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ORIGINAL ARTICLE

Green Preparation of Citric Acid Crosslinked Starch for Improvement of Physicochemical Properties of *Cyperus* Starch

Cyperus Nişastasının Fizikokimyasal Özelliklerinin İyileştirilmesi için Sitrik Asit Çapraz Bağlanmış Nişastanın Yeşil Hazırlanması

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ABSTRACT

Objectives: This study was undertaken to assess the properties of *Cyperus esculentus* tuber starch crosslinked with citric acid from liquid substrates of orange peel derived via the natural solid-state fermentation process; a green approach.

Materials and Methods: The flow properties of the prepared starches were evaluated using standard methods. Water-holding capacity, swelling capacity, moisture sorption capacity, gelatinization temperature using differential scanning calorimetry, morphology, fourier infrared spectroscopy, and pH of the starches were evaluated.

Results: Results showed that the pH of the crosslinked starches were lower (3.39-4.07) than that of the native starch (5.25) and the flow profile was found to improve. The crosslinked starches' water-holding capacity (90.67%-96.69%) were higher, whereas its emulsion capacity (15.33) was similar to that of the native starch (15.33). No change was observed in the morphology of the crosslinked starches' granules. The infrared spectra of the native and crosslinked starches showed identical peaks; however, the enthalpy of gelatinization (ΔHgel) of the crosslinked products were found to differ from that of the native starch. Modified starches show propensity of being exploited as binding agents in food and pharmaceutical industries.

Conclusion: The green modification process proved to be a valuable addition to the available starch modification processes.

Key words: *Cyperus esculentus* tuber starch, crosslinking, citric acid, orange peel, crosslinked starch

ÖZ

Amaç: Bu çalışma yeşil bir yaklaşımla, doğal katı hal fermantasyon işlemi yoluyla portakal kabuğunun sıvı substratlarından elde edilen sitrik asit ile çapraz bağlanan *Cyperus esculentus* yumru nişastasının özelliklerini değerlendirmek için yapılmıştır.

Gereç ve Yöntemler: Hazırlanan nişastaların akış özellikleri standart yöntemler kullanılarak değerlendirildi. Nişastaların su tutma kapasitesi, şişme kapasitesi, nem tutma kapasitesi, jelatinleştirme sıcaklığı, diferansiyel tarama kalorimetresi kullanılarak morfolojisi, fourier kızılötesi spektroskopisi kullanılarak değerlendirildi ayrıca pH'leri belirlendi.

Bulgular: Bulgular, çapraz bağlı nişastaların pH'nin, doğal nişastanın (5,25) pH'sinden daha düşük olduğunu (3,39-4,07) ve akış profilinin iyileştiğini gösterdi. Çapraz bağlanmış nişastaların su tutma kapasitesi (%90,67-%96,69) daha yüksekti, oysa emülsiyon kapasitesi (15,33) doğal nişastanınkine (15,33) benziyordu. Çapraz bağlanmış nişasta granüllerinin morfolojisinde hiçbir değişiklik gözlenmedi. Doğal ve çapraz bağlı nişastaların kızılötesi spektrumları özdeş zirveler gösterdi; bununla birlikte çapraz bağlanmış ürünlerin jelatinleşme entalpisinin (ΔHgel) doğal nişastanınkinden farklı olduğu bulunmuştur. Değiştirilmiş nişastalar, gıda ve ilaç endüstrilerinde bağlayıcı maddeler olarak sömürülme eğilimi gösterir.

Sonuç: Yeşil modifikasyon prosesinin, mevcut nişasta modifikasyon proseslerine katkı sağlayacağı kanıtlandı.

Anahtar kelimeler: *Cyperus esculentus* yumru nişastası, çapraz bağlama, sitrik asit, portakal kabuğu, çapraz bağlı nişasta

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INTRODUCTION

Starch has been widely sourced from plant roots, seeds, leaves, and fruits in recent past, however, modern trends in technologies and processes have moved on to adopt other sources in obtaining starch. In the development of starch, non-conventional sources with wide applicability in pharmaceutical formulations as tablet binders and disintegrants, glidants, suspending, and emulsifying agents have been exploited.[1-6]

Although starch in its native form has many potentials, its poor flow properties, instability when exposed to high temperature and under high shear rate, variations in pH, high retrogradation, its limited solubility in common organic solvents, and freeze-thaw processes make them less desirable for industrial applications.[7-9] Therefore, to find means of overcoming these limitations while developing versatile starch-based excipients that have multifunctional applications is the goal of many formulation scientists. One solution to this demanding technological need is to modify native starch properties. This involves tweaking its physical and chemical characteristics to improve its functionality with useful structural attributes via physical, chemical, enzymatic, and genetic means.[10-13]

In the last century, the most prevalent means employed in modifying starch was chemical modification, and the developed products have found several applications in the food, textile, and pharmaceutical industries.[14] This type of adaptation involves the incorporation of functional groups through chemical reactions that can alter the starch molecule or substitute some functional groups. However, the modification outcome is influenced by factors like starch source, reactant type, reaction concentration and time, and the reacting medium's pH. Chemical modification through etherification, esterification, decomposition, grafting, and crosslinking techniques yields starch products.[12]

A covalent interconnection is introduced by crosslinking through hydrogen bonds in starch granules, which provides strong bonds between molecules and restrict polymer chain movement.[15,16] This decreases the rate of starch retrogradation, which consequently increases its gel rigidity and hardness, in addition to enhancing its solubility in organic solvents.[8,17]

In the crosslinking reaction, various reagents are used, which determines the property of the crosslinked product. Hirsch and Kokini[18] showed that the reaction of waxy maize starch with phosphorous oxychloride produced starch with high viscosity and minimum swelling. In Wattanachanta et al.[19] crosslinking sago starch with a mixture of sodium trimetaphosphate and sodium tripolyphosphate (STPP) produced starch with high resistance to acids, rigid gels, and high freeze-thaw stability. The reaction of chlorinated corn starch with formaldehyde was investigated in another study and it was observed that increasing the quantity of formaldehyde while reducing oxidation extent improved the reaction process' efficiency and the products.[20]

In a completely different study, Chowdary and Chaithanya[21] found that the product was insoluble in aqueous, alkaline, and acidic media, the process of crosslinking potato starch gelatinized with urea imparted better flow on the starch. However, prolonged drug release for up to 24 h was observed in the tablet formulations produced by the crosslinked starch; thus concluding that the product could be used in the preparation of extended-release tablet formulations.

The crosslinking effect of the *Icacina trichantha* tuber starch was investigated by Omojola et al.[7] and they observed that water absorption capacity in the modified starch was higher than in the native starch, with no gelatinization occurring upon heating. They proposed that the modified starch could be a more effective tablet disintegrant than the native *I. trichantha* tuber starch. In another study by Omojola et al.[22] the reaction of *Cola nitida* starch with acetic anhydride and STPP as explored, and results showed lower viscosity, solubility, and swelling in the obtained crosslinked starch than in the unreacted starch, and was thus suggested for use as a food thickener.

Isah et al.[9] reported that *Digitaria exilis* starch crosslinked with citric acid decreases the oil and water absorption capacities, foam capacity, and pH of the modified starch. By using scanning electron microscopy, they observed that the starch granule's shape and morphology were not altered by crosslinking; whereas the presence of an additional peak was shown using fourier transform infrared (FTIR). This suggested the use of the crosslinked *D. exilis* starch in the food and pharmaceutical industry.

In the crosslinking process, agents like citric acid have been commonly used. Citric acid is a very essential organic acid that is widely utilized in the food and pharmaceutical industries. It is an intermediate of the tricarboxylic acid cycle, and is found in a variety of acidic fruit juices, particularly the citrus family.[23]

Citrus fruits are readily available in most regions of the world. Annually, after the extraction of the citrus juice, processing industries generate tons of residues, including peels and segment membranes, for commercial purposes. However, these wastes are usually not put to use/reasonable use, thus creating environmental hazards. For example, the burden already present at landfills where solid wastes are deposited is increased; whereas liquid wastes disposed into the rivers could cause nutrient imbalance, loss of oxygen, and, consequently, death of the aquatic life. Failure to exploit these wastes' potential could also impact negatively on the economy. Citric acid, with an estimated annual production of about 10,000,000 tons, has one of the highest level of production worldwide among fermentation products.[24]

In recent years, various manufacturing processes and products with marginal likelihood of causing harm have been explored by researchers and scientists while also improving the sustainability of product development.[25,26] This can be achievable through the green approach system, which, in addition to reducing waste, entails manufacturing processes that create technologies that are safer for the environment and those working in those environment. Innovations through green synthesis has led to the development of diverse products for different applications, including the production of citric acid by fermentation of citrus byproducts using microorganisms.[27,28]

Submerged, surface, and solid-state are the different techniques employed in the production of citric acid through fermentation, with the latter being the simplest of all the techniques.[29,30]

To obtain citric acid from fruit wastes, we intend to use the green approach, which would serve as a precursor for the development of a pharmaceutical excipient. Therefore, this study aims to crosslink starch obtained from *Cyperus esculentus* seed using liquid substrates of orange peel derived by means of the natural solid-state fermentation process, and evaluate its physiochemical properties.

MATERIALS AND METHODS

Materials

Tigernut starch (TS) from *C. esculentus* tuber [extracted from the Department of Pharmaceutical Technology and Raw Material Development, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria] and oranges (obtained from Karmo market, Abuja, Nigeria).

Orange peel substrate preparation

Fresh oranges were procured from the Karmo market in Abuja. They were washed with water, and the skin was separated from the fruit's pulpy segments. Orange peel of 900 g was soaked in 2.5 L of water, and the mixture's pH was recorded. The soaked peels' pH was subsequently recorded on days 1, 5, and 15.

Starch preparation

The method of Kunle et al.[31] was used to extract the TS.

Preparation of modified starch

The procedure described earlier[32] was adopted with some modifications. On day 5, the orange peel substrate's pH was determined, and 50 mL of the filtered substrate was poured in a beaker. Placed in another beaker was 50 g TS, where the filtered substrate was then added in aliquots and mixed until a smooth paste was obtained. The resulting paste was spread on a tray and left to dry at room temperature for about 18 h. The air-dried product was then oven-dried at 60°C for 1 h and 30 min. Using a mortar and pestle, the oven-dried product was size-reduced and after which it was further dried in the oven for 2 h at 130°C. The product (TS1) was then cooled, weighed, and then stored in the desiccator for further analysis.

Other products of the modified starch (TS2 and TS3) were prepared according to the procedure previously stated using orange peel substrate obtained on days 10 for TS2 and 15 for TS3.

Physicochemical properties evaluation of the modified starch

Morphology

Under a light microscope (Leica DM light microscope) at a magnification x400, a small quantity of TS was mounted on the microscope slide and was examined. The starch product's shape was extrapolated from the photomicrograph.

pH determination

The pH of a 10% w/v slurry of the starch product was determined at room temperature (28°C) using the pH meter. Triplicate determinations were made and the mean was computed.

Angle of repos

The funnel method was used; 20 g each of the dried products (TS0, TS1, TS2, and TS3) was allowed to flow through a funnel clamped at a fixed height from a flat surface. The powder heap's height (h) and radius (r) were measured, and the repose angle (A) was calculated as follows,

$A = \tan - 1\ {}^{h}/_{r}$.

Bulk and tapped densities

In a graduated cylinder, the volume occupied by the 20 g starch product was noted; and the bulk density (g/mL) was calculated as the ratio of the powder's weight to the volume occupied in the cylinder. Similarly, the tapped density (g/mL) was computed as the ratio of the powder's weight to the volume it occupied after tapping the cylinder 100 times.

Carr's compressibility index (CI) and Hausner ratio (HR)

Carr's CI, %) was calculated as follows:

$^{(\text{tapped density-bulk density})}/_{(\text{tapped density})} \times 100$.

HR was computed as the ratio between the tapped and bulk densities.

Swelling capacity

A slurry of the starch product (1% w/v) was made in distilled water. With intermittent stirring, the dispersion was heated on a water bath (Karl kolb, Dreiech West Germany) at 30°C for 30 min. This was then centrifuged at 1500 rpm for 30 min; the supernatant was discarded, the starch paste weight was determined, and the swelling capacity was calculated as follows:

$S\ (\%) = {}^{(\text{weight of wet starch})}/_{(\text{initial weight of dry starch})} \times 100$.

This procedure was carried out for each of the starch products (TS0, TS1, TS2, and TS3) at 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, and 90°C.

Gelatinization temperature

Differential scanning calorimetry (DSC) was used to determine the gelatinization temperature. The starch samples (5 mg) were placed in an aluminum pan, and a drop of water was added. At a heating rate of 10°C under constant nitrogen flow, the samples were scanned between 60°C and 300°C.

Water-holding capacity

To obtain the water-holding capacity, the method of Kornblum and Stoopak[33] was adopted. The crosslinked starch dispersion (1% w/v) was placed in preweighed, stoppered centrifuge tube and shaken intermittently for 10 min and was left to stand for another 10 min. It was then centrifuged at 1,500 rpm for 5 min; the supernatant was discarded and the weight of the hydrated starch was determined. Hydration capacity was calculated using the equation below,

$H\ (\%) = {}^{(\text{weight of dry starch})}/_{(\text{weight of starch sediment} \times 100)}$.

Foam capacity

For foam capacity, the method by Isah et al.[9] was adopted. The starch product's water dispersion of 2% w/v was homogenized for 5 min. It was poured into a measuring cylinder (50 mL), and the volume was recorded after 30 s. The percentage increase in volume expressed is the foam capacity.

Emulsion capacity

Emulsion capacity was also carried out based on the method of Isah et al.[9] The starch product was dispersed in water (2 % w/v) and homogenized for 2 min. After which, vegetable oil (2.5 mL) was gradually added to the mixture and stirred continuously for 30 sec. The suspension was centrifuged at 1,600 rpm for 5 min, and the volume of oil floating was determined. The computed quantity of oil emulsified per gram of the sample is the emulsion capacity.

Moisture content

The oven-dry method was used to determine moisture content. The starch product (0.5 g) was placed into a dry crucible (W_1) and was placed in an oven at for 10-12 h at 100-105°C until constant weight was obtained. The crucible was then allowed to cool in a desiccator for 30 min and was reweighed (W_2). Moisture content (%) was calculated as follows:

W_1-W_2 / W_1 x100.

Moisture sorption capacity

The starch product (1 g) was placed in a tarred porcelain dish and was weighed (W_1). The dish was then placed in a desiccator containing distilled water. The sample and dish were weighed daily (W_2) for 5 days, and the moisture sorption capacity was determined as follows,

W_1-W_2 / W_1 x 100.

FTIR spectral studies

Native TS and the crosslinked products (TS1, TS2, and TS3) were triturated with potassium bromide and were made into pellets (1 ton/cm^2). The Nicolet iS10, Thermo Scientific (USA), FTIR spectrometer was used to take the IR spectra between the scanning ranges of 4,000 and 400 cm^{-1}.

Statistical analysis

Results were obtained from at least three determination and were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Organoleptic properties of TS and its crosslinked derivatives

Table 1 shows the organoleptic properties of TS and its crosslinked products. Native TS (TS0) was a white, tasteless, and odorless smooth powder, whereas the crosslinked productsthat had been reacted with orange peel substrates from days 5 (TS1), 10 (TS2), and 15 (TS3) were observed to be off-white in color, odorless with a characteristic taste, and is coarse to touch. The color change observed in the crosslinked products can be attributed to the color of the orange peel substrate used.

Table 1. Organoleptic properties of Tigernut starch products (TS0, TS1, TS2, and TS3)

Parameters	TS0	TS1	TS2	TS3
Color	White	Off-white	Off-white	Off-white
Odor	Odorless	Characteristic	Characteristic	Characteristic
Taste	Tasteless	Bland	Bland	Bland
Texture	Smooth	Coarse	Coarse	Coarse

Number of determinations (n)=6, TS: Tigernut starch

pH of orange peel substrate

Table 2 presents the pH of the orange peel substrate on days 0, 5, 10, and 15. Results show that during the study period, the substrate's pH was not consistent; the pH decreased to 2.00 by day 5 and increased to 2.79 by day 10 but decreased again to 2.30 by day 15. This could be a result of fermentation where microorganisms use carbohydrates from the substrate as a source of energy, thus influencing the substrate/medium's chemical environment. This process causes lactobacillus growth, which produce organic acids that lowers the medium's pH. The absence of a trend in the substrate's pH could be associated with the fact that microorganisms consumed the nutrients from the orange peels at the beginning fo the fermentation process and released organic acids into the medium, thus lowering the pH; however, shortage of these nutrients after a period of time could have resulted in the consumption of the organic acids, which led to an increase in the medium's pH. Because the initial decrease in pH is attributed to citric acid production, it implies that the crosslinking effect on starch products treated with substrate on day 5 (TS1) would be greater than that on day 10 (TS2) when the pH was observed to increase again. Factors that influence the medium's pH kinetics are the substrates' nature in the fermentation medium and the method by which the organic acids are produced.[34]

Flow properties of TS and its crosslinked derivatives

Flow properties results of TS products (TS0, TS1, TS2, and TS3) are shown in Table 3. A material's repose angle is a reflection of its flow ability; values less than 30° are an indication of excellent flow, whereas those between 31° and 35°, 36° and 40°, and those greater than 40° are indicative of good, fair, and poor flow, respectively.[35] Obtained values for TS0 (33.06°) and TS1 (34.66°) were observed to be similar, showing that they possess good flow; whereas TS2 (26.96°) and TS3 (30.39°) were lower, which reveal that they have good to excellent flow ability.

Table 2. pH of orange peel substrate

Day	pH
0	5.23±0.05
5	2.00±0.02
10	2.79±0.01
15	2.30±0.02

Number of determinations (n)=3, ± standard deviation

An increase in citric acid probably resulted in better modification as a substitution result of more OH groups on starch, leading to a less amorphous and better flowing product. Carr's CI is a parameter that assesses a material's ability to deform under pressure; whereas HR measures powdered material's cohesiveness by determining that material'sthe densification degree.[35] A material has excellent, moderate, or fair flow when CI ≤10%, CI: 16%-20%, or CI: 21%-25%, respectively. Consequently, the material is cohesive when HR ≤1.11, and become less cohesive when values are between 1.12 and 1.2.

The results presented in Table 3 show that increasing the fermentation period improved the flow of the crosslinked starch products (TS1, TS2, and TS3), with TS3 having lower CI (25.24%) and HR (1.34) than TS2 (CI, 29.24%; HR, 1.42) and TS1 (CI, 30.36%; HR, 1.45). Moreover, these values were also observed to be lower than those obtained for native starch (32.13% and 1.47), indicating that treating the starch with the orange peel substrate improved the flow of TS.

This indicates that when pressure is applied during tablet compression, the crosslinked starch (TS3) would produce good compacts. It also shows that there would be reproducible product manufacture and performance with consequent uniform drug distribution in the tablet batch.

Physicochemical properties of TS and its crosslinked derivatives

The pH of native TS (TS0, 5.25) was similar to that reported (5.60) by Kenneth et al.[36] in their characterization of TS. For the crosslinked products (TS1, TS2, and TS3), the values obtained were found to be lower (4.07, 3.39, and 3.87, respectively) and could be linked to the varying pH of orange peel substrate used. This may also be attributed to the presence of carboxylic acid on the starch molecule leading to increased acidity.

However, these values were within the range (3-9) specified for most starches used in pharmaceutical, cosmetics, and food industries.[37] This result is also similar to some earlier reports[7,34] where the pH of *I. trichantha* tuber starch and *Tacca involucrate* tuber starch crosslinked with citric were found to be 4.59 and 4.68, respectively. In addition, pastes from various modified starches have been documented to be acidic, therefore, the results of this study is consistent with earlier reports.[38-40]

Presented in Table 4 are the water-holding capacities of TS products. The crosslinked products' water-holding capacity (TS1, TS2, and TS3) were observed to increase with the orange peel substrate's length of stay. They were also found to be appreciable (91.37%, 90.37%, and 96.69%, respectively) compared with the native starch TS0 (85.55%). Water-holding capacity is a material's capacity to retain water when pressure is applied or when heated; it is also the measure of that material's hydration capacity.[41] The observed increase in water-holding capacity may be ascribed the carboxyl groups' attachment on the starch molecules, thus, improving its ability to imbibe water. Specifically in the application of starch products as disintegrants in solid dosage formulations or as drug carriers for controlled drug delivery systems, this phenomenon is desirable.[39] This result is in tandem with those of *Icacina* and *Tacca* starch citrate.[7,32] In Jyothi et al.,[42] cassava starch citrate was also reported to have higher water-holding ability than the native cassava starch.

Foam and emulsion capacities are vital functional properties that determine the behavior and applicability of these starches in processing, consumption, and storage. The crosslinked products' foam capacity were observed to be the same (3.33) but slightly higher than that of the native starch (3.07). On the other hand, their emulsion capacities were found to be the same (15.33%), indicating that their emulsifying property was not affected by starch modification. These results demonstrate the modified starches' ability (TS1, TS2, and TS3) to decrease

Table 3. Flow properties of Tigernut starch products (TS0, TS1, TS2, and TS3)

Parameters	TS0	TS1	TS2	TS3
Angle of repose (°)	33.06±1.79	34.66±1.05	30.39±1.01	26.96±1.02
Bulk density (g/mL)	0.46±0.01	0.49±0.01	0.48±0.01	0.50±0.01
Tapped density (g/mL)	0.68±0.01	0.71±0.02	0.68±0.01	0.67±0.01
Hausner ratio	1.47±0.02	1.45±0.01	1.42±0.04	1.34±0.03
Carr's index (%)	32.18±1.03	30.36±1.57	29.24±2.06	24.25±1.30

Number of determinations (n)=3, ± standard deviation, TS: Tigernut starch

Table 4. Physicochemical properties of Tigernut starch products (TS0, TS1, TS2, and TS3)

Parameters	TS0	TS1	TS2	TS3
pH	5.25±0.02	4.07±0.02	3.39±0.06	3.87±0.02
Water-holding capacity (%)	85.55±1.05	91.37±5.42	90.67±3.40	96.69±1.88
Foam capacity (%)	3.07±1.01	3.33±1.15	3.33±1.15	3.33±1.15
Emulsion capacity (%)	15.33±0.58	15.33±0.58	15.33±0.58	15.33±0.58
Moisture content (%)	8.00±0.30	7.80±0.10	8.10±0.10	9.20±1.00
Moisture sorption capacity (%)	5.87	5.42	5.84	5.54

Number of determinations (n)=3, ± standard deviation, TS: Tigernut starch surface tension, and as such may be used as emulsifiers in food, cosmetic, and pharmaceutical products.

The crosslinked starches' moisture content increased with the increase of the crosslinking substrate's concentration (7.8%, 8.1%, and 9.2%, respectively) and were generally comparable with that of the native starch (8%). These values are within the limit specified for pharmaceutical starches[43] as presence of excessive moisture can impact negatively on the flow and mechanical properties of the material.[44] In addition, it can promote microorganism growth in the formulation during packaging and storage and decrease the formulation's stability and shelf-life.[45]

Moisture sorption capacity is a measure of moisture sensitivity and an indication of a material's physical stability under storage.[46] The crosslinked starches showed similar sorption capabilities (5.42%, 5.84%, and 5.54%, respectively) with the native starch (5.87%). Materials with low values less tendency to undergo hydrolytic degradation, thus, are beneficial in formulations containing moisture-sensitive drug components.

Table 5 shows the swelling capacity of TS0, TS1, TS2, and TS3 over a range of temperatures (30°C-90°C). Starch granules' ability to absorb water when exposed to heat is portrayed by its swelling characteristics. Swelling over different temperature ranges is important because it evaluates the starches' behavior under industrial conditions. Although swelling was found to be higher at higher temperatures (60°C-90°C) than at lower temperatures (30°C-50°C), the results show a general increase in starch volume as the exposure temperature increased for the native and crosslinked starches. This pattern is ascribed to intrinsic binding forces that tend to weaken at varying temperatures,[47] and as reported by Omojola et al.[48] However, an exception to this trend was observed with TS2, where swelling was observed to appreciably increase at 80°C but decreased at 90°C. TS1, TS2, and TS3 showed similar swelling patterns at 30°C, 40°C, 50°C, and 60°C. However, less swelling was observed in TS2 at 70°C than TS1 and TS3; at 90°C, no further swelling was observed in TS2 and TS3. Of all the starches, TS1's swelling profile was observed to be the most consistent (progressively increasing).

Table 5. Swelling power Tigernut starch products (TS0, TS1, TS2, and TS3)

Temperature (°C)	TS0	TS1	TS2	TS3
30	2.33±0.43	2.38±0.11	2.09±0.07	2.55±0.03
40	2.52±0.11	2.79±0.09	2.64±0.17	2.70±0.43
50	2.44±0.19	2.43±0.17	2.22±0.04	2.63±0.09
60	2.68±0.13	2.69±0.05	2.54±0.17	2.97±0.17
70	5.59±0.40	5.53±0.14	3.56±0.10	5.22±0.42
80	8.77±0.93	8.03±0.08	8.00±0.30	8.03±0.11
90	7.44±0.34	9.18±0.76	7.35±0.24	8.01±0.17

Number of determinations (n)=3, ± standard deviation, TS: Tigernut starch

Nonetheless, the swelling profile of the native and crosslinked starches were similar. This suggests that crosslinking did not have any effect on granule swelling and could be attributed to increased resistance to starch granule swelling because of higher bond strength conferred by crosslinking, which has also been reported by Xie and Liu.[49]

These starches may not be good disintegrant candidates for solid dosage formulations because of their limited water uptake into starch granules, which implies that structural granule break down will not take place. However, our result is different from those of Adebiyi et al.,[32] where *Tacca* starch citrate was observed to have higher swelling power than that of the native *Tacca* starch. This can be attributed to differences in the biological sources of starch materials and starch extraction methods employed.

Figure 1 illustrates the morphology of the native and crosslinked starches. The native starch (TS0) have small and large-sized, oval-shaped, smooth granules occurring mostly in single un-aggregated forms. The crosslinked starches (TS1, TS2, and TS3) are more uniform in size, oval-shaped, and occur in aggregates that are observed to increase with the time of crosslinking (TS1 > TS2 > TS3). Here, application of the orange peel substrate to modify TS did not rupture or change the granule morphology, which shows that under applied pressure resulting in higher bond strength, modified starch would pack more closely and consolidate better than the native starch. Starch granules with small and uniform sizes are widely used in pharmaceutical and food industries,[50] as such, this cheap substrate crosslinked starches could find application in these industries.

FTIR of TS and its crosslinked derivatives

Figure 2 presents the FTIR spectra of the starch products. The native starch shows significant peaks at 3240 cm^{-1}, whereas those of TS1, TS2, and TS3 are 3257.7, 3235.3, and 3250.2 cm^{1}, respectively, all indicating OH stretching. The band 2929.7 cm^{-1} of the native starch is characteristic of C-H stretches associated with ring hydrogen atoms and was observed to slightly decrease to 2926 cm^{-1} in all the crosslinked products. The change in

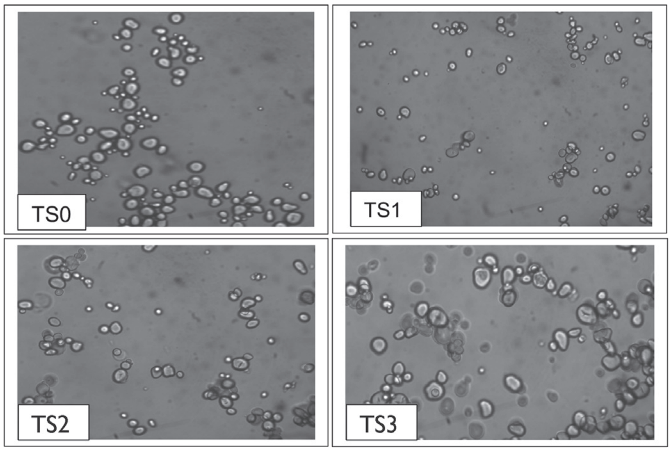

Figure 1. Photomicrograph of native Tigernut starch (TS0), crosslinked starches from day 5 (TS1), day 10 (TS2), and day 15 substrate (TS3)
TS: Tigernut starch

intensity in the C-H stretch could be because of the possible change in starch's amylose and amylopectin content, which is known to be responsible for variation in the physicochemical properties of starch. All the starches showed peaks at about 1640, 1338, and 1140 cm^{-1}, indicating the presence of intra-molecular hydrogen bonds, C-H bending, and C-O stretching. Bands at 708-928 cm^{-1} portray the characteristic identity of the starch.[50]

DSC of TS and its crosslinked derivatives

Presented in Figure 3A-C are DSC thermograms of the behavior of the starch granules of all the samples (TS0, TS1, TS2, and TS3) when subjected to heat. A series of processes that occur when starch is heated and converted to paste is referred to as starch gelatinization; and the temperature at which gelatinization occurs is dependent on the starch molecules' crystalline nature. This transitional phase of starch granules is an important characteristic that makes them applicable in industrial processes.

Thermal properties of the native and modified starches are presented in Table 6. The onset temperature of gelatinization (T_o) of TS0 is observed to be lower (60.35°C) than those of TS2 and TS3 (104.16°C and 113.18°C, respectively). This is attributable to increased granule strength conferred as a result of crosslinking, thus higher temperature is required to initiate starch gelatinization. Increase in gelatinization temperature also indicates better binding ability of the starch, which shows that crosslinked starches possess better binding capability than native starch. Some studies have also reported an increase in gelatinization temperature with other crosslinked starches, relating to decreased mobility of amorphous chains in the starch granule.[16,38] Similarly, peak (T_p) temperature and conclusion temperature (T_c) of the modified starches were observed to be generally higher than those of TS0 (125.59°C and 134.44°C, respectively). This suggests that modified starches acquire more crystalline forms than the native starch; this is in tandem with literature where positive correlation between onset and peak temperature of gelatinization and the materials' amorphous/crystalline nature have been reported.[50,51]

The enthalpy of gelatinization (ΔH) is directly related to the starch granules' crystalline nature (amylopectin), and demonstrates the extent to which the bond order within the

Table 6. Differential scanning calorimetry (DSC) parameters of the native and modified starches

Parameter	TS0	TS1	TS2	TS3
Onset temperature (°C)	60.35	59.84	104.16	113.18
Peak temperature (°C)	125.59	141.60	124.47	132.88
Conclusion temperature (°C)	134.44	293.41	129.52	132.92
Enthalpy of gelatinization [J/(g*K)]	1926.95	2120.73	488.44	1096.20
ΔT (°C)	74.09	233.57	25.36	25.74
Peak height index (PHI)	26.01	9.07	19.26	42.59

TS: Tigernut starch, ΔT: Gelatinization temperature range

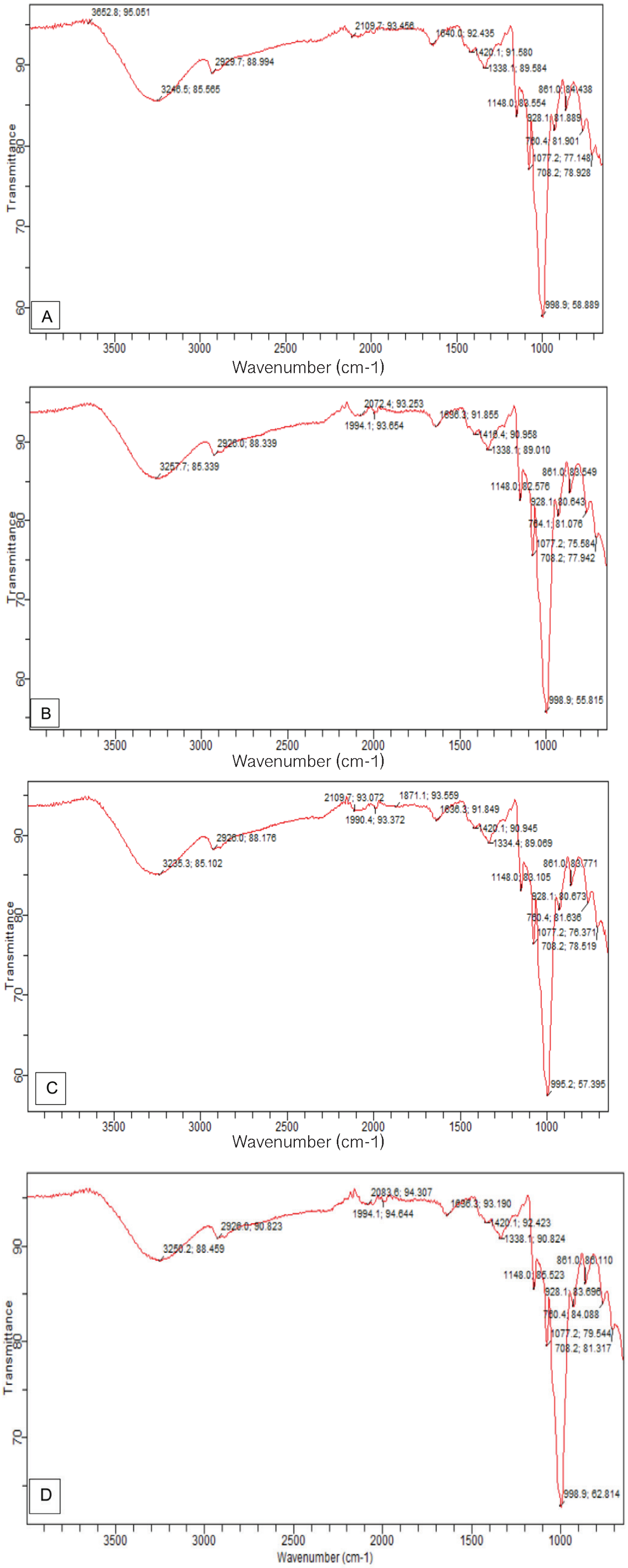

Figure 2. Fourier transform infrared (FTIR) spectra of (A) native Tigernut starch, (B) crosslinked starch from day 5 substrate, (C) crosslinked starch from day 10 substrate, (D) crosslinked starch from day 15 substrate

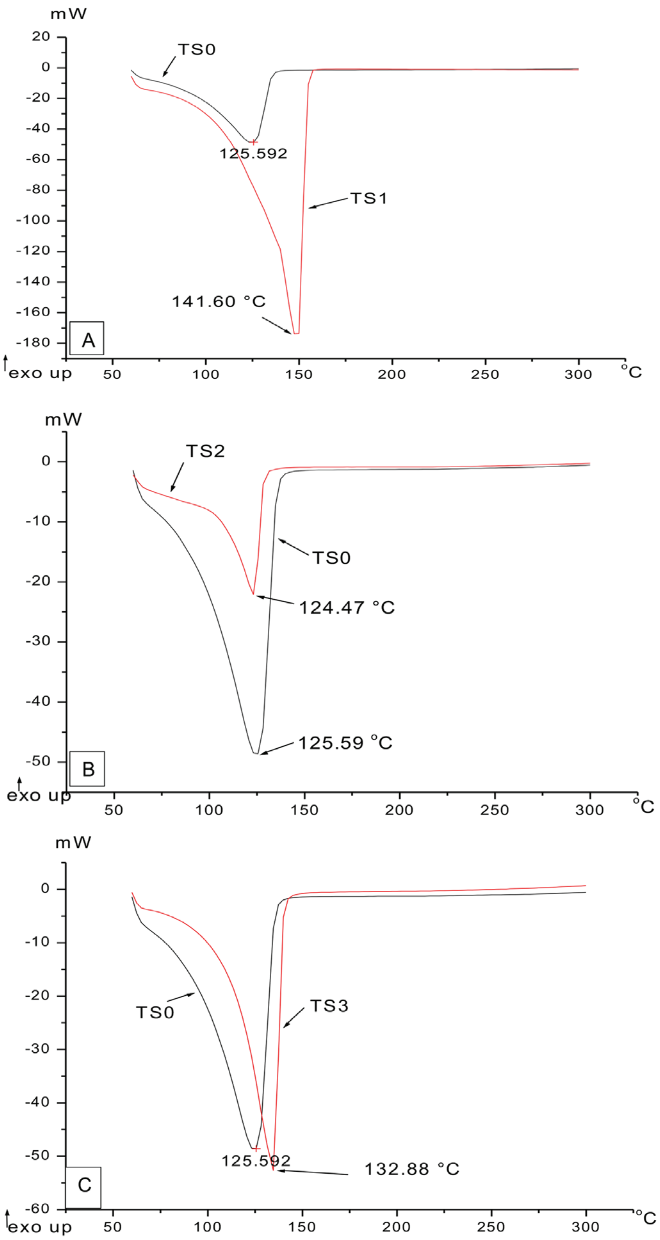

Figure 3. (A) Native Tigernut starch (TS0) and crosslinked starch from day 5 substrate (TS1); (B) TS0 and crosslinked starch from day 10 substrate (TS2); (C) TS0 and crosslinked starch from day 15 substrate (TS3)

starch molecule is broken and melted during the heating process. Considered to be the result of dissimilar bond forces within the starch granules are the differences in ΔH.[52] Higher gelatinization enthalpies as observed with TS0 and TS1, 1926.95 and 2120.73 J/(g*K), respectively, shows that higher energy is required to disentangle these bonds compared with those of TS2 and TS3; thus, showing the crosslinking effect on bond rearrangement within the starch granules. Therefore, TS1 would function as a better binder than TS2 and TS3.

Generally, higher T_o, T_p, and T_c of the modified starches suggest high degree of crystalline association within those granules than in TS0; these resulted in higher structural stability and resistance to gelatinization as previously reported.[53] Low temperatures observed in TS0 could be an indication of low stability of starch molecules because of weak molecular arrangement, which corroborates with the theory that native starches are generally unable to withstand industrial temperatures.

Peak height index (PHI) is an indication of starch granules distribution and its uniformity during gelatinization. Table 6 shows a reduction in the peak height of TS1 and TS2 in relation to TS0, which could be attributed to the effect of increasing the concentration of the crosslinking substrate. PHI and ΔT are observed to be negatively related; the wider the temperature range as a result of higher gelatinization temperature, the smaller the PHI as observed with TS1. This suggests that the modified starches could be effective as a binding agent in solid dosage formulations.

CONCLUSION

We proved in this study that agricultural waste constituting environmental nuisance, such as orange peels, could be employed as raw material for the green synthesis of starch obtained from *C. esculentus* tubers. Results obtained shows that modified TS had better water-holding capacity and thermal stability while retaining its microstructural properties, which suggests that it may perform well as binding agents in food and pharmaceutical industries.

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