

## Physico-Chemical Properties of Cross-linked and Hydroxypropyl White Sorghum (*sorghum bicolor*) Starch

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### ABSTRACT

The study investigated the effects of cross-linking and hydroxypropylation on some physico-chemical properties of white sorghum (*sorghum bicolor*) starch. The sorghum starch (NSS) was isolated and chemically modified by hydroxypropylation with propylene oxide and by cross-linking with epichlorohydrin. The swelling, solubility and other physico-chemical properties of the starch was determined. The pasting property and the granule morphology of the starches were also determined using Rapid Visco-Analyzer and Scanning Electron Microscope respectively. Changes in chemical structure of the starch molecule were confirmed by FT-IR. The amylose content (27.01%) of the native sorghum starch decreased after modifications to (20.11 and 18.81) % for cross-linked, and hydroxylpropyl sorghum starches while the starch solubility and swelling increased in the temperature range (50 – 90°C), with the native starch having the highest values. Hydroxylpropyl sorghum starch (PSS) however has better swelling power in all the temperature range when compared with cross-linked sorghum starch (CSS). The shape of the starch granules was mainly spherical and polygonal with various diameters ranging from 6 to 22 µm, however modification does not have any pronounced effect on the starch granules. Hydroxypropylation reduced the breakdown viscosity of the native sorghum starch from 1630 to 1048 RVU, the peak viscosity of sorghum starch decreased from 2779 RVU for the native starch (NSS) to 2731 RVU in CSS. In conclusion, the modifications employed improved the physiochemical properties of sorghum starch and hence the modified starch could be used industrially.

**Keywords:** Sorghum, Modification, Swelling, Starch, Granules

### Introduction

Starch is the major carbohydrate storage material in many higher plants, and after cellulose, the second largest natural biopolymer. Among food carbohydrates, starch occupies a unique position. It contributes to the physico-chemical properties of food products made from cereals, tubers, roots,

legumes, and fruits (Amaraweera *et al.*, 2021; Ammala *et al.*, 2011). Starch is formed during the process of photosynthesis in green plant (Blilck *et al.*, 2010). In human nutrition, it is the basic source of energy for the majority of the world's population, it plays a key role in supplying the metabolic energy that enables the body to perform

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its different functions and it is one of the most important raw materials used in industries.

Starch, a renewable biopolymer, is cheap, readily available and has wide application in various industries for food and non-food applications. However, the native form of starch has some limitations in its physico-chemical properties thereby reducing its wide industrial applications. Native starch contains many hydroxyl groups which are hydrophilic in nature, resulting in its moisture sensitivity. Due to amorphous (amylose) regions, starch exhibits low mechanical properties such as low tensile strength, elongation at break and brittleness (Amaraweera *et al.*, 2021). Physico-chemical properties of starch mainly depend on the amylose and amylopectin ratio.

Hence, modification is often carried out in order to reduce these limitations. This is based on the fact that various industries where starch is applicable require specific functional properties to get the desired end product. In modification, the properties of starch is improved upon to meet the requirement of end user, giving rise to a range of specialty products. Consequently, starch can be modified by physical, chemical, or enzymatic methods to promote specific functional properties (Krithika and Ratnamala 2010; Jacobs and Delcour 1998).

However, the origin of starch plays significant role in its physico-chemical properties (Amaraweera *et al.*, 2021; Singh *et al.*, 2011). It can be gotten from several botanical sources including potato, maize, wheat, sorghum, cassava, yam, rice etc (Adebowale *et al.*, 2005). Corn starch is one of the most popular cereal starches widely used in the industry and account for about 60% of the industrial starch used worldwide. Other cereal starch sources with industrial appeal include wheat, rice, etc (Singh *et al.*, 2011). Nevertheless, there are some lesser-known cereals with promising potentials for the industrial application of its starch. One of such cereal is sorghum. Sorghum is a grass specie cultivated for its grain, it

can grow under various environmental stresses and is very eco-friendly (Singh *et al.*, 2011).

Starch is one of the major industrial raw materials in plastic, food, cosmetics, textiles, adhesives,

pharmaceutical and paper industry. The demand for starch as raw material for food and non-food industry in Nigeria is rapidly increasing, this demand is currently being met largely by importation of starch from other countries; the major challenges arising from this are high cost, inadequate supply, late deliveries and transit damages. These problems can be solved by exploiting locally grown crops with good adaptations and yield efficiency as an alternative; cereal such as *sorghum bicolor* could offer this opportunity.

This study was undertaken to exploit the unique characteristics of the starch from *sorghum bicolor*. The physico-chemical, functional and structural properties of the starch were investigated.

## Materials and Methods

### *Proximate analysis of sorghum starch*

The starch was isolated using the method of Afolabi, (2012). Moisture content of the starch was determined with a moisture analyzer (Pertentecmaster). Ash, crude protein, carbohydrate, and lipid contents were determined according to Ranganna, (1987).

### *Determination of Amylose content of sorghum starch*

The method of Juliano, (1971) was employed with slight modification. 100 mg of the starch sample was weighed into a 100 mL volumetric flask. Approximately 1ml of 95 % ethanol and 9 mL of 1 N NaOH was added to gelatinize the sample and liberate amylose respectively. Iodine solution (2 mL) was added to form amylose-iodine complex and the absorbance was read at a wavelength of 620 nm (PG instruments, T60-U UV-visible spectrophotometer, USA) Standard starch solution

containing 0, 20, 40, 60, 80, 100 % amylose was prepared and transmittance read at 620 nm and standard graph plotted. Amylose content of the sample was then determined with reference to the standard curve and expressed on percentage basis.

#### ***Hydroxypropylation of sorghum starch***

The native sorghum starch (100 g) was weighed into 500 mL screw cap jar and 200 ml distilled water was added followed by the addition of 20 g of Na<sub>2</sub>SO<sub>4</sub>. The slurry was mixed for 30 min and the pH was adjusted to 11.5 with 1 M aqueous NaOH, 40 ml of propylene oxide was added and the suspension was mixed thoroughly while the jar is closed. The reaction was allowed to proceed at 40°C for 24 h. The starch suspension was neutralized with dil. HCl (0.1M) and the slurry was centrifuged for 15 min at 10,000 rpm. The starch cake obtained was washed with distilled water until it gave a negative sulphate reaction with BaCl<sub>2</sub>. The propyl starch was dried in an oven at 40°C to 10% moisture content and kept in a sealed container.

#### ***Cross-linking of sorghum starch***

Native sorghum starch was weighed (100 g) and suspended in 60 mL of distilled water, it was agitated for 2 h using high speed impeller, 2 mL of epichlorohydrin was added and the pH was adjusted to 10.5 with NaOH. The mixture was stirred for 24 h at room temperature using magnetic stirrer. At the expiration of the 24 h period the pH of the slurry was adjusted to 5.5 with acetic acid, filtered and washed with distilled water and subsequently washed with 80 % acetone. The cross-linked starch was dried in an oven at 40°C for 48 h to constant weight. It was then stored in a sealed container.

#### ***Determination of solubility and swelling power of sorghum starch***

The starch (1.0 g) was dispersed in 50 mL of distilled water; it was manually shaken and heated in a water bath at 50, 60, 70, 80 and 90 °C for 30

min, it was then cooled to 30 °C and centrifuged at 500 rpm for 15 min. The supernatant clear liquid was carefully transferred to a container of specified weight and dried at 110 °C for 4 h. The centrifuge tubes were weighed along with the sediments deposited in them after the supernatant was removed. After drying, the samples were weighed. The swelling power and water solubility index were determined using the relations below (Abdorreza *et al.*, 2012):

$$\text{Swelling power} = \frac{\text{Weight of sediment}}{\text{Sample weight} - \text{Weight of soluble}} \times 100 \quad (1)$$

$$\text{Solubility (\%)} = \frac{\text{Weight of soluble}}{\text{Weight of sample}} \times 100 \quad (2)$$

#### ***Characterization by Fourier transformed infra-red (FT-IR) spectroscopy***

The FT-IR spectra of unmodified and modified starches were obtained using Perkin Elmer spectrum one spectrometer coupled to an Auto Image light microscope. Spectra were taken with a resolution of 2 cm<sup>-1</sup>. Samples were thoroughly grounded with exhaustively dried KBr and pellets were prepared by compression under vacuum (Rajan and Emilia, 2006).

#### ***Pasting properties of sorghum starches***

The pasting properties were measured using Rapid visco analyzer (Perten RVA Tec master version 633). 2.5 g of starch samples were dispersed in 25 mL distilled water, mixed in the RVA Aluminium sample bin thoroughly and was then fitted into the RVA. The slurry was heated from 50°C to 95°C at a rate of 1.5°C/min by means of a thermoregulator. At 95°C the sample was held constant for 20 min (first holding period) while being stirred continuously. The paste was cooled to 50°C at 1.5°C/min and held at that temperature for 15 min (second holding period). The RVA recorded the viscosity continuously as each were reached. Final viscosity, trough, breakdown, setback viscosity, peak time and pasting temperature were read from the pasting profile.

### **Morphology of sorghum starches**

Granule morphology of the starches was studied by Scanning Electron Microscopy (SEM). Samples were sputter-coated with Au/Pd using a vacuum evaporator and examined using a scanning electron microscope at 10 kV accelerating voltage using the secondary electron techniques (Reddy and Bhotmange, 2013).

### **Results**

#### **Proximate composition of sorghum starch**

The proximate composition of sorghum starch is presented in Table 1. Moisture content of the sorghum starch ranges from 8.39 % -12.27%. The moisture content increased on modification to 9.38 % (PSS) to 12.27 % (CSS). The moisture level is within the level (maximum 13.50 %) recommended for safe storage (Abdullah *et al.*, 2000). When moisture content is high it could lead to microbial growth with subsequent deterioration in quality of the starch (Moothy 2002). The protein content of the starches ranges from (8.34 to 9.3), 9.34 % protein content for native starch is close to 9.50 % reported by (Awika and Rooney 2004), but higher than a number of cereals (usually below 8 %), which suggest why sorghum is considered suitable as weaning or food supplement for infants.

The amylose content of NSS (27.10 %) is higher than the value for corn starch and most cereals (Ma and Li 2011). The amylose content reduced after modification of the starch. The reduction may be due to breaking of covalent linkages by thermal degradation, and also as a result of amylose leaching. Reduction in amylose content after chemical modification of cereal starches was also reported by (Singh *et al.*, 2011; Chung and Liu 2009; Lu *et al.*, 1997) for sorghum, rice and corn starches respectively.

### **FT-IR analysis of sorghum starch**

The infra-red spectra for native (NSS) and modified sorghum starches are shown in Figures 1(a-c). The characteristic absorption frequency for starch include; O-H stretching (3214 – 3976)  $\text{cm}^{-1}$ , C-H stretching (1250 – 900)  $\text{cm}^{-1}$ . Three characteristic peaks between 998 and 1150  $\text{cm}^{-1}$  are attributed to C-O bond stretching; The peak around 2930  $\text{cm}^{-1}$  is attributed to  $\text{CH}_2$  symmetrical stretching vibration, while the peak at 1642  $\text{cm}^{-1}$  is assigned to scissoring of two O-H bonds of absorbed water molecules. A new peak at 1119  $\text{cm}^{-1}$  was observed in the PSS spectra (Figure 1c). The peak at 999  $\text{cm}^{-1}$  is associated with ether group and the intensity of the band after hydroxypropylation show that the functional groups which have a C-O-C ether linkage are newly formed. The band at 1012  $\text{cm}^{-1}$  in figure 1b is associated to an ester group which confirms cross-linking reaction in CSS. This is because both ether and ester groups exhibited strong intensity absorptions near 1000 – 1300  $\text{cm}^{-1}$ . Woggum *et al.* (2014) reported that peaks at these ranges are sensitive to moisture. The peak at 1242  $\text{cm}^{-1}$  corresponds to  $\text{CH}_3$  deformation vibration of the ester moiety (Tupa *et al.*, 2015; Woggum *et al.*, 2014). Changes in chemical structure of the starch molecule were confirmed.

#### **Swelling power and Solubility of the Starch**

The results of the swelling power and solubility of the sorghum starch is shown in Table 2. The swelling power of native and modified sorghum starches increased with increase in temperature from 50–90 °C. The swelling power increased from 3.21 to 9.61 for NSS, 3.05 to 5.05 for CSS and 3.55 to 8.08 for PSS. The swelling power of starches was in two-stage process, an initial slow swelling at a temperature range 50–60 °C followed by a significant increased swelling power as the temperature range of 60–90 °C. Hydroxylpropyl sorghum starch (PSS) had highest swelling power in the temperature range (50 – 90) °C compared with the native and cross-linked sorghum starch (CSS). The enhanced swelling capacity with

increase in temperature of the starch is as a result of increased water penetration into the amorphous region of the starch granule as temperature increased (Olayinka *et al.*, 2008). Increase in swelling power of a starch makes it suitable to be used as a disintegrant in the pharmaceutical industry (Chowdary and Enturi, 2011).

The solubility of the native and modified starches also increased as the temperature increased which indicates the dependence of solubility on temperature. The solubility increased from 3.21 to 9.61 for NSS, 3.05 to 5.05 for CSS and 3.55 to 8.08 for PSS. Increase in the starch hydrophilicity after modification facilitated water percolation into the starch granules which is capable of enhancing leaching of amylose granules from the amorphous region. Cross-linked starch exhibit lower solubility than native starch because cross-linking strengthens the boundaries between the starch chains thereby causing an increase in the resistance of the granules (Sun *et al.*, 2013; da Rosa Zavareze and Dias 2011). Cross-linking treatment adds intra and intermolecular bonds at random locations in the starch granule that stabilize and strengthen the granule (Hoover *et al.*, 2010).

The observed increase in starch solubility as the temperature increased suggests higher amylose contents due to amylose leaching during heating process (Lawal 2004).

### **Pasting Properties of sorghum starch**

The peak viscosity of sorghum starch decreased from 2779 RVU for the native starch (NSS) to 2731 RVU in CSS. Reduction in peak viscosity and cold paste viscosity following modification is a direct result of structural reorganization within the granule of the modified starches. Enhancement of crystallinity after modification limits the starch swelling and structural disintegrations which contribute to starch viscosity in a significant way. These observations are in agreement with similar reports on other starches such as pigeon pea,

smooth pea, and rice. (Puncha-arnon and Uttapap 2013; da Rosa Zavareze *et al.*, 2012).

Furthermore, set-back value, which is the difference between final viscosity and peak viscosity follows the order PSS > CSS > NSS. The maximum viscosity at a given concentration reflects the ability of the granule to swell freely prior to the physical breakdown. Starches that are capable of swelling to a high degree are also less resistant to breakdown. The decrease in breakdown value recorded can be attributed to the degradation of the starch granules, which further reduced the degree of cross-linking among the starch molecules (Hoover *et al.*, 2010).

The pasting temperature of CSS is higher than that of PSS, cross-linking increased the peak viscosity owing to increased swelling power. The substitution of the bulky functional group interferes with the nature of inter-molecular binding forces present between the starch chains and thereby weakens the starch granules. Surface erosion of the starch granule after modification may further ease the process of gelatinization. Breakdown viscosity reflects the resistance of starch granules against heat and shear; hydroxypropylation increased breakdown viscosity as compared to CSS, because degradation of amylose chains within amorphous regions of starch granule results in increased solubilisation of starch polymer. Ali and Hasnain, (2013) also reported significant reduction in the breakdown viscosity of white sorghum after modification. The pasting profiles of the starches are presented in Figures 2(a-c). Antarlina, *et al.*, (2021) classified viscosity patterns from Brabendar amylograph as follows; Type A; high swelling starches that show a high pasting peak followed by rapid and major thinning during cooking. Type B; moderate swelling starches that show lower pasting peak and much less thinning; Type C; restricted swelling starches that show no pasting peak by high viscosity that remains constant or increases during cooking; Type D; highly restricted swelling starches that do not swell sufficiently to give a

viscous paste when cooked at normal concentrations. Based on these classifications PSS are similar to Type B (characteristic of normal cereal starches), while CSS is Type C (characteristic of cross-linked or legume starches).

### Morphology of sorghum starch granules

The results of scanning electron microscope (SEM) are presented in Figure 3 (a-c). The starch granules are mainly spherical and polygonal in shape with various sizes ranging from 6 - 22  $\mu\text{m}$ . Pores are visible on the surface of the granules and the surface of the starch granule appears smooth. No damage was observed, suggesting that the method of extraction did not cause any significant damage to the starch granule. Singh *et al.* (2011) also reported the presence of pores on the surface of sorghum starches. Similar pores were reported on the surface of maize and millet starch granules (da Rosa Zavareze *et al.*, 2012).

The alkaline environment during the hydroxypropylation process accounts for the

structural changes. This result also suggests that starch crystallinity was altered and allowed the etherifying agents to have more access to the starch molecules. Changes in appearance of starch granules after hydroxypropylation have also been reported for pigeon pea starch. However, no change in surface and shape characteristics was observed.

**Table 1:** Proximate composition of native and modified sorghum starches

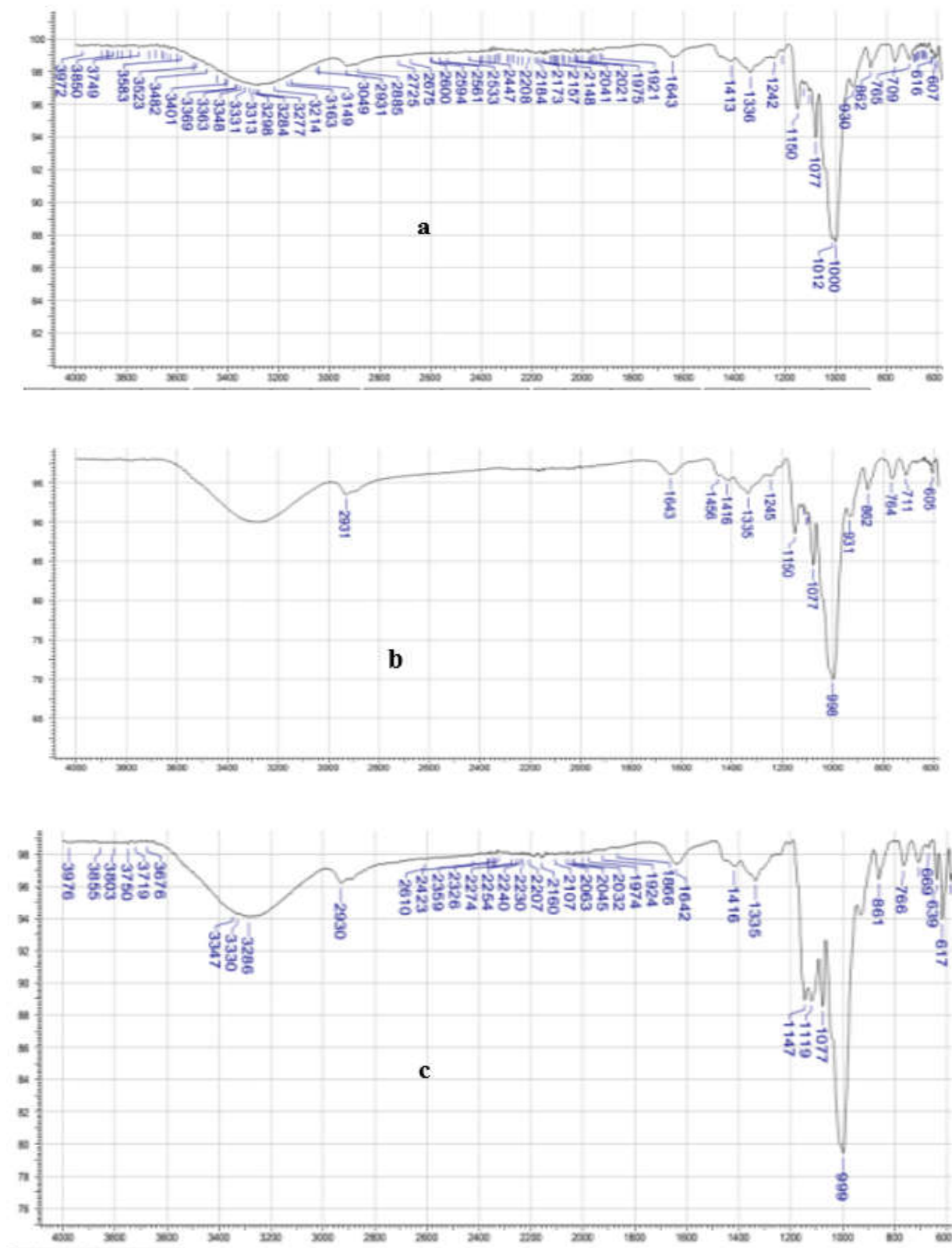
	NSS	CSS	PSS
Ash content (%)	0.45 $\pm$ 0.02	0.43 $\pm$ 0.01	0.35 $\pm$ 0.08
Moisture content (%)	8.39 $\pm$ 0.00	12.27 $\pm$ 0.07	9.38 $\pm$ 0.07
Fat content (%)	1.65 $\pm$ 0.01	1.62 $\pm$ 0.01	1.64 $\pm$ 0.08
Protein content (%)	9.34 $\pm$ 0.00	8.41 $\pm$ 0.00	8.35 $\pm$ 0.07
Crude fibre (%)	2.96 $\pm$ 0.01	2.72 $\pm$ 0.01	2.61 $\pm$ 0.01
Carbohydrate Content (%)	79.01 $\pm$ 0.01	68.51 $\pm$ 0.01	65.06 $\pm$ 0.06
Amylose content (%)	27.10 $\pm$ 0.00	20.11 $\pm$ 0.07	18.81 $\pm$ 0.01

NSS- native sorghum starch, PSS – hydroxypropylated sorghum starch, CSS – cross-linked sorghum starch

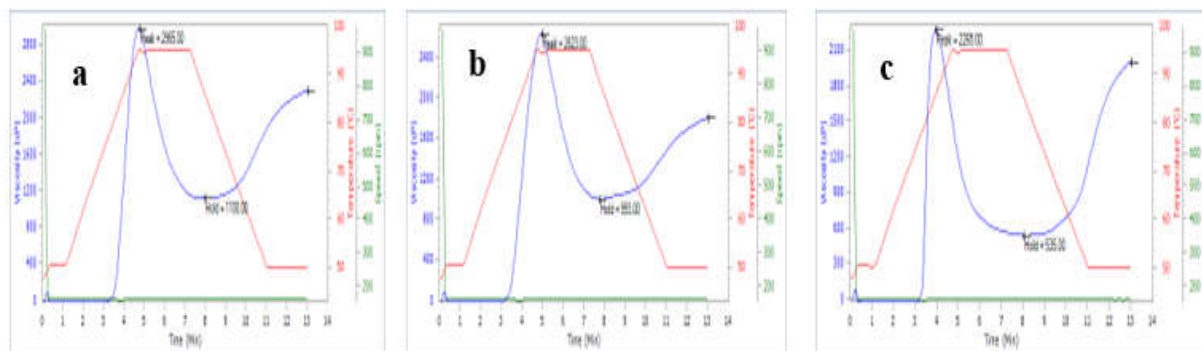
**Table 2:** Swelling and Solubility of native and modified sorghum starches

Sample/ T( $^{\circ}\text{C}$ )	50	60	70	80	90
<b>Swelling power</b>					
NSS	3.55 $\pm$ 0.02	4.35 $\pm$ 0.02	5.66 $\pm$ 0.00	7.05 $\pm$ 0.00	8.08 $\pm$ 0.01
CSS	3.05 $\pm$ 0.00	3.68 $\pm$ 0.01	4.25 $\pm$ 0.01	4.77 $\pm$ 0.01	5.05 $\pm$ 0.02
PSS	3.21 $\pm$ 0.01	4.31 $\pm$ 0.02	5.21 $\pm$ 0.02	9.11 $\pm$ 0.01	9.61 $\pm$ 0.23
<b>Solubility</b>					
NSS	2.88 $\pm$ 0.23	2.97 $\pm$ 0.01	4.11 $\pm$ 0.23	5.04 $\pm$ 0.01	5.76 $\pm$ 0.00
CSS	2.21 $\pm$ 0.02	2.75 $\pm$ 0.00	3.45 $\pm$ 0.01	3.87 $\pm$ 0.01	4.75 $\pm$ 0.00
PSS	2.08 $\pm$ 0.01	2.82 $\pm$ 0.23	5.15 $\pm$ 0.01	6.15 $\pm$ 0.01	8.17 $\pm$ 0.23

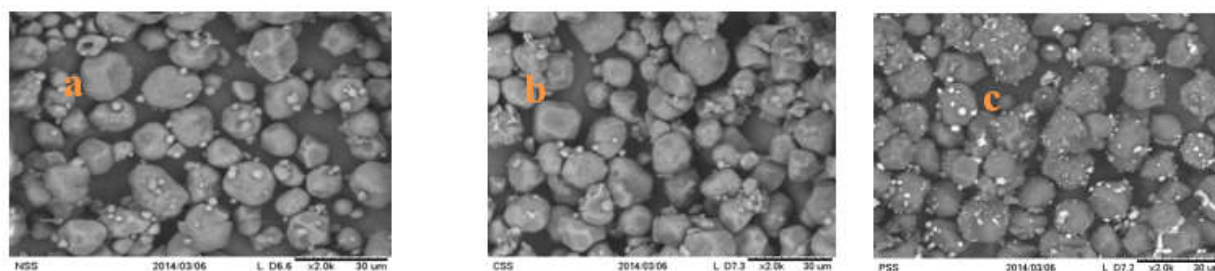
NSS- native sorghum starch, PSS – hydroxypropylated sorghum starch, CSS – crosslinked sorghum starch



**Fig 1:** FT-IR Spectra of (a) native starch, (b) cross-linked starch and (c) Hydroxylpropyl starch



**Fig 2:** Pasting profiles of (a) native starch, (b) cross-linked starch and (c) hydroxypropyl starch



**Fig 3:** SEM of (a) native sorghum, (b) cross-linked and (c) hydroxypropyl sorghum starch

## Conclusion

The study focused on the effect of modification on the physico-chemical, functional and pasting properties of sorghum starch. From the results, cross-linking and hydroxypropylation of the sorghum starch significantly improved the physico-chemical and pasting properties of the starch. Swelling and solubility of the starch reduced significantly after modification, it was observed that temperature had significant effect on the swelling capacity and solubility of the starches. The pasting properties were also affected with the reduction in final viscosity, setback and breakdown values after modification. These properties of the modified starches studied indicate that the starches may be suitable in various industrial applications such as in food products as a thickening agent, stabilizer or emulsifier and in pharmaceuticals as

carriers for controlled release of drugs and bioactive agents.

## Competing Interest

There is no conflict of interest

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