PHYSICOCHEMICAL PROPERTIES OF HYDROGEN PEROXIDE– MODIFIED AFRICAN BREADFRUIT STARCH

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Abstract

In this study, starch was isolated from African breadfruit (Treculia africana) and modified using hydrogen peroxide. The physicochemical properties of native and oxidised starches were examined through Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction patterns, oil and water absorption capacities, gelation studies, and the effect of temperature on swelling power and solubility. The FT-IR revealed the formation of carbonyl and carboxyl groups in the modified starch. XRD demonstrated the granules structure of the native and oxidised starches as Type-A. The oil and water absorption capacity ranged from 165.6 ± 18.4 – 239.2 ± 48.7 g/100 g and 306.7 ± 30.6 – 280 ± 28.3 g/100 g. Oxidized starch showed most significant increase in oil absorption capacity while the native starch had the highest water absorption capacity. Gelation ranged from 6-16 % with oxidation not improving starch gelation. Swelling power measured from 55 to 95^{o}C increased as temperature increased. While the oxidised starch had better swelling at lower temperatures of 55-65oC, native African breadfruit starch had higher swelling power at higher temperatures. The solubility of the starches also increased as the temperature was increased with the oxidized starch having the highest solubility values. It was concluded that the native starch could serve as thickener, filler and gelling agent while the oxidised starch could serve as flavor retention and mouth feel agent.

Keywords: Treculia africana, modification, hydrogen peroxide, oxidation, characterisation

Introduction

African breadfruit *(Treculia africana*) belongs to the family *Moraceae*. It is found widely in the Western and Tropical Africa. In Nigeria, African breadfruit is widely distributed in Southern states. It is a berry containing edible seeds with high nutritional value of 73% carbohydrate (Osabor *et al*., 2009). Different regions of Nigeria call it by different names, which show it is widespread. The tree is evergreen producing 30-50 fruits yearly with each fruit yielding about 5-15 kg seeds after processing. The seed can be boiled and the kernels combined with maize, rice, or yam. The fresh seeds are also salted, roasted, shelled and eaten as snacks or ground and used as thickeners in traditional soups and in the preparation of pudding (Akubor *et al.*, 2000). The high carbohydrate content of makes African bread fruit a potential source of starch that can augment existing conventional sources.

Starch is a biopolymer of great importance in the food and non-food industry. Its major constituents are amylose and amylopectin. It is a valuable raw material in the food industry because of its gelling, filling, thickening and food system stabilizing capacities (Waterschoot *et al.*, 2016) making it suitable in noodles and pasta, salad dressing, and pie filling, adhesive, fuel, binding agents and stabilizing agents (Zuo *et al.*, 2017) among other uses. Starch is majorly obtained from staple foods such as wheat, maize, rice, potatoes and cassava. There is a growing functional need of starch with attendant possible overburdening of the staple foods which currently serve as starch sources. With the fear of possible food insecurity in the nearest future due to the rate of population growth, continual dependence on staple foods is unsustainable. That has led to researches on unconventional starch sources to augment existing ones for the prevention of food insecurity.

Despite its value in the industry, starch use in the native form is usually limited by industrial operation conditions (Zuo *et al*., 2017). It does not dissolve in cold water, and exhibit poor shear properties, exhibits poor thermal stability, low light transmittance, poor freeze-thaw stability, sensitivity to excessive pH conditions and high tendency for retrogradation (Xiao *et al.*, 2012). To overcome these shortcomings and obtain better physicochemical performance and functional characteristics, starch modifications using chemical, physical, genetic and enzymatic methods are applied. Different modifications such as esterification, etherification, oxidation, and cross-linking have been reported (Tharanathan, 2005).

Chemical modification such as oxidation can alter starch gelatinization, thermal properties, stability, paste clarity, pasting, and starch expansion performance by introducing carbonyl and carboxyl moieties into the starch chain. The oxidized starches therefore finds use industrially in paper, textile, laundry finishing, film product and binding materials industries to provide surface sizing, coating properties and adhesion characteristics (Yu *et al.*, 2017). Suitable reagents used for starch oxidation include sodium hypochlorite, permanganate, periodate, chromic acid, nitrogen dioxide ozone, hydrogen peroxide (Zhang *et al.*, 2009) bromine, and ambient oxygen (Dias *et al.*, 2011), with sodium hypochlorite being the most widely used industrially. The use of hydrogen peroxide is however gaining attention because it is cheap, environmentally friendly, suitable for no chlorine residuals processes, non-toxic to human at about 3% ingestion (Pereira *et al.*, 2017; Sangseethong *et al.*, 2010). Compared with hypochlorite, limited information exist on hydrogen peroxide oxidation and none is known, to the best of our knowledge, on *Treculia africana* seed starch oxidation. The aim of this study therefore was to isolate starch from *Treculia africana* seeds, enhance its properties through hydrogen peroxide oxidation and determine the effect of modification on the starch physicochemical properties.

Methodology

Sample collection

African Breadfruit was bought from a local market (Sayedero), in Yewa South, Ogun State Nigeria. Extraneous materials such as stones, stalks and non-viable seeds were removed and then oven-dried for 3 h at 100^{o}C. The sample was deshelled using sheaving, the shells winnowed and the kernel ground into fine flour using local mill. The fine flour was cooled, and stored in polythene bag at room temperature until required. Hydrogen peroxide (30 %), n-hexane (60-80 ^{o}C general purpose), Sodium hydroxide (Analar, BDH Chemical Ltd, Poole, UK), Hydrochloric acid (BDH, Poole UK), Vegetable oil (Power oil, Nigeria)

Starch isolation

Isolation and purification of starch was done by the method of Lawal and Adebowale (2005) with modification. 1 kg of fine seed flour was separately defatted by cold extraction for 72 h, with intermittent shaking using n-hexane. Defatted sample flour was separately soaked in 4 L of distilled water and the pH was adjusted to 8.0 using NaOH solution (0.05M) at room temperature for 4 h with continuous stirring. The suspension obtained was screened using muslin cloth and centrifuged for 30 mins at 4500 rpm (Hettich, North America). The starch obtained was washed twice, before drying for 48hours at 30± 2^{o}C (Gen Lab) and labelled ABna.

Preparation of oxidized starch

Oxidized starch prepared by hydrogen peroxide was produced according to the method of Han (2016). A 50 g African breadfruit starch and 750 mL distilled water were mixed and heated at 80^{o}C for 30 min with mild stirring. 25 ml H_2O_2 (30%) was added to the mixture after the temperature dropped to 40^{o}C for 4 h. The slurry was separated by slow-speed centrifugation and the precipitated product was washed with five 200 ml portions of distilled water before drying for 24 h at 50^{o}C in a vacuum oven. The starch obtained was labelled ABox.

Physicochemical properties

Fourier-Transform Infrared Spectroscopy (FT-IR) analysis

In order to characterize the chemical changes, the method of tabletting with KBr was used with FTIR (530 M, Buck Scientific, USA). To remove the moisture completely, the native starch and ATDS were further dried in the oven (at 50 ^{o}C) for 48 h. the tested samples were obtained after grinding fully with the weight ration of sample: KBr = 1:100. FTIR curves of samples were obtained in a range of 1000-4000 cm^{-1}.

X-ray Diffraction

Diffraction measurements of the powder sample were performed on the crystallography beam line of the ELLETRA storage ring as reported by Lawal *et al.* (2005). The powder was manually ground and loaded in glass capillaries of 0.3 mm diameter. Capillaries were sealed with wax and immersed in a sonic bath to obtain a more compact sample. The x-ray beam emitted by the wiggler source on the 2Gev electron storage ring was monochromatized by a Si (111) double crystal monochromator and focused on the sample. Data were collected by rotating the sample to achieved better homogeneity with a photon wavelength of Å using a Mar 345 imaging plate as a detector.

Oil and Water Absorption Capacity

The method of Beuchat as reported by Lawal *et al.* (2005) was used to determine oil and water absorption capacities of the starch. 10 mL of distilled water or oil (power oil, Nigeria) was added to 0.5 g of sample. The mixture was mixed thoroughly for 30seconds and allowed to stand for 30 min. The volume of the supernatant was recorded.

Gelation Studies

Gelation studies were performed by the method of Lawal *et al.* (2005). Different concentrations of 2-18 w/v of starch sample were prepared in test-tube with 5 mL of distilled water. The suspensions were mixed with for 5 min. The-tube was heated for 30 min at 80 ºC in a water bath, followed by rapid cooling under running cold tap water. The test-tube were further cooled at 40 ºC for 2 h. Least gelation concentration was determined as that concentration from the inverted test-tube did not fall down or slip.

Swelling Power and Solubility

Effect of temperature on swelling power and solubility determination was carried out in the temperature ranging from 55-90ºC using Lawal *et al.* (2005) method. 0.3 g of starch sample was weighed and was transfer into a clean and dried test-tube and weighed (W_1). 10 mL of distilled water was added to the test-tube and the mixture was mixed thorough for 30 sec. The slurry were heated at varies temperature between 55 to 90 ºC for 30 min (using a temperature regulated water bath). The mixture was cooled to room temperature and centrifuged (5000 rpm, 15 min). The residue gotten and the water retained were weighed (W_2).
Swelling power can be calculated using Eqn 3.1

$$\text{Swelling power (g/100 g)} = \frac{W2 - W1}{\text{Weight of sample}} \qquad (3.1)$$

Aliquots (5 ml) of the supernatant obtained after centrifugation were dried to a constant weight at 110 ºC. The residue obtained after drying the supernatant represented the amount of starch solubilized in water. Solubility was calculated as grams per 100 g of starch on dry weight basis (Eqn 3.2).

$$\text{Solubility (g/100 g)} = \frac{\text{Mass of residue x Vol. of distilled water x 100}}{\text{Vol. of supernatant x Weight of sample}} \qquad (3.2)$$

Statistical Analysis

Analyses were done in triplicate. Analysis of variance was performed to calculate significant differences in treatment means, and LSD ($p < 0.05$) was used to separate means using Sigma Plot for Windows 14.0 (Systat Software, Inc.)

Results

The results of the FTIR spectra of the native and oxidised African breadfruit kernel starches are presented in Fig. 1 Absorption was measured at a wavenumber between 650 and 4000 cm^{-1}. Fig. 2a and 2b present the X-ray diffraction patterns of native and oxidised African breadfruit kernel starches with prominent 2θ peaks values observed being 15.3°, 15.6°, 18.4°, 23.5°, 27.0°, 15.5°, 17.1 °, and 18.2°. Oil and water absorption capacities of native and oxidised African breadfruit kernel starches ranged from 165.6 ± 18.4 – 239.2 ± 48.7g/100 g and 306.7 ± 30.6 – 280 ± 28.3g/100 g, respectively. The results of the least gelation concentrations of native and oxidised African breadfruit kernel starches are presented in Table 1 and ranged from 6 – 16% w/v. The results of the effect of temperature on the swelling power and solubility of native and oxidised African breadfruit kernel starches are presented in Fig. 3 and 4. Swelling power ranged from 233.16 ±10.49 – 844.64 ± 9.25 g/100 mL while solubility ranged from 1.93 ± 0.11 – 4.64 ± 0.45 g/ 100 mL.

Discussion

FTIR spectra of native and oxidized African breadfruit starches (Fig. 1) showed broadband between 3000–4000 cm^{-1} and strong peaks in the 2936 cm^{-1}. This was as a result of complex vibration stretches associated with free and inter/intra-molecular hydroxyl groups (OH) and CH, respectively. Peaks at 1799 and 1031 cm^{-1} are characteristic of C=O and C-O groups (stretching vibration), produced by oxidation of hydroxyl groups to aldehyde and carboxyl groups. FTIR spectra of oxidized and native starches differed insignificantly and similar absorption peaks have been reported for Lima bean starch with adsorptions corresponding to the stretching of the principal group characteristic of

the starch molecule (Okekunle *et al.*, 2020). Oxidation resulted in the conversion of hydroxyl groups on starch molecules into carbonyl and carboxyl groups. The successful introduction of these groups into starch molecules enabled the starch granules to absorb water and swell. Similarities observed in absorption bands of FTIR spectra of native and oxidized starches were because of small carbonyl and carboxyl groups formed during oxidation. Similar results have been reported for oxidized barley starch over the native barley starch. (El Halal *et al.*, 2015). Han (2016) also reported that, the FT-IR spectra of oxidized starch prepared with H_2O_2 exhibited a higher intensity of this absorption peak than other starches correlating with its higher carbonyl content.

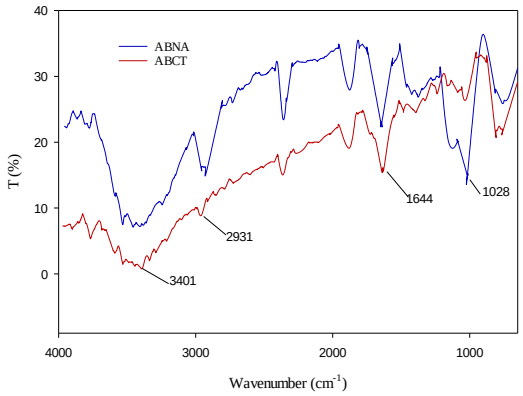
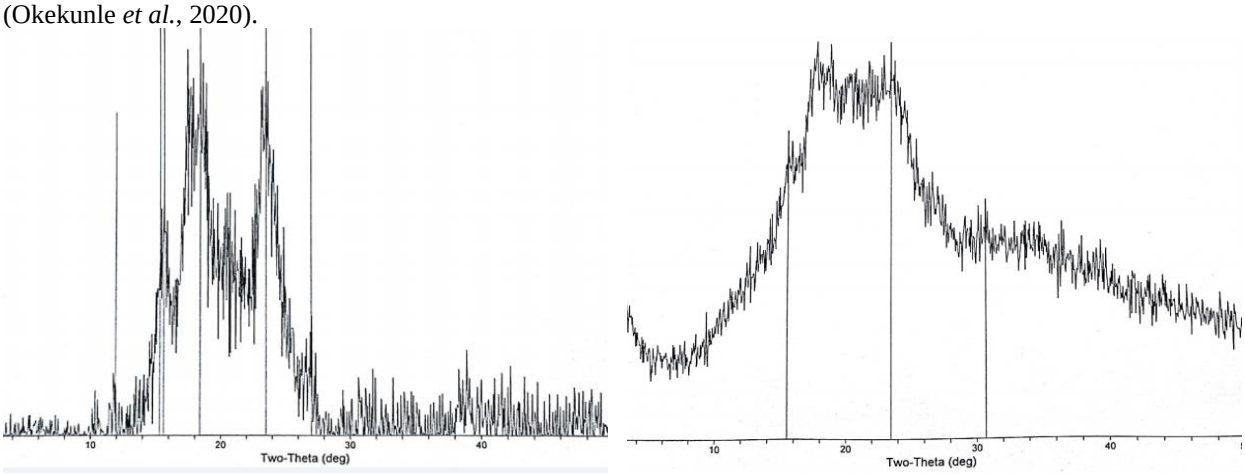


Fig. 1 FTIR spectra of native and oxidised African breadfruit starches

The X-ray diffraction patterns of the native, ABna and oxidized, ABox, African breadfruit starches (Fig. 2) presented strong peaks at around 2 θ value of 15.3°, 15.6°, 18.4°, 23.5°, 27.0° and 15.5°, 23.5° as typical of A-type diffraction pattern. According to Madruga *et al.* (2014), type-A starches show strong signals in 2 θ equal to 15.3°, 17.1°, 18.2° and 23.5°. Han *et al.* (2013) reported that ACS granules showed peaks at 15°, 18°, 20° and 23° but gradually disappeared and can be ascribed to the decreased crystallinity with esterification. While crystallinity of the starch was considered a major determinant of the extent of oxidation, the starch oxidation level in terms of the carboxyl content was strongly related to the degree of relative crystallinity and the formation of carbonyl and carboxyl groups. Also, peaks at 13 and 19.88° are indicative of the presence of V-type polymorphs while those at 16.9 and 27.0° are characteristic of A-type (Shamai *et al.*, 2003). X-ray diffraction pattern may depend on starch origin as well as environmental growth conditions. Similar diffraction pattern were observed in varieties of native lima bean starches (Okekunle *et al.*, 2020).



(a) (b)

Fig. 2 X-ray diffraction pattern of native (a) and oxidised (b) African breadfruit starches

The results of the oil and water absorption capacities of native and oxidised African breadfruit starches are presented in Fig 3. Oil and water absorption capacities ranged from 165.6 ± 18.4 – 239.2 ± 48.7g/100 g and 306.7 ± 30.6 – 280 ± 28.3g/100 g, respectively. Modifications led to increase in oil absorption capacity (OAC) of native African breadfruit starch, Abna. Similar observation of increase in OAC following oxidation has been reported for Sword bean (Adebowale *et al.*, 2006) and Lima bean (Okekunle *et al.*, 2020) starches. Increased oil absorption capacity of

African breadfruit starch increased the tendency of the use of the oxidised starch as flavour retention palatability improvement agent. Native African breadfruit starch however, had better water absorption capacity (WAC) which was significantly greater than that of the oxidized starch.

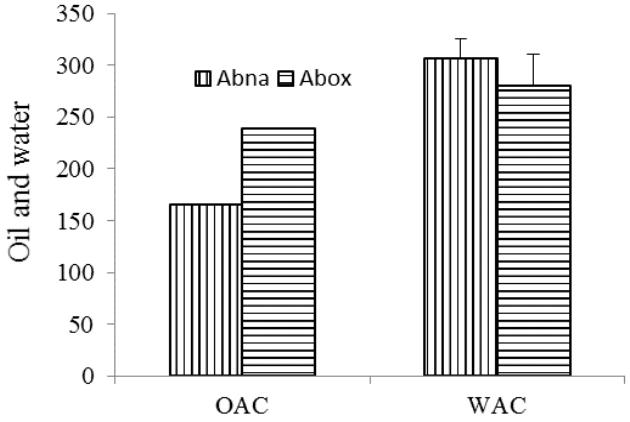


Fig. 3 Oil and water absorption capacities of native and oxidised African breadfruit kernel starches

A measure of gelation ability, the least gelation concentration, LGC, represents the minimum concentration of starch suspension in water that produced gel (Table. 1). From the results obtained, gelation tendency increased with increase in the concentration of starch suspension in water. LGC ranged from 6 to 16% w/v with oxidation increasing gelation least concentration (16%w/v). The ability of starch to readily form gel when heated is a desirable quality in the food industries. Although, the African breadfruit starch has good gelation profile in the native form, chemical modification worsened this property.

Table 1. Gelation properties of native and oxidised African breadfruit kernel starches

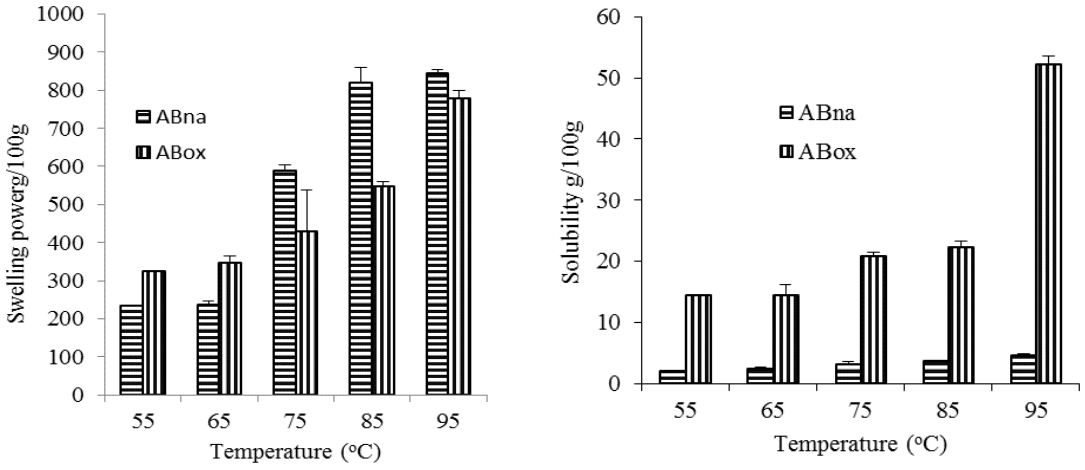
Concentration (% w/v)	Native	Oxidised
2	L	L
4	V	L
6	G	L
8	G	V
10	G	V
12	G	V
14	G	V
16	G	G
LGC	6	16

LGC= Least Gelation Concentration; L = liquid; V = viscous; G = gel

The ability of starch to absorb water is referred to as swelling (the degree of water absorption), while the percentage of molecules leached (the degree of dissolution) from starch granules during swelling is referred to as solubility. As temperature increased, the swelling power of native and oxidised African breadfruit starches measured from 55 ^{o}C to 95 ^{o}C increased (Fig. 4a). High swelling power with increase in temperature has been attributed to weakening of the bindings in intra-granular starches, which facilitated unrestricted swelling as temperature increased (Oderinde *et al.*, 2020). When compared to their native starch, oxidized starches had reduced swelling power, suggesting a reduction in the strength of water-binding forces within the starch granules. According to Pereira *et al.* (2017), the oxidized starch granules were able to absorb water, during heating but were not able to retain water under centrifugation in the same way as native starch. However, when compared with the native starch at 55°C and 65 °C, oxidized African breadfruit starch had higher swelling power. There was a sharp increase in swelling power between 75 ^{o}C to 95 ^{o}C. Okekunle *et al.* (2020) also reported that native starch had higher swelling powers over modified starches. Adebowale *et al.* (2006) reported also that, the oxidized starch of Sword bean had lower swelling power than its native counterpart, attributing that to highly associated starch granules with an extensive and strongly bonded micellar structure displaying relatively great resistance towards swelling.

The solubility of the African breadfruit kernel starch at different temperatures (Fig 4b) showed that as the temperature increased, there was an increase in the solubility of the starches, with the oxidized starches having highest solubility at all the temperatures of study. A sharp increase was observed between 85 and 95 ^{o}C. El Halal *et al.* (2015) studied the oxidation of barley starch and reported that when compared to native starch, there was an increase in the

solubility of the oxidized starch. That was attributed to the weakening of the internal structure of the starch granules and to amylase depolymerisation when part of the amylose of oxidized barley starch was cleaved. Adebowale *et al.* (2006) and Okekunle *et al.* (2020) also observed that with increase in temperature, solubility of oxidized starches increased. Also, when compared to the native starches, oxidized starches had higher solubilities which can be attributed to the weakening of starch granules to facilitate access of water to amorphous areas, increased leaching of the amorphous region and structural disintegration of starch granule.



(a) (b)

Fig. 4 Effect of temperature on swelling power (a) and solubility (b) of native and oxidised African breadfruit kernel starch

Conclusion

Native African breadfruit kernel starch was oxidised using hydrogen peroxide. The physicochemical properties of the native and oxidised starches were determined. Some important effects of oxidation on the properties of African breadfruit starch were observed. The result of FTIR showed the formation of carbonyl and carboxyl functionalities on the starch following oxidation. The X-ray diffraction patterns of the native and oxidized African breadfruit starches showed that both were Type-A. The oxidised starch can serve as a flavor retention agent because it had better oil absorption capacity while the native starch can be used as a thickening agent because it had better water absorption capacity. Native African breadfruit starch can serve as a good gelling agent considering its least gelation concentration and a better filler due to its swelling power. Oxidation increased African breadfruit starch solubility.

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