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Characterisation and Pasting Properties of Native and Oxidised Cowpea *(Vigna unguiculata)* Starch

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ABSTRACT

Starch was isolated from brown cowpea and chemically modified via oxidation employing sodium hypochlorite as the oxidizing agent. 20 ml of N10 g NaOCl was added over a period of 30 min while maintaining pH at 9.0 – 9.5. Physicochemical properties, pasting properties and FTIR characterization of the native and oxidized starch were studied. The cowpea starch had a yield of 21.4 %. Moisture content, water absorption capacity and oil absorption capacity increased following oxidation. Chemical modification reduced pH, bulk density, dispersibility and foam capacity of cowpea starch. FTIR analysis revealed similar peaks for both modified and native starches. Rapid viscosity analysis revealed that oxidation had pronounced effect on the pasting properties of cowpea starch. Oxidation increased peak viscosity, breakdown, final viscosity while hot paste clarity, pasting temperature and setback reduced following oxidation. A reduction in the setback value after oxidation indicates that starch retrogradation would minimize after modification and a reduction in the pasting temperature shows that oxidised cowpea starch may find application in products that are susceptible to high temperature.

KEY WORDS: cowpea starch, oxidized starch, pasting properties, physicochemical, retrogradation,

INTRODUCTION

Starch is the main reserve carbohydrate synthesized by superior plants. It constitutes an essential source of energy to many living organisms, especially to man [1]. Starch represents an important component of a large number of agricultural products like cereals (corn, wheat, rice) whose polysaccharide content ranges from 30 to 80%; legumes (beans, pea, faba) with 25 to 50%; tubers (potato, tapioca) with 60 to 90%, as well as of some tropical fruits like banana which

when green may contain up to 70% on a dry basis [2]. Native starch is relatively inexpensive, a versatile product and the raw material for production of many derivatives, sweeteners and ethanol [3]. The applications of unmodified starches are often limited by the extremes of certain conditions such as pH, temperature and shear during processing. However, certain properties of native starches could be harmful in effect to the original concept of the processor's idea of a good product. However, these limitations could be addressed by modification of the native starch.

Recently, chemically modified starches have been prepared by acid hydrolysis, oxidation, etherification and cross linking [4]. Oxidation is an important modification applied to starch. Oxidised starches have found applicationin many industries, especially in the paper, textile, laundry finishing and binding materials industries to provide surface sizing and coating properties due to its low viscosity, high stability, clarity, film-forming and binding properties [5, 6]. Several methods have been used to prepare oxidised starch; some of which include the use of hydrogen peroxide, ambient oxygen, ozone, bromine, chromic acid, permanganate, nitrogen dioxide, ultraviolet radiation and hypochlorite oxidation [7,8]. Oxidised starch is produced by reacting starch with a specific amount of oxidising reagent under controlled temperature and pH conditions [9]. The oxidation of starch gives room for carboxyl and carbonyl groups to substitute hydroxyl groups on the polymer backbone. The number of carboxyl and carbonyl groups on oxidised starch indicates the level of oxidation; this oxidation primarily occurs at the hydroxyl groups on the C2, C3 and C6 positions in a d-glucopyranosyl unit [10, 11]. According to Kuakpetoon and Wang [11], oxidation occurs mainly in the amorphous regions, as no changes were observed in the X-ray patterns and intensities of the oxidised products. In this study, the effect of oxidation on the properties of cowpea starch is presented.

MATERIALS AND METHODS

Starch Isolation

Brown Cowpea (*Vigna unguiculata*) was purchased from a local market in Ijebu-Igbo, Ogun state, Nigeria. The method of Ashogbon and Akintayo [12], was used for the starch isolation. 700 g of Cowpea seeds were steeped in distilled water for 2 h. The seed coats were manually removed and the inner endosperm blended for 5 min at slow rotation using a laboratory blender. The slurry was diluted with distilled water and allowed to stand for 1 h. The supernatant was

decanted and distilled water added to the starch residue. Repeated dilution and decantation continues until the pH was neutral. The cowpea starch residue was collected and air dried for 72 hrs and packed in a polythene bag prior to use. All reagents used in this work were of analytical grade.

Preparation of Oxidised Starch

This was performed according to the method described by Forssel *et al*. [13]. 100 g of native cowpea starch was mixed with 500 ml of distilled water and the pH of the mixture was adjusted to 9.5 with 2.0 M NaOH. 10 g of NaOCl was added to the slurry over a period of 30 min with constant stirring while maintaining a pH range of 9.0 – 9.5. The reaction was allowed to proceed for 10 min after all the NaOCl had been added. Subsequently, the pH was adjusted to 7.0 with 1M H_2SO_4 and the oxidized starch was filtered, washed four times with distilled water and air dried for 48 h

Determination of Moisture Content

About 5 g of cowpea starch was weighed in an automatic moisture analyzer and regulated to 105 ^oC temperature for a period of 35 minutes. The moisture percentage of the cowpea starch was display on the screen of the moisture analyser machine.

Physicochemical properties of starch

pH

The method reported by Benesi *et al.* [14] was used for pH determination. Approximately 5 g of starch sample was added to 20 ml of distilled water in a beaker. The contents were stirred for 5 min. Starch was allowed to settle and the pH of the water phase was measured using a calibrated pH meter.

Loose and tapped bulk densities

About 2 g of the powdered sample was placed in a 10 ml measuring cylinder and the volume (V0) occupied by the sample without tapping was noted. After 100 taps on the table, the filled volume (V100) was read. The bulk loose and tapped densities were calculated as the ratio of weight to volume (V0 and V100, respectively) [15].

Dispersibility

This was determined by the method described by Kulkarni *et al*. [16] as recently modified by Akanbi *et al*. [17]. 10 g of starch was suspended in 100 ml measuring cylinder and distilled water was added to reach a volume of 100 ml. The setup was stirred vigorously and allowed to settle for 3 hrs. The volume of settled particles was recorded and subtracted from 100. The difference was reported as % Dispersibility.

Oil and water absorption capacity

The method of Beuchat [18] was used with a little modification to determine oil and waterabsorption capacity of the starch. 10 ml of distilled water or Power oil (a commercial vegetable oil) was added to 1 g of sample. The mixture was mixed thoroughly with a glass rod for 5 mins and allowed to stand for 30 mins. Then, the volume of the supernatant was recorded.

Foaming Capacity

About 2 g of native and modified cowpea starch were each homogenized in 100 ml of distilled water using a magnetic stirrer for 5 minutes. The homogenate was poured into a 250 ml measuring cylinder and the volume occupied was recorded after 30 seconds. The foam capacity is expressed as the percent increase in volume.

Fourier Transform Infra-Red (FTIR) Spectra of Starch

About 200 mg of Pottasiumbromide (KBr) was added to 2 mg of starch sample and the mixture was crushed together. The mixture was then transferred into a pellet forming-die, and then compressed using a pressing machine to form a pellet. After forming a pellet, it was transferred in a cell holder which was then inserted in a FTIR (Perkin-Elmer Spectrum 100 FT-IR spectrometer Walth man, MA, USA) machine, and the results of the graph showed on the monitor.

Pasting Properties

About 3.5 g of the starch sample was weighed and 25 ml of distilled water was dispersed in a canister; paddle was placed in the canister and was joggled for few seconds and then inserted into the Rapid Visco Analyser (RVA) (Model RVA 3D+, Network Scientific, Australia). The measurement cycle was initiated by pressing the motor towerof the instrument. The profile can be seen as it is running on the monitor of the computerconnected to the RVA machine. The 13 min profile was used, time temperature region used wasidle temperature 50 ^{o}C for 1 min heated

from 50 ^{o}C to 95 ^{o}C in 3 min 45 secs, then held at 95^{o}C for 2 min 30 secs. The sample was subsequently cooled to 50 ^{o}C over a 3 mins 45 secs period, followed by a period of 2 min where the temperature was controlled at 50 ^{o}C. After these, the pasting characteristics of the starch was displayed on a graph.

RESULTS AND DISCUSSION

Yield, moisture and physicochemical properties of Native and Oxidized Cowpea Starches

Starch yield was derived using the following equation:

$$Starch\ yield = \frac{Weight\ of\ starch\ (g) \times 100}{Weight\ of\ edible\ portion}$$

The yield of Native cowpea starch (NCS) was 21.4% and this is lower than 40 and 38% reported for white and brown cowpea starch [12] but higher than 12.3% reported for beach pea [19]. The low yield of isolated cowpea starch could be attributed to the presence of highly hydrated fine fiber fraction [20] which is derived from the cell wall enclosing the starch granules. Additional reasons might be due to presence of some insoluble proteins and compact association of cowpea starch granules with other biomolecules that could be present. The moisture content increased after modification of (NCS) from 15.3% to 16.24%. This observation is consistent with similar report on breadfruit starch [21]. Oxidation reduced the pHof the native starch and this reduction in pH after oxidation could be due to the formation of carboxyl groups on starch molecules which partly dissociate to make the starch more acidic. Lawal *et al*., [7] also reported a reduction in pH after modification for hybrid maize. The bulk density of NCS and OCS are presented onTable 1.The bulk density (Loose and tapped) is in the range 0.50 to 0.80. A reduction of bulk density was observed after oxidation of native cowpea starch which might be attributed to increased crystallinity following oxidation, Increase crystallinity is characteristic of more ordered state and this might impact greater stability on the oxidized sample. This observation is consistent with similar report on acha starch following oxidation [22]. This improved physical property following oxidation of NCS is desirable in food and pharmaceutical applications as good dispersant and preparation of biopolymer based flocculants.

Table 1: Physicochemical properties of native and oxidized cowpea starch.

Physicochemical Properties	NCS	OCS
pH	6.493±0.012	6.400±0.116
Bulkdensity before tapping (g/ml)	0.560±0.019	0.573±0.008
Bulk density after tapping (g/ml)	0.758±0.023	0.751±0.034
Dispersibility (%)	84.667±1.155	83.533±0.503
WAC (g/ml)	1.000±0.500	2.267±0.643
OAC (g/ml)	1.500±0.300	2.100±0.100
Foam capacity	8.667±1.155	6.667±1.155

Values are means of triplicate determinations. NCS: native cowpea starch, OCS: oxidized cowpea starch, WAC: water absorption capacity, OAC: oil absorption capacity

Dispersibility is a measure of reconstitution of starch flour in water, the higher the dispersibility, the better the flour reconstitutes in water [16]. NCS has a value of 84.667 % which is higher than that observed for OCS with a value of 83.533 %. Since the higher the dispersibility the better the starch flour reconstitutes, therefore the values obtained for NCS were better than that of OCS. The values obtained for NCS and OCS falls within the range reported for bambarra groundnut and cassava starches versus cocoyam and wheat starches (83-87%) [23] and it was higher and better than 40.67% reported for breadfruit starch [17]. High dispersibility starches such as NCS and OCS could find useful application in adsorptive removal of ions from contaminated water system [24]. Water absorption capacity (WAC) and oil absorption capacity (OAC) increases after oxidation. Similar observations have been reported for hybrid maize [7] and new cocoyam starch [25] following oxidation, which was attributed to the electrostatic repulsions resulting from the interactions of the bulky functional groups on oxidized starch molecules facilitating percolation of water molecules within the starch granules. The increase in water absorption capacity following oxidation is a very important property especially in the application, either as a drug carrier or disintegrant in tablets and capsule formulation [15]. The foam capacity of OCS

was reduced to 6.67% from 8.67% observed for NCS. Reduction in foam capacity following oxidation could find application as an emulsifier in the food industries [26].

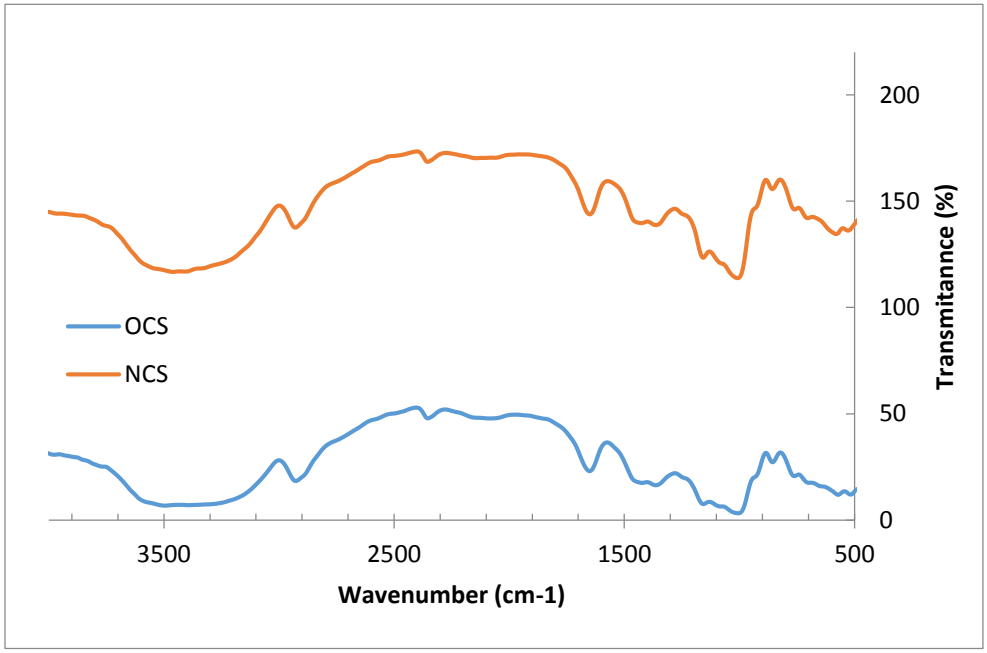

Figure 1: Infra-red spectra of native and oxidized cowpea starch.

FTIR Analysis

The FT-IR data of NCS and OCS are shown in Figure 1. NCS shows strong peaks in the 3000-3600 cm^{-1} and 2950 cm^{-1} region, which correspond to O-H and C-H respectively. Peaks at 1650 cm^{-1} and 1420 cm^{-1} correspond to O-H and C-H bending [27]. Similar peaks were observed and no major differences were detected when NCS and OCS spectra were compared. Similar observation has been reported for oxidized barley starch [28].

Pasting Properties of NCS and OCS

The pasting properties of the native cowpea and oxidised cowpea starches are presented in Table 2. Peak Viscosity (the highest viscosity attainable during heating) corresponds to the point when the numbers of swollen but still intact starch granules are maximal, which indicates the water binding capacity of the starch granules [29] and it is also frequently correlated with final product quality. The peak viscosity increased after oxidation from 381.8 RVU to 482.2.RVU. Afolabi *et*

al., [30] reported an increase in the peak viscosity for oxidized finger millet (*Eleusine coracana*) starch and attributed the increase to the incorporation of carbonyl and carboxyl groups into the starch structure which promotes swelling of the granules [31]. Fonseca *et al*., [32] also reported that oxidation of potato starch at low levels (0.5 and 1.0g/100g active chlorine) increased the peak viscosity while the potato starch oxidized with the highest active chlorine concentration (1.5g/100g) presented a reduction in peak viscosity as compared to the native starch and they concluded that the increase in viscosity of starches with low degree of oxidation may be due to partial depolymerization of the starch, thus facilitating swelling [8]. The trough (hot paste viscosity) reduced following oxidation from 317.3 RVU to 301.8 RVU. This indicates that modification greatly affect shear thinning during the holding period and NCS which has the highest value (317.3 RVU) will have more ability to withstand breakdown during cooling than the oxidized starch.The reduction in hot paste viscosity after oxidation have been reported earlier [7,21, 25,33,34]. The decrease in viscosity of hypochlorite-oxidised and peroxide-oxidised starches can be attributed to the oxidative cleavage of starch chains, which results in starch with a lower molecular size [11].

The Breakdown Viscosity is a measure of the vulnerability of cooked starch to disintegration. The breakdown value of a starch paste, defined as the difference between the peak viscosity and the viscosity after holding for 30 min at 95 ^{o}C (35), a measure of fragility of the starch [36]. It is possible to assess the starch stability at high temperatures based on the results of the breakdown which is the resistanceof starch to the heating and mechanical stirring.The breakdown viscosity increased in oxidized cowpea starch when compared to the native from 164.6 RVU to 180.5 RVU. The higher the breakdown in viscosity, the lower is the ability of the starch sample to withstand heat and shear stress during cooking. Therefore, NCS (164.6 RVU) starch might be able to withstand more heat and shear stress compared to OCS (180.5 RVU) because of their lower breakdown values. An increase in breakdown viscosity after oxidation has been reported by earlier reports [7, 25,30, 33,34]. Fonseca *et al*., [32] reported that samples of oxidized potato starches with 0.5 and 1.0 g/100 g of active chlorine showeda higher breakdown as compared to native starch. However, oxidation at the highest active chlorine concentration reduced the breakdown of potato starch, therefore showing a greater stability to heat and mechanical agitation and a lower retrogradation compared to other oxidized starches.

Table 2: Pasting properties of native and oxidized cowpea starch

Pasting Properties	NCS	OCS
Peak viscosity	381.8	482.2
Trough	317.3	301.8
Breakdown	164.6	180.5
Final viscosity	345.3	408.4
Setback	128.1	106.6
Peak time	4.7	4.6
Paste temperature	82.5	81.6

Final Viscosity indicates the ability of the starch to form a viscous paste. The final viscosity of OCS is higher than that of NCS. Similar observation was reported by Afolabi*et al*., [30] foroxidized finger millet (*Eleusinecoracana*) starch. A high final viscosity (FV)of starch indicates that the paste is more resistant to mechanical shear and may easily form a more rigid gel [37]. Therefore OCS will be more resistant to mechanical shear and may easily form rigid gel than NCS. OCS may be desired in many food products such as in soups, sauces and dressings. Setback value is a measure of retrogradation tendency or syneresis of the starch. The setback of NCS reduced after oxidation. This reduction in NCS after oxidation indicates that new substituent groups have been introduced into the modified derivatives and this restricted the tendency of the starch molecules to realign after cooling, thereby encouraging a lower setback value for the modified starches [33]. The pasting temperature of NCS was reduced after oxidation from 82.5 ^{o}C to 81.6 ^{o}C. This reduction in pasting temperature of NCS following modification indicates granular fragility [38] and it is not only economical in terms of cooking energy, but also may find greater application in products that are susceptible to high temperature [39].

CONCLUSION

Starch was isolated from brown cowpea and it was subjected to oxidation. Changesinphysicochemical properties after oxidation were investigated. The study revealed that oxidation had pronounced effect on the physicochemical and pasting properties of the cowpea starch. Oxidized cowpea starch had a higher final viscosity than the native cowpea starch which shows that it may be desired in many food products such as in soups, sauces and dressings. Being an underutilized source of starch this study shows that starch could be successfully isolated from cowpea thereby suggesting its potential as a source of starch for industrial purposes.

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