Published by The College of Science & Information Technology (COSIT), TASUED, Vol. 18, No. 2, pp. 63-71.

COSIT, TASUED
Journal of Science and Information Technology (JOSIT)

Functional and Pasting Properties of Citric Acid Cross-Linked Tigernut Starch

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Abstract

Tigernut (*Cyperusesculentus L.*) is an underutilised crop though widely cultivated in several countries. The starch was isolated and cross-linked with citric acid. The functional properties of the starch were altered and characterized. Characterization using FTIR yielded a characteristic peak at 1720 cm-1 suggesting the presence of starch citrate esters. Modification of Tigernut starch was also shown to affect its functional properties, such as swelling power, solubility and viscosity. Starch water absorption and emulsion capacity increased from 80.0 % and 15.33 % to 94.0 % and 18.34 %, respectively. Cross-linked starch (CTS) had better swelling power and solubility in all the temperature range 50 – 90°C compared with native starch (NTS). The result shows that cross-linking treatment drastically increased the lipophilic power of tiger nut starch. The formation of starch-citrate bonds accounted for the effects observed. The properties shown by the modified starch synthesized make them good candidates for application in food processing as thickening agents and stabilizers in ice screams yoghurts etc.

Keywords: Tigernuts, Starch, Solubility, Cross-linking, Characteristics, Emulsion

INTRODUCTION

Since life began polymers have been existing. Polymers such as DNA, RNA, protein and polysaccharides all have crucial roles in plant and animal life. Polysaccharides are naturally occurring polymers derived from aldoses and ketoses by polymerization of remove water. The main polysaccharides are cellulose alongside starch which is formed by green plants through photosynthesis using carbon dioxide and water and starch consists of d-glucose units.

Yet another material of industrial interest is starch. It is a natural polymer, a polymer of α-D glucose, the main energy reservoir of green plants and a diet source for humans and animals. Leaves of all green plants, seeds, fruits, stems, roots, and tubers contain starch. The granules of this starch are composed of linear amylose molecules and highly branched amylopectin molecules (Apriyanto *et al.*, 2022). The α-1,4 glycosidic bond linked amylose and the α-1,4 and α-1,6 glycosidic bond for amylopectin which is responsible for the branching of the molecule. The botanical source of the starch determines the relative proportion of amylose to amylopectin.

Starch, unlike cellulose, is more soluble in water, readily hydrolysed and readily digested. it is a semi-crystalline biopolymer that is odourless and tasteless, formed in the amyloplasts of higher plants as well as in the chloroplasts, (Chung *et al.*, 2010). There are several sources starch has been extracted from, the various source includes banana, rice, barley, maize, chestnuts, corn, wheat, millet, chickpeas, mung beans, peas, yam, cassava,

Cite as:

Ogundiran, A. A.; Ogundiran, O.O (2024). Functional and Pasting Properties of Citric Acid Cross-Linked Tigernut Starch. *Journal of Science and Information Technology (JOSIT)*, Vol. 18 No. 2, pp. 63-71.

potatoes, cocoyam and so on (Awokoya *et al.*, 2012; Bello *et al.,* 2010; Robbert *et al.*,1998; Salwa *et al.,* 2010; Bente *et al.,* 2007).

Tigernut (Cyperus esculentus) is a crop which has restricted application notwithstanding its nutritious and rich carbohydrate content (Bamigbola *et al.,* 2016; Temple *et al.*,1990). It has a starch content of 26%- 30% (Liu *et al.,* 2019). Tigernut in Nigeria is called Aya in Hausa, Ofio in Yoruba and Akiausa in Igbo.

Tigernut starch has been used in flour composite formulation due to the high amount of carbohydrate present in it (Awolu, *et al.,* 2017; Bamigbola *et al.,* 2016). The results of previous studies showed that tigernut starch has excellent gelling properties, and is also classified as slow-digesting starch which is useful in functional food formulation for lowering glucose in the blood (Lv *et al.,* 2022; Wang *et al.,* 2022).

Starch is easily produced, convenient to use, cheap and long-lasting. Starch-based films exhibit good physical characteristics; it is odourless, tasteless, colourless and impermeable to oxygen. Starch crystallinity is reduced with a high amount of amylose that is present in the starch and therefore will increase the ease of penetration of water into the starch granule (Biliaderis, 2009). However, previous studies have shown that one major shortcoming of starch as a biopolymer is its tendency to absorb water, degrade at a fast rate and poor mechanical properties (Demirgoz, 2000). Starch is often modified to circumvent these limitations.

The starch can be available for several types of modifications and may be used to broaden and increase its industrial applications (Harinder *et al.,* 2011). Starch modifications have been known for many years, in recent years there have been reports by several authors on modification of starches to a higher degree of substitution. This is because starch in its native form has some inherent limitations which includes; retrogradation, instability of paste under stress, poor paste clarity and change in its appearance during storage due to breakdown, and inability to withstand stress during processing (Lawal, 2011).

In starch modifications, structural alterations are carried out and new groups are sometimes introduced into the starch. Through modification, the functionality of starch is improved upon in order to meet up with industrial applications (Lawal, 2011).

Cross-linking is a starch chemical modification where the granules require stabilization by chemical bonds acting as a bridge between the molecules. During the cross-linking reaction, intra- and inter-molecular bonds are added at random locations, which stabilises and strengthens the granule (Majzoobi *et al.,* 2014). Cross-linked starch pastes exhibit high viscosity and show less viscosity breakdown during prolonged cooking time, additional acidity or vigorous shear (Kaur *et al.,* 2012). It has been shown to be a useful tool, delivering high film strength and reducing granule failure. This changes the paste characteristics of the swollen granules, changes the viscosity and texture (rheological properties) of the paste and can also help to reduce the sensitivity of the paste of the swollen granules to acidic environments and shear forces. It has been used to enhance starch paste film-forming properties (Singh *et al.,* 2007).

Starch cross-linking agents are most commonly bi-functional and poly-functional reagents for forming ether or other bonding forms with hydroxyl groups of starch molecules. The commonly used cross-linking agents are sodium trimetaphosphate (STMP), sodium tripolyphosphate, epichlorophydrin and phosphoryl chloride. There are two approaches to starch cross-linking: covalent and non-covalent interaction. The non-covalent crosslinking, i.e., hydrogen bonding is highly flexible and, in some cases, reversible to obtain a controllable polymer network. Starch cross-linking could be controlled by adjusting the cross-linker degree, starch concentrations, pH and temperatures (Singh *et al.,* 2007).

The cross-linking treatment of starches can be performed in a granule state or paste state. Typically, the reaction conditions are performed under neutral or basic conditions. Cross-linked starches have better textural properties and increased resistance to high temperatures, low pH, and high shear force; their mechanical thermal and chemical resistance is also higher than that of native starches (Leila *et al.,* 2019). Starch hydrogels with cross-linking have a variety of applications in the areas of food, oilfields, pharmaceuticals others.

Thus, the aim of the research was to isolate and modify starch extracted from tigernut

tubers as potential biomaterial for industrial application.

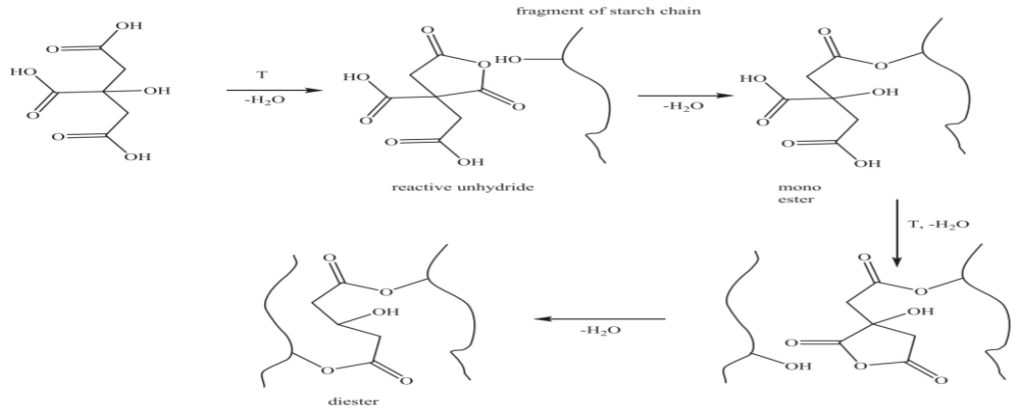

Figure 1. Citric acid Cross-linking reaction modification of starch. (Kapelko-Zeberska *et. al*, 2016).

MATERIALS AND METHOD

Tiger nuts tubers were purchased from a local market in Ijebu-Ode, Ogun state, Nigeria. All reagents used were Analar grades.

Extraction of the Starch

Tiger nuts starch was extracted using the method of Afolabi, (2012) with modifications. Tiger nut tubers were washed and soaked in distilled water for 24 h. Thereafter, the tigernut changed and then milled. The slurry was stirred and run through a muslin sieve fabric to screen the shaft away from it. The suspension thus obtained was allowed to stand for 24 h. (during this time the supernatant was replaced regularly to prevent fermentation). The starch obtained was thoroughly washed with distilled water, air dried and kept in a sealed container for further use.

Proximate Analysis

Proximate analysis of food is the determination of the major food components of food, which includes: moisture content, lipids (fats) ash, mineral, protein, carbohydrates and fibre contents. The method described by AACC (2013) was used to determine the proximate composition of the Tiger nuts.

Cross-Linking of the Starch

Tigernut starch (10 g) was incorporated in 10%, 20 mL citric acid. The synthesis was stirred well until a complete homogeneous mixture was obtained. A dilute NaOH solution was added to the mixture to adjust the pH to 3.5. The final volume of the mixture reached 50 mL. The blended solution was allowed to sit for 16 h at 28 °C, and after the time of reaction was up the mixture was filtered. The water was discarded and the solid was washed with distilled water to remove the unreacted citric acid. It was then dried at 55 °C in a hot air oven.

Fourier Transform Infrared Spectroscopy Characterisation

The FT-IR characterisation was performed using the Perkin Elmer spectrum 1 spectrometer, which was coupled to the Auto Image light microscope. The spectra were collected at 2 cm-1 resolution. The starch samples were finely pulverized with an excess of dry KBr and pellets were made by compression under vacuum.

Determination of Emulsion, Water and Oil Absorption Capacities

5 mL of the tigernut starch sample dispersion was mixed with 5 mL of vegetable oil. Homogenization of the mixture was done under 500 rpm for 1 min and the mixture was then centrifuged for 20 min. The ratio of the height of the emulsified phase to the height of the total liquid was expressed as emulsion capacity (%) (Abdul-Hamid and Luan, 2000).

$$Emulsion\ capacity = \frac{\text{height of the emulsified phase}}{\text{height of total liquid}} x\ 100$$

Water Absorption Capacity of Tigernut Starch was determined by the method of (Claver *et al.,* 2010). 1 g of the starch was suspended in 10mL distilled water and centrifuged at 4000rpm for 10 min. Water absorption capacity was determined using the equation below:

$$\textit{Water Absorption Capacity} = \frac{\text{weight of residue (g)}}{\text{weight of sample (g)}}$$

The oil absorption capacity of tigernut was determined by the method of (Ganiyat *et al.*, 2017). Vegetable oil (10 mL) and starch (1 g) were added together and allowed to rest for 30 min at room temperature and centrifuged at 3000 rpm for 15 min. The value of oil absorption capacity was then obtained using the equation below:

$$\textit{Oil Absorption Capacity} = \frac{\text{weight of residue (g)}}{\text{weight ofsample (g)}}$$

Solubility and Swelling Power of the Starch

In a test tube, a starch (1.0 g) was added to 50 mL of distilled water, shaken manually and heated in a water bath for 30 min at temperatures of 50, 60, 70, 80 and 90 °C. It was allowed to cool to 30 °C after 30 min and centrifuged at 500 rpm for 15 min. The difference between the residue weight is the swelling power of the starch. This was achieved by drying 5 mL of the supernatant to constant weight at 110 °C in an oven. The remains demonstrate the quantity that was solubilized of the starch. Solubility and swelling power were expressed in g per 100 g of the starch on a dry weight basis. The equation is expressed as:

$$\textit{Solubility} = \frac{weight\ of\ soluble\ starch}{weight\ of\ sample}$$

$$\textit{Swelling Power} = \frac{weight\ of\ sediment\ paste}{waeight\ of\ sample - weight\ of\ soluble\ starch}$$

Pasting properties of sorghum starches

The pasting properties were determined using a Rapid Visco Analyzer (Perten RVA Tec Master version 633) 2.5 g of starch samples were dispensed in 25 mL distilled water, and mixed thoroughly in the RVA Aluminium sample bin and then connected to the RVA. The slurry was heated from 50 °C to 95 °C for 2 min then cooled to 50 °C with 2min holding time. The heating and cooling were carried out at a constant rate of 11.25 min/ °C. The viscosity was recorded continuously by the RVA while the temperature increased to 90 °C which was held constant for a prescribed time before/after that the temperature was reduced to 50 °C. Final viscosity, setback, trough, peak time, breakdown and pasting temperature were read from the pasting profile.

RESULTS AND DISCUSSION

The result of the functional properties and the proximate composition of the native and cross-linked Tiger nuts starch are shown in a Table. 1. It was found that modification changed the proximate composition of the starch. The moisture content reduced to 8.05 %, the fat content and the ash content were elevated by the modification. The starch's emulsion capacities and water absorption increased from 80.0 % to 94 % and 15.33 % to 18.34% respectively. The result also proved that cross-linking treatment drastically increased the lipophilic tendency of tiger nuts starch. Similar observations were reported by Zavareze *et al.*, (2012) and Olayinka et al. (2008) for white sorghum. The water absorption capacity increased from 80% to 94 % after modification, this implies high amylopectin content in the modified starch (Alimi *et al.*, 2017) and also implies a low level of crystallinity of the crosslinked tigernut starch (Kaur *et al.*, 2011). Emulsion capacity also increased after the cross-linking which is an indication of the high fat content and it can be used as an emulsifier (Adama *et al.*, 2014).

SWELLING AND SOLUBILITY

Table 2 indicated that swelling power and solubility of Cross-linked starch (CTS) were higher than values from native starch (NTS) in all temperature ranges of 50 – 90°C tested. Cross-linking successfully stimulated the interaction within the starch chains by preventing the close networking of chains by steric hindrance and limiting the formation of inter-chain hydrogen bonds by altering the hydrophilicity of the molecules and thus influencing the bonding with water molecules. The greater the swelling power seen for a starch the lower the amylose content and the smaller the granule size (Table 2). Olayinka *et al.*, (2008) reported that acetylation led to an increase in the swelling power of the waxy rice starches due to the more open structure mainly amylopectin than in non-waxy starches, thus facilitating the rapid water penetration of waxy starches and increased swelling power and solubility. The swelling power of a starch indicates the rate at which the amorphous chain and crystalline domain interact within the starch structure (Mehboob *et al.*, 2015).

FT-IR Spectra of Tiger Nuts Starch

The infra-red spectra for native Tiger nuts and cross-linked starches are shown in Figures 2 and 3 respectively. The characteristic absorption frequencies for starch include O-H stretching (3214 – 3976 cm-1), and C-H stretching (1250 – 900 cm-1). Three feature peaks between 998 and 1150 cm-1 are attributed to C-O bond stretching; the peak around 2930 cm-1 is attributed to CH2 symmetrical stretching vibration, and the peak around 1642 cm-1 is assigned to scissoring of two O-H bonds of absorbed water molecules. After modification, there were significant changes in the intensity peaks in terms of the height and base values. The band at 1012 cm-1 in Figure 3 corresponds to an ester group which indicates the cross-linking reaction in CTS. This is due to both ether and ester groups having high absorption intensity in the range of 1000 –1300 cm-1. Thewika et al. (2014) found signals from peaks within these ranges may be moisture-laden. The peak at 1242 cm-1 is related to the CH3 deformation vibration of ester moiety. (Tupa *et al.*, 2015; Thewika *et al.*, 2014). It could be concluded that NTS was successfully crosslinked with the changes observed from the absorbance values after the modification of tigernut starch.

Table 1. Proximate Compositions and functional Properties.

Parameters	NTS	CTS
Moisture Content (%)	11.11 ±0.08	8.05 ±0.05
Fat (%)	3.76 ±0.02	4.42 ±0.02
Ash (%)	12.03 ±0.03	13.05 ±0.03
Fibre content (%)	0.1 ±0.01	0.13 ±0.01
Protein content (%)	11.47 ±0.02	12.08 ±0.12
Carbohydrates (%)	61.6 ±0.11	62.27 ±0.15
WAC (%)	80 ±0.06	94 ± 0.06
OAC (%)	63.04±0.07	88.9 ±0.06
EC (%)	15.33 ±0.05	18.34 ±0.05

NTS- native tiger nut starch, CTS- cross-linked starch, WAC- Water absorption capacity OAC- Oil absorption capacity, EC- emulsion capacity.

Table 2. Swelling Power and Solubility of Tiger nuts starch.

Temperature (℃)	50	60	70	80	90
Swelling					
NTS	2.45	2.66	5.56	6.82	7.7
CTS	2.5	3.73	6.44	8.02	8.12
Solubility					
NTS	1.82	1.85	2.24	2.42	2.84
CTS	2.25	2.43	2.5	2.54	3.02

NTS- native tiger nut starch, CTS- cross-linked starch.

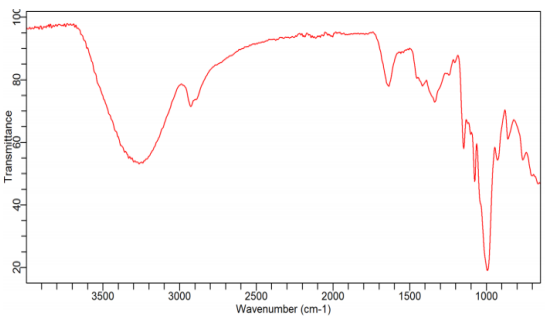

Figure 2. The FTIR spectrum of native tigernut starch.

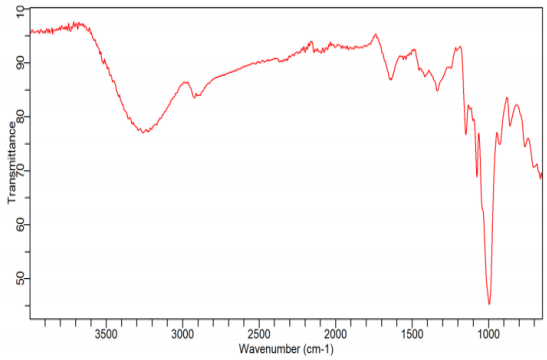

Figure 3. FTIR spectrum of cross-linked Tigernut starch.

Pasting Properties of Tiger Nuts Starch

The pasting properties are used to determine how starch behaves during and after it has been cooked. Reduction in peak viscosity to 3232 RVU for CTS from 5462 RVU for NTS (Table 3) following modification is a consequence of structural reorganization in the starch granule post-modification. The post-modification increase of crystallinity restricts alterations in the swelling and structural disintegrations of starch, which can account for a substantial portion of the starch viscosity (Zavareze *et al.*, 2011; Adebowale *et al.*, 2005). These observations are in agreement with similar reports on other starches such as pigeon peas, smooth peas, and rice (Puncha -arnon and Uttapap, 2013).

The set-back value reduced from 914.0 RVU (NTS) to 831.0 RVU (CTS) starches. The set-back value is a measure of tendency retrogradation associated with amylose and amylopectin structure. Small amylose molecules and low-chain amylopectin molecules respond quickly to retrograde (Afolabi, 2012). The reduction in set-back values obtained for the modified starch indicates a reduction in the retrogradation tendency in the starch paste. This may be explained by the diffusion of amylose chains outside the starch granules at the cooling stage. The breakdown viscosity of 1376.5 for NTS is reduced to 559.5 after modification (Table 3). The breakdown covers the difference between peak and trough viscosity and also proves that starch is stable during the heating and cooking process (Otegbayo *et al.*, 2014).

Pasting temperature is the temperature where the viscosity of the starch starts to develop. The native starch pasting temperature was 96 °C but slightly increased after to 98°C which indicates that it will require a higher temperature for it to cook during processing. However, with the lower peak time, it will require a shorter processing time. This makes the crosslinked starch suitable as stabiliser and binders. Starch pasting is a phenomenon that takes place after gelatinization in the process of starch dissolution; it is affected by parameters like amylose content, granule size distribution, granule volume fraction, granule morphology, granule–granule interaction and continuous phase viscosity (Lawal, 2011).

Table 3. Pasting properties Tiger nuts starch.

Parameters/samples	Peaks (RVU)	Through(hold) (RVU)	Breakdown (RVU)	Final visco (RVU)	Setback viscosity (RVU)	Peak time (S)	Peak tempt (°C)
NTS	5462.5	4086.0	1376.5	5000.0	914.0	5.5	96.0
CTS	3232.0	2667.5	559.5	3250.0	831.0	6.0	98.0

NTS- native tiger nut starch, CTS- cross-linked starch

CONCLUSION

Tigernut starch was effectively cross-connected with citric acid and the modification was depicted alongside the starch functional properties changes. FTIR characterization results indicated that starch citrate esters give rise to a specific peak at 1720 cm-1. However, the modification greatly influences the functional properties of tigernut starch including swelling power, solubility, and viscosity. The effects were associated with the establishment of starch–citrate linkages. The pasting properties being shown by the modified starch synthesized make them good candidates for application in food processing as thickening agents and stabilizers in ice screams yoghurts and so on.

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