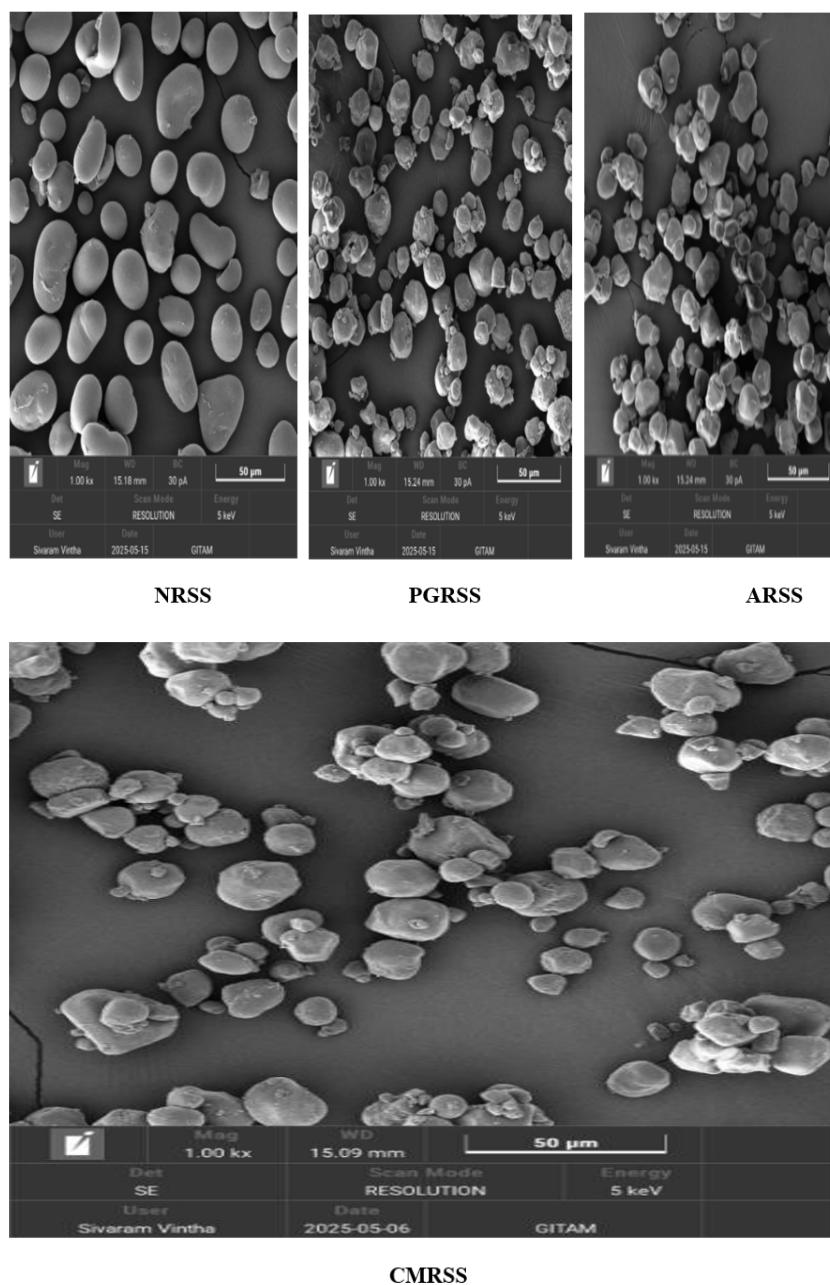


Figure 3: The SEM Spectra of native and modified red sorghum starches.

factor in food industries for making food products. The tapped density of a powder represents its random dense packing. The low tapped density indicates the non-cohesive properties of the material. Higher tapped density is suitable to the packaging and the greater amount of material is packed with in constant unit volume. Hausner value less than 1.25 indicates good flow and greater than 1.25 indicates poor flow. This result in comparison with (Verma, 2022) who stated Hausner ratio of cassava flour was at 1.68.

The ability of starch to absorb water increases with the degree of gelatinization. According to (Zhen *et al.*, 2011; Hoover and Sosulski, 1986) gelatinized starch contains more hydrophilic groups, which enhance its capacity to bind water, while increased

flour porosity further facilitates water absorption. Similarly, (Herlina, 2017) observed that pre-gelatinized sweet potato starch shows higher water absorption capacity (2.98) compared to native sweet potato starch (2.32), further supporting this result.

The WAC of the yam starches was affected in different ways by annealing; however, the WAC of native white yam starch was considerably raised; this was not the case for the other native starches. Weakened associative forces between the starch polymers in the granules of annealed white yam starch were indicated by an increased WAC. Annealing increased the WAC of African yam bean starch (Mweta, 2009) red sorghum starch (Adebawale *et al.*, 2009; Adebawale *et al.*, 2005) but had varying effects on cocoyam and sweet potato starches. Annealed starch

increased slightly compared to the water absorption capacity of the native starch. Increased water absorption capacity can be caused parts of amorphous experienced little development and few hydrogens bonding between the amorphous and crystalline broke (Adebawale *et al.*, 2005).

Compared to native and annealed starches, acid-hydrolyzed and citrate-modified starches most likely possessed more hydrophilic groups. Additionally, higher WAC could be caused by the weakening of associative interactions between the starch polymers in the granules of acid-modified starches. The modification procedures most likely enhanced the density of the hydrophobic residues on the surface of the starch granules while reducing the hydrophilic groups of the starch molecules that would have permitted water retention (Moorthy, 2002).

The OAC of native white, water, and bitter yam starches was marginally decreased by citric acid modification, but the OAC of white yam flour starches treated with citric acid increased (Akubor, 2013). According to (Morrison *et al.*, 1993) the higher density of lipophilic residues on the surface of ARSS's granules is likely the cause of its increased oil absorption ability. According to (Surendra *et al.*, 2018) the increased hydrogen bonding ability of amylose and amylopectin molecules, the altered gelatinization temperature, and the increased diffusion of water molecules to the amorphous phase could all contribute to the significant swelling power after annealing. The decreased solubility could be caused by less amylose leaching as a result of more amylose chains interacting with one another inside the starch granules on annealing.

CONCLUSION

Based on the results of this study, it is concluded both pre-gelatinization and annealing processes increase the swelling power. A Hausner ratio for PGRSS and ARSS that is lower than the standard values indicates that both materials possess good flow properties. pre-gelatinized (126.35 ± 0.81) and annealed starches (91.7 ± 1.43) was a higher water absorption capacity than native starch (88.66 ± 5.15). Pre-gelatinized (52.61 ± 1.08) and annealed starches (50.36 ± 4.61) was a higher oil absorption capacity than native starch (49.59 ± 3.85). The amylopectin content of ARSS (91.96 ± 0.62) was higher than that of NRSS (90.28 ± 0.44) and PGRSS (89.63 ± 1.46). Only the pre-gelatinization processes increased the swelling power, solubility, amylose, amylose/amylopectin ratio. Three distinct crystallinities A-, B-, and C-type can be seen in the XRD. The FTIR of NRSS distinct peaks were identified in the region of 3269.87 cm^{-1} represents the O-H stretch, free hydroxyl bonding of starches and it indicates the functional groups of alcohols and phenols. The PGRSS of FTIR characteristics peak at 3286.95 cm^{-1} denotes the alkynes and $\text{-C}=\text{C-H}$: C-H stretching of bond, 2925.71 cm^{-1} represents the C-H stretch of bond and it indicates the alkanes group. The ARSS of FTIR structural characteristics peak at 3271.47 cm^{-1} it represents

the O-H stretch, H-bonded and O-H bond and it indicates the phenols, alcohols and primary, secondary amines and amides of functional groups, 2927.01 cm^{-1} peak denotes the C-H stretch, O-H stretch and it indicates the alkanes and carboxylic acids. The scanning electron microscope of native and modified starches shows the spherical, polygonal, irregular shapes, shrinking in granule size, disrupted the hydrogen bonds between starch molecules, smooth, non-porous granule surface with an oval and elongated shape. Annealing Modification can improve the water absorption, increased crystallinity, reduced swelling Power and solubility. It can use for food industry like bread, cakes, noodles and pharmaceutical industry.

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ABBREVIATIONS

RSS: Red sorghum starch; **NRSS:** Native red sorghum starch; **PGRSS:** Pre-gelatinization red sorghum starch; **ARSS:** Annealing red sorghum starch; **CMRSS:** Citric acid modified red sorghum starch; **PGS:** Pre-gelatinization starch; **A:** Absorbance value; **XRD:** X-ray Diffraction; **SEM:** Scanning Electron Microscopy; **FTIR:** Fourier transform infrared spectroscopy; **WAC:** Water absorption capacity; **OAC:** Oil absorption capacity, **BD:** Bulk density; **TD:** Tapped density; **CI:** Carr's index; **HR:** Hausner Ratio; **SPSS:** Statistical Package for the social science; **LSD:** List significant difference; **L* value:** Lightness; **a* value:** Redness; **b* value:** Yellowness.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: KJ and DPK; Experiments: DPK; Data analysis: KJ, DPK, MM, BS, ASB, SRJ; Supervision: KJ; Funding: DPK, MM and BS; Manuscript writing: KJ and DPK. All authors Contributed to and approved the final version of the manuscript, ensuring its accuracy and integrity.

SUMMARY

The present study investigated the effects of pre-gelatinization, annealing, and citric acid modification on the physicochemical, structural, and functional properties of red sorghum starch. The modification treatments significantly influenced the functional and structural characteristics of red sorghum starch. Pre-gelatinization markedly increased the water absorption

capacity, oil absorption capacity, swelling power, and solubility, indicating enhanced hydration and functional properties. Annealing improved the flow properties of the starch, with moderate changes observed in its physicochemical characteristics. Citric acid modification resulted in the highest apparent amylose content and amylose-to-amylopectin ratio, suggesting alterations in the molecular organization of the starch. X-ray diffraction analysis revealed a transformation in the crystalline pattern from A-type in native starch to C-type in pre-gelatinized starch and B-type in annealed and citric acid-modified starches. FTIR spectra confirmed structural alterations following the modification treatments, while SEM micrographs demonstrated noticeable changes in starch granule morphology. Among the modification methods, pre-gelatinization was the most effective in improving the functional properties of red sorghum starch, whereas citric acid modification was more effective in increasing the apparent amylose content. The findings indicate that physical and chemical modification techniques can effectively enhance the functional characteristics of red sorghum starch, thereby expanding its potential applications in food and other starch-based industries.

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