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Physicochemical characteristics and thermal properties of chemically modified jack bean (*Canavalia ensiformis*) starch

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Abstract

Starch isolated from jack bean was subjected to chemical modification through oxidation, acetylation and acid thinning. Moisture content, ash, protein, fat, fibre, amylose and pH reduced after chemical modifications. Wide angle X-ray diffractograms showed conventional 'C' pattern, characteristic of legume starches. Except increase in intensity observed in acid thinned starch (Atjs), no significant differences were observed between the X-ray pattern of native starch and modified derivatives. Scanning electron microscopy and light microscopy revealed that starch granules were oval and round shape with heterogeneous sizes. The range of the granule size for width was 12–30 μm and 12–34 μm for length. Both swelling power and solubility increased with increase in temperature. And swelling power increased after acetylation but reduced following acid thinning and oxidation. All chemical modifications increased solubility of native starch. Both water and oil absorption capacities improved following acetylation and oxidation, but reduced following acid thinning. The result obtained indicates that LGC (least gelation concentration) increased following oxidation but reduced after acid thinning while both acetylated starch and native starch had LGC value of 8%w/v. Gelatinization enthalpy reduced from 0.75 J/g in native starch to 0.54 and 0.56 J/g in Ojs (Oxidised jack bean starch) and Ajs (Acetylated jack bean starch), respectively. However, enthalpy of gelatinization increased in acid thinned starch compared with native starch. Retrogradation tendency reduced following acetylation and oxidation but increased in acid thinned starch derivative.

Keywords: Jack bean; Acetylation; Oxidation; Acid thinning

1. Introduction

Various domestic and industrial demands for starch rank this biopolymer among vital biomaterials relevant to economic advancement, particularly in developing countries all over the world. With wide applications in food, textile, pharmaceutical, paper and recently in synthetic polymer industries, it plays prominent role even in technological developments. However, a large percentage of starch that serve this purpose are from crops that equally serve as sources of staple foods. This development has necessitated research on alternative means of sourcing starch for domestic and industrial uses. Focus on underutilized plant resources for starch production has stimulated research on crops such as mucuna beans (Adebowale & Lawal, 2003a,b), bambarra groundnut (Adebowale, Afolabi, & Lawal, 2002; Adebowale & Lawal, 2002), new cocoyam (Lawal, 2004), black Gram (Sathe, Rangnekar, Deshpande, & Salunkhe, 1982), Great Northern Bean (Sathe & Salunkhe, 1981), sago (Cui & Oates, 1999), pigeon pea (Akintayo, Oshodi, & Esuoso, 1999), yambean (Agunbiade & Longe, 1999), field pea (Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001) and lentil (Hoover & Manuel, 1996).

The source of starch production varies all over the world depending on local traditions and climatic conditions, but it is more or less only starch and starch derivatives of maize and potato that are of commercial interest (Swinkels, 1985). Among underutilized plant sources that could be of alternative use are legumes such as Jack bean, which belongs to the family fabacea. It has been used as high protein food crop by natives of the South-Western USA,

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Mexico, Central American coutries, Brazil, Peru, Ecuador and the West Indies for many centuries. Its high carbohydrate content (59%) makes it an invaluable source of starch for both domestic and industrial uses (Doku & Karikari, 1971

The use of natural starch is restricted by the extremes of certain conditions such as pH, temperature and shear during processing. As a result of this development, certain properties of native starch could be inimical to the original concept of the processor's idea of a good product. Chemical modifications have been used to prevent most of these problems. In recent times, many types of chemically modified starches have been prepared by acid hydrolysis, oxidation, etherification, esterification and cross-linking (Santacruz, Koch, Svensson, Ruales, & Elisson, 2002).

Oxidised starch is produced by reacting starch with a specified amount of oxidizing reagent under controlled temperature and pH (Kuakpetoon & Wang, 2001; Wurzburg, 1986). Oxidation causes depolymerisation, which results in a lower dispersion viscosity and introduces carbonyl and carboxyl groups, which retard recrystallization (Rutenberg & Solarek, 1984).

In acid modification, the hydroxonium ion attacks the glycosidic oxygen atom and hydrolyses the glycosidic linkage. An acid acts on the surface of the starch granule first before it gradually enters the inner region. Acid modification changes the physicochemical properties of starch without destroying its granule structure and the properties of acid-thinned starches differ according to their origin (Bentacur, Chel, & Canizares, 1997). The gelatinization temperature, and the breadth of the gelatinization endotherm have also been shown to increase on acid hydrolysis. The retrogradation rate of acid thinned starch increased as hydrolysis proceeded (Kang, Kim, Lee, & Kim, 1994). The method for the manufacture of acid thinned starch entails treating concentrated starch slurry with mineral acid at temperatures below gelatinization temperature for specific period depending on the desired viscosity or degree of conversion. Effect of different acids (HCl, HNO_3, H_2SO_4 and H_3PO_4) under similar conditions of treatment on molecular weight, alkali fluidity number, iodine binding capacity and intrinsic viscosity of various starches has also been studied (Singh & Ali, 2000).

Starch acetates are derivatives of starch obtained through esterification. In acetylation, hydrophilic hydroxyl groups are substituted with hydrophobic acetyl groups. Acetylation makes starch more hydrophobic and prevents the formation of hydrogen bonding between hydroxyl groups and water molecules. Since the tendency of an aqueous starch dispersion to increase in viscosity on cooling and finally to gel is related to the association of amylose molecules, a treatment such as acetylation which retards or eliminates this crystallisation or retrogradation will effect stabilisation of the starch sol.

Acetylation also prevents or minimizes association of amylopectin outer branches. This is of practical value in many industrial and food applications because such associations can cause cloudiness and syneresis in aqueous dispersions of starches (Bentacur, Chel, & Canizares, 1997).

The objective of this study was to investigate the effects of oxidation, acetylation and acid thinning on physicochemical and thermal properties of a starch isolated from jackbean, (*Canavalia ensiformis*). A secondary objective was to study the influence of pH and temperature on some physicochemical properties of native and modified starches.

2. Materials and Methods

2.1. Materials

Jack beans seeds were obtained from the seed section of agronomy department, University of Ibadan. All other chemicals used in the experiments were of analytical grade.

2.2. Isolation of starch and purification of starch

One kilogram of the jack bean seeds was soaked in 4 l of distilled water and the pH was adjusted to 8.0 using solution of 1 M NaOH at 4 °C for 12 h Coats of the seeds were removed by manual abrasion. The dehulled seeds were blended for 30 min using warring blend. (Braun Multimix de luxe MX40, type 2291). The slurry obtained following blending was re-suspended in 5 l of distilled water and the pH was adjusted to 8.0,using 0.5 M NaOH solutions. While keeping the pH at 8.0–8.5, the mixture was stirred manually for 30 min. The suspension obtained was screened using 75 μm sieve and centrifuged for 30 minutes at $10{,}000 \times g$ (Type GLC-1 Ivan sovall, Inc., USA). The starch obtained was washed twice before drying in the air for 48 h at 30 ± 2 °C. It was stored in polythene bag until use.

2.3. Starch acetylation

The method of Sathe and Salunkhe (1981), was used for starch acetylation. 100 g of starch were dispersed in 500 ml of distilled water; it was stirred magnetically for 20 min. The pH of the slurry obtained was adjusted to 8.0 using 1 M of NaOH. 10.2 g acetic anhydride was added over a period of one hour, while maintaining a pH range of 8.0–8.5. The reaction proceeded for 5 min after the addition of acetic anhydride. The pH of the slurry was adjusted to 4.5 using 0.5 M HCl. It was filtered, washed for four times with distilled water and air-dried at 30 ± 2 °C for 48 h.

2.4. Starch oxidation

The method of Forssel, Hamunen, Autio, Suortti and Poutanen, 1995, was employed with modifications. 50% slurry of starch was prepared by dispersing 100 g of starch in 200 ml of distilled water. The pH was adjusted to 9.5 with 2 M NaOH. 10 g of NaOCl were added to the slurry over a

period of 30 min, while maintaining pH range of 9–9.5, with constant stirring 30±2 °C. The reaction proceeded for 10 min after addition of NaOCl. After the reaction, the pH was adjusted to 7 with 1 M H_2SO_4 and the oxidized starch was filtered, washed four times with distilled water and air dried at 30±2 °C for 48 h.

2.5. Acid thinning

One hundred grams of native starch were slurried in 500 ml of 0.15 M HCl It was stirred magnetically for 8 h, while maintaining a temperature of 50 °C. The acid modified starch was filtered and the residue obtained was washed four times with distilled water. It was dried in the air for 48 h at 30±2 °C.

2.6. Degree of acetylation

The content of acetyl groups (expressed as percentage in dry basis) and the degree of substitution of acetylation were determined according to Smith (1967). 5 g of acetylated starch was placed in a 250 ml flask, and 50 ml distilled water were added upon mixing. A few drops of phenolphthalein indicator were added and the suspension titrated with 0.1 M sodium hydroxide to a permanent pink end point. After addition of 25 ml 0.45 M sodium hydroxide solution, the flask was sealed tightly with a rubber stopper and shaken vigorously for 30 min. After shaken, the stopper was removed carefully and washed down, together with the walls of the flask with distilled water. The saponified mixture, containing excess alkali was then titrated with standard 0.2 M HCl solution until disappearance of the phenolphthalein colour. The native starch was treated in the same manner to obtain a blank value.

Percent acetyl (dry basis)

$$= \frac{(\text{Blank titre} - \text{Sample titre})\ \text{ml} \times \text{Acid Molarity} \times 0.043 \times 100}{\text{Sample weight in g (Dry basis)}}$$

$$\text{Degree of substitution (D.S)} = \frac{162A}{4300 - 42A}$$

In which A = percent acetyl (dry basis).

2.7. Carboxyl and Carbonyl contents

The method of Parovuori, Hamunen, Forssel, Autio, and Poutanen (1995) was used for the determination of Carboxyl contents. 5 g of oxidized starch sample were slurried in 25 ml of 0.1 M HCl. It was stirred for 40 min. The slurry was filtered through a medium porosity fritted glass crucible and the residue was washed with distilled water until it was free of chloride, using silver nitrate test. The chloride free sample was dispersed in 300 ml of distilled water. The dispersion was heated in a steam bath and stirred continuously until the starch gelatinized. The hot sample was titrated with 0.1 M NaOH to a phenolphthalein end point. To quantify acidity due to other sources, mainly fatty acids complexed with amylose, a blank titre was determined. 5 g of native starch were titrated to provide for a blank value.

Percent carboxyl

$$= \frac{(\text{Sample titre} - \text{Blank titre})\ \text{mlxAlkali molarity} \times 0.045 \times 100}{\text{Sample weight (g).}}$$

The hydroxylamine method described by Smith (1967) was used for the determination of carbonyl content. 2 g of oxidised starch were dispersed in 100 ml of distilled water and the suspension was gelatinized by heating in a boiling water bath and then cooled to 40 °C. The pH was adjusted to 3.2 and 15 ml of hydroxylamine reagent was added (The hydroxylamine reagent was prepared by dissolving 25 g of reagent grade hydroxylamine hydrochloride in water and adding 100 ml of 0.5 M NaOH. The solution was made to 500 ml with distilled water). The sample was covered with aluminium foil and placed in a water bath at 40 °C. After 4 h, the excess hydroxylamine was determined by rapid titration of the reaction mixture to pH 3.2 with 0.1 M hydrochloric acid.

Percent carbonyl (C = O)

$$= \frac{\text{Blank titre} - \text{Sample titre})\ \text{ml x Acid molarity} \times 0.028 \times 100}{\text{Dry sample weight (g)}}$$

2.8. Chemical composition

Standard Association of Official Analytical Chemistry method, AOAC (1996) were adopted for estimating moisture, ash, crude fibre, amylose, protein and fat contents.

2.9. Wide-angle X-ray diffraction of starch granules

Diffraction measurements of the powder samples were performed on the crystallography beamline of the Elletra storage ring at Trieste, Italy. The powder was manually ground and loaded in glass capillaries of 0.3 mm of 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 2 Gev 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 achieve better homogeneity, with a photon wavelength of 1.0 Å, using as a detector a Mar 345 Imaging plate (MarResearch GmbH, Norderstedt, Germany). Sample to detector-to-detector distance and wavelength were calibrated using Lanthanum Hexaboride.

2.10. Granule morphology

Scanning electron microscopy (SEM) and light microscopy (LM) was used for granule morphology studies. Using SEM, a thin layer of starch granule was mounted on aluminium specimen holder by double-sided tape. The specimen holder was loaded in a polaron SC 7610 sputter coater (fison Instrument, England.) It was coated with gold palladium, to a thickness of about 30 nm. The specimen holder was then transferred to XL-20 series (Phillips) scanning electron microscope and starch samples were examined at 10 KV. Light micrographs of the starch samples were taken with a Diplan-Microscope model GF (Leitz Wetzler, Germany). The method of Sathe and Salunkhe (1981) was used for analysis of size of the starch granules.

2.11. Physico chemical properties

2.11.1. Swelling power and solubility

Swelling power and solubility determinations were carried out in the temperature range of 55–95 °C, using the method of Leach, McCowen and Scoch (1959). 0.1 g of starch samples were accurately weighed and quantitatively transferred into a clear dried test tube and weighed (w_1). 10 cm^3 of distilled water was added to the test tube and the mixture was mixed thoroughly with a Variwhirl mixer for 30 s. The resultant slurries were heated at desired temperatures, varied between 55 °C and 95 °C for 30 minutes in a water bath (using temperature regulated water bath). The mixture was cooled to room temperature and centrifuged ($5000 \times g$, 15 min).

The residue obtained from the above experiment (after centrifugation) with the water it retained and the test tube was weighed (W_2).

Swelling of starch $= W_2 - W_1$/Weight of starch

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

2.12. Effect of pH on swelling power and solubility

Effect of pH on solubility and swelling was investigated using the method of Sathe & Salunkhe, 1981. 1.0 g of starch sample was weighed and quantitatively transferred into a clear dried test tube and weighed (w_1), after which the starch was mixed with 10 cm^3 of distilled water. The pH was adjusted to desired value (2–12) with 0.1 M HCl or 0.1 M NaOH. The slurries were allowed to stand for 1 h, at 30 ± 2 °C, centrifuged at $5000 \times g$, for 15 min. The residue obtained after centrifugation with the water it retained and the test tube was weighed (W_2).

2.13. Swelling of starch $= W_2 - W_1$/Weight of starch

Five millilitre of the supernatant were dried to constant weight at 110 °C to determine percentage solubility of the starch as stated earlier

2.14. Oil and water absorption capacity

The method of Beuchat (1977), was used to determine oil and water absorption capacity of the starch. 10 mL of distilled water or oil (Executive Chef Oil, Lever Brothers (Nigeria) Plc, Lagos, Nigeria) was added to 1 g of sample. The mixture was mixed thoroughly with a Variwhirl mixer for 30 s and allowed to stand for 30 min. Then the volume of the supernatant was recorded. The mass of oil or water absorbed was expressed as g/g starch on a dry weight basis.

2.15. Gelation studies

Sample of starch, 2–14% (w/v) were prepared in test tube with 5 ml of distilled water. The starch suspensions were mixed with Vari-whirl mixer for 5 min. The test tubes were heated for 30 min at 80 °C in a water bath, followed by rapid cooling under running cold tap water. The test tubes were further cooled at 4 °C for 2 h. Least gelation concentration was determined as that concentration when the sample from the inverted test tube did not fall down or slip.

2.16. Brabender Viscography

Brabender viscographic pattern of 8% starch paste (36 g of starch on dry weight basis in 450 ml of water) was obtained on a brabender viscograph (Type 8012003 W-G) equipped with 700 cmg sensitivity cartridge. The starch suspension was heated from 50–95 °C. It was kept at this temperature for 30 min, then cooled to 50 °C and held at this temperature for 30 min. The speed of the rotor was fixed at 75 rpm and the heating as well as cooling rate was 15 °C per minute through out the range of gelatinization holding and cooling steps.

2.17. Differential scanning calorimetry and retrogradation studies

Gelation and retrogradation of starches were measured using a Perkin–Elmer DSC6 (Norwalk, CT) differential scanning calorimeter, equipped with thermal analysis software, Pyris windows (perking-Elmer). 6.0 μl distilled water was added to 2.0 mg of starch in DSC pans (BO14-3017). It was sealed, reweighed and kept at 30 ± 2 °C for 24 h to ensure equilibration of the starch sample and water. The samples were scanned from 30 °C to 130 °C at 10 °C/min using empty pans as reference. The heated pans were then

cooled immediately and kept at 4 °C inside a refrigerator for 24 h, following which they were kept for one or six days at 30 ± 2 °C, to make complete storage days of two and seven respectively. Following these periods of storage, the samples were scanned under the same condition with the first scanning. Indium and Zinc were used for calibration, while empty pan was used as the reference scale. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH, J/g) for gelatinization and retrogradation were determined. The enthalpy (ΔH) was estimated by integrating the area between the thermogram and the base line under the peak. Experiments were replicated three times.

2.18. 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 (SAS, 1988).

3. Results and discussion

3.1. Chemical composition

The chemical composition of the starches is presented in Table 1. All parameters studied, which include the moisture content, ash, protein, fat, fibre, amylose and pH reduced after chemical modifications. Since the starch molecules were dried under the same condition, reduction in moisture content could probably be as a result of the substitution of the hydroxyl groups on the starch molecules. Similar observation has been reported by Wootton and Bamunuarachchi (1979). Reductions observed in ash content after modifications could be attributed to washing away of the mineral contents of the starches during modifications. Also, reductions in protein and fibre content could be attributed to degradative effect, particularly in amylose fraction of starch granules after modifications. Following oxidation, the introduction of carboxyl groups on starch molecules probably accounted for slight increase in acidity as observed. It is also reasonable that introduction of acetyl groups on starch molecules also increased the acidity of starch molecules after acetylation, while reduction in pH after acid thinning was probably a consequence of acid hydrolysis of the starch granules. It is however noteworthy that reduction in pH values of the modified derivatives were insignificant at $P < 0.05$. Similar observations on reductions in ash, fat, crude fibre and protein content have been observed in oxidised and acetylated starch derivatives of mucuna bean (Adebowale & Lawal, 2003a), bambarra groundnut (Adebowale et al., 2002), new cocoyam starch (Lawal, 2004), cassava starch (Agboola, Akingbala, & Oguntimehin, 1991), rice starch (Gonzalez & Perez, 2002).

3.2. Wide-angle X-ray diffraction patterns

The wide-angle X-ray diffraction patterns of the native and modified jack bean starches are presented in Fig. 1. The starches show the conventional characteristic 'C' pattern of legume starches (Colona, Buleon, & Mercier, 1981; Hoover & Sosulski, 1985; Gernat, Radosta, Damaschun, & Schierbaum, 1990; Hoover & Manuel, 1996; Chavan, Shahidi, Hoover, & Perera, 1999). 'C' crystalline polymorphs of legume starches are mixtures of 'A' and 'B' polymorphs, which are characteristic polymorphs of cereals and tuber, starches, respectively (Gernat et al., 1990). Both 'A' and 'B' type starches are based on the parallel stranded double helices, in which the double helices are closely packed in the 'A' type starch but loosely packed in the 'B' type starch. In a study conducted on DSC and X-ray of pea starch gelatinized in 0.6 M KCl solution, Borgracheva, Morris, Ring, and Hedley (1998) concluded that the 'A' and 'B' polymorphs in pea starch are present in the same granule and that the 'B' polymorph is situated in the centre of all granules and is surrounded by the 'A' polymorph. Hizukuri (1986) and Hizukuri, Kaneko, and Takeda (1983) have shown that starches with amylopectin of short chain length (<20 residues) exhibit 'A' type of crystallinity, whereas those with amylopectin of longer average chain length show the 'B' pattern. In this study, no pronounced difference was observed between the native starch and modified starches. However, acid thinned derivatives showed slightly sharper peaks at $2\theta = 18.8°$ and 25.0°. This is possibly as a result of cleavage of starch chains in the amorphous region, which

Table 1
Chemical composition of native, oxidised, acetylated and acid thinned starches of jack bean

Sample	Parameter (%)						
	Moisture	Ash	Protein	Fat	Fibre	Amylose	pH
Native	12.35 ± 0.04[a]	0.33 ± 0.07[a]	1.02 ± 0.04[a]	0.10 ± 0.04[a]	0.61 ± 0.04[a]	18.42 ± 0.06[a]	7.07 ± 0.01[a]
Ojs	9.86 ± 0.01[b]	0.14 ± 0.09[b]	0.76 ± 0.08[b]	0.04 ± 0.04[b]	0.51 ± 0.05[b]	16.76 ± 0.07[b]	6.93 ± 0.05[a]
Ajs	10.45 ± 0.01[b]	0.30 ± 0.08[a]	0.61 ± 0.02[b]	0.06 ± 0.01[b]	0.35 ± 0.01[c]	15.65 ± 0.04[b]	6.12 ± 0.04[a]
Atjs	9.21 ± 0.05[b]	0.12 ± 0.01[b]	1.03 ± 0.01[a]	0.02 ± 0.01[b]	0.14 ± 0.05[d]	13.20 ± 0.61[c]	6.10 ± 0.05[a]

All Values are means of triplicate determinations ± standard deviation. Means within columns with different letter are significantly different ($P < 0.05$). Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch. Ojs: COOH = 0.39(%); CHO = 0.21 (%). Values are numbers of carboxyl and carbonyl groups per 100 anhydroglucose unit, AGU. Ajs, degree of substitution (moles of acetyl substituent per mole of D-glucopyranose unit) = 0.43

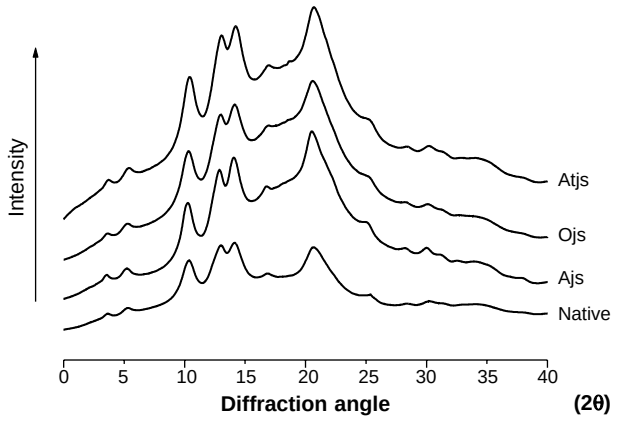

Fig. 1. Wide angle X-ray diffraction pattern of native, acetylated, oxidised and acid thinned jack bean starch.

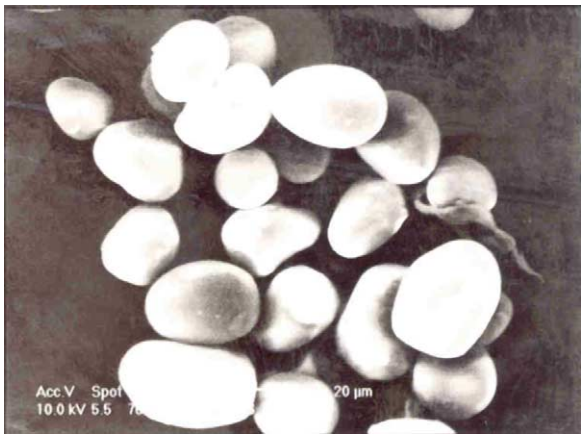

Fig. 3. Scanning electron micrographs of native jack bean starch.

allows reordering of chain segments to give more crystalline structure with a sharper X-ray pattern. The result lends credence to the report of Wang and Wang (2001) on the structures and physicochemical properties of acid-thinned Corn, Potato and Rice starches.

3.3. Granule morphology

Light micrograph and scanning electron micrograph of native jack bean starch are presented in Figs. 2 and 3. Starch granules were oval and round shape with heterogeneous sizes. The range of the granule size for width was 12–30 and 12–34 µm for length. In previous publications, oval and round shape has been reported for pinto bean and native bean (Gujska, Reinhard, & Khan, 1994) while oval and spherical shapes were reported for field pea (Galvez & Resurreccion, 1993). As revealed by the light micrograph, presence of hyla on some of the starch granules was observed with different shapes. Similar observation has been reported for black gram starch (Sathe, Rangnekar, Deshpande, & Salunkhe, 1982). They appear as dark band fissures or cracks on the starch granules (Naivikul & D'Appolonia, 1979), which are due to internal cracking during air-drying of the starch (Hall & Sayre, 1971). In the present investigation, no significant differences were observed in shape and appearance of native and modified (micrographs not shown) starch derivatives this is probably due to physiology of the starch granules and the level of modifications.

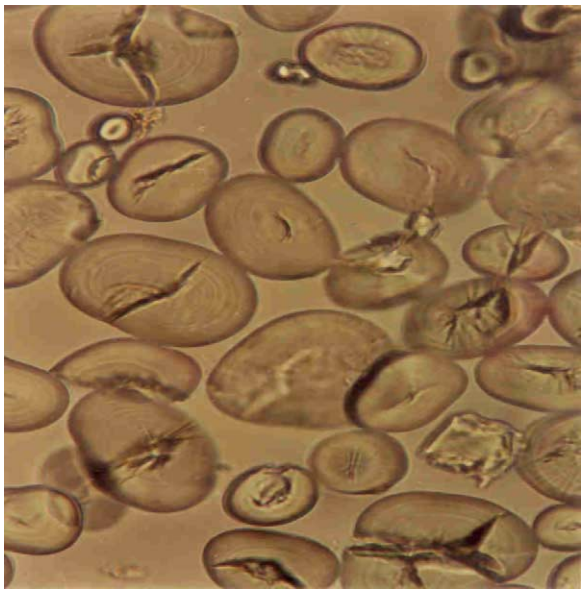

Fig. 2. Light micrographs of native jack bean starch.

3.4. Swelling power and solubility

Swelling power and solubility of starches were temperature dependent as indicated in Figs. 4 and 5. Both swelling power and solubility increased with increase in temperature.

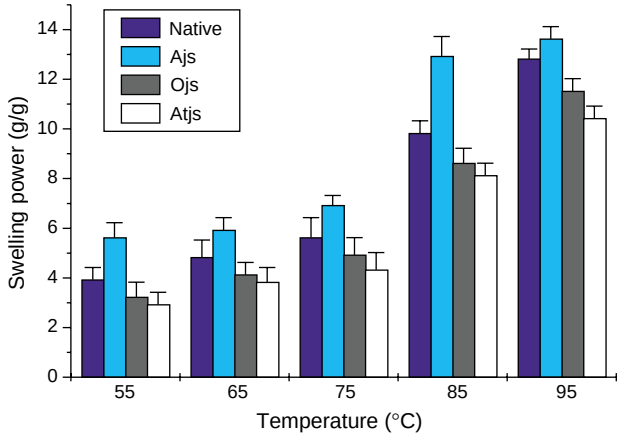

Fig. 4. Effect of temperature on swelling power of native, acetylated, oxidised and acid thinned jack bean starch Error bars: Standard deviations Results are means of triplicate determinations Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch.

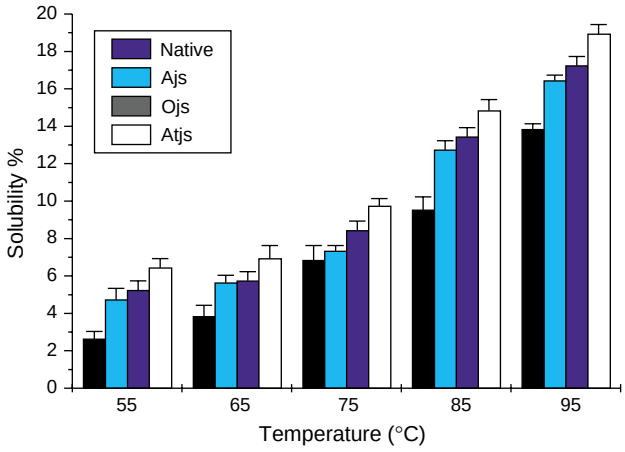

Fig. 5. Effect of temperature on solubility of native, acetylated, oxidised and acid thinned jack bean starch. Error bars: Standard deviations Results are means of triplicate determinations Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch.

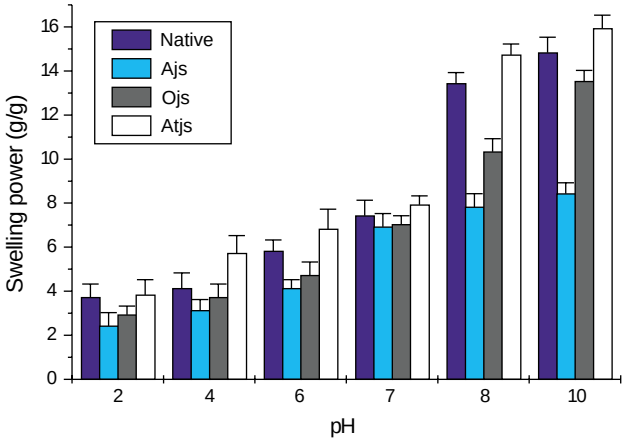

Fig. 6. Effect of pH on swelling power of native, acetylated, oxidised and acid thinned jack bean starch. Error bars: Standard deviations Results are means of triplicate determinations Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch.

Increase in temperature weakened the intragranular binding forces of native and modified starches, thus facilitating less restricted swelling as the temperature increased. Similar observations were reported for Great Northern bean starch (Sathe & Salunkhe, 1981). The result also indicates that swelling power increased after acetylation but reduced following acid thinning and oxidation. All chemical modifications increased solubility of native starch. Among the derivatised starch samples, at all temperatures, solubility was highest in oxidised jack bean starch, followed by acid thinned starch. These observations are consistent with previous reports on swelling and solubility of rice starch (Gonzalez & Perez, 2002), cassava starch (Aiyeleye, Akingbala, & Oguntimehin, 1993), bambarra ground nut starch (Adebowale et al, 2002), wheat starch (Wootton & Bamunuarachchi, 1979) and potato starch (Kim & Noh, 1992). During the process of acid thinning, the hydroxonium ion (H_3O^+) breaks down the glycosidic oxygen atoms and hydrolyses the glycosidic linkages. Acid gradually degrades the surface of the starch granule first before entering the inner region. It preferentially breaks down the amorphous region because the crystalline area is not freely accessible to the acid and this makes it remain intact. As a result of this development, % relative crystallinity increases following acid thinning. Increase in crystallinity probably accounts for reduction in swelling capacity of the acid thinned starch since swelling is restricted by stiffness of entangled amylopectin network in the crystalline region of the starch (Kanuma, & French, 1971; Cairns, Leloup, Miles, Ring, & Morris, 1990).

Effect of pH on swelling capacity and solubility of native and modified starches are presented in Figs. 6 and 7, respectively. At all pHs studied (2–10), both acid thinning and oxidation reduced the swelling capacity, however, swelling capacity improved after acetylation. This result agrees with the earlier report on acetylated black gram starch (Deshpande, Sathe, Rangnekar, & Salunkhe, 1982). The result also showed that swelling capacity of all the starches improved as the pH increased. Minimum solubility of native starch was observed at pH 6, while modified derivatives had minimum solubility at pH 2. Except pH 2, highest solubility was observed in Ojs at all other pHs. At alkaline pHs, partial gelatinization of starches may occur and this probably resulted in increased swelling and solubility at these pHs.

3.5. Water and oil absorption capacity

Both water and oil absorption capacities of native jack bean starch improved following acetylation and oxidation. Contrarily, water and oil absorption capacities reduced in acid thinned starch derivative compared to the native starch

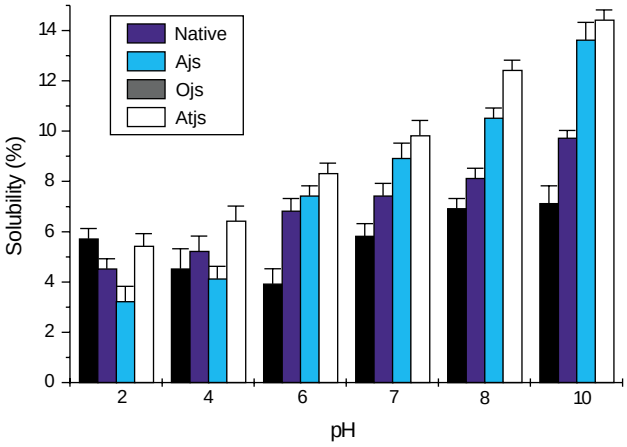

Fig. 7. Effect of pH on solubility of native, acetylated, oxidised and acid thinned jack bean starch. Error bars: Standard deviations Results are means of triplicate determinations Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch.

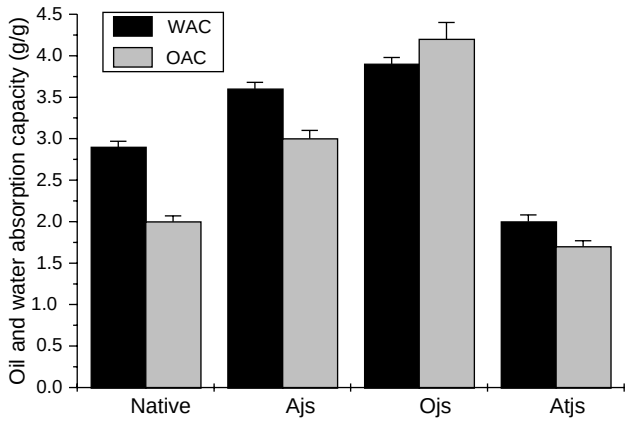

Fig. 8. Water and Oil absorption capacity of native, acetylated, oxidised and acid thinned jack bean starch. Error bars: Standard deviations. Results are means of triplicate determinations. Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch.

(Fig. 8). This result agrees with observations reported on the water and oil absorption capacities of modified bambarra ground nut starch (Adebowale et al., 2002) cocoyam starch (Lawal, 2004) and rice starch (Gonzalez & Perez, 2002). Following acetylation, acetyl groups were introduced on the starch molecules and following oxidation, carboxyl and carbonyl groups were also introduced. We believe that the introduction of these bulky groups caused electrostatic repulsion among starch molecules, thereby facilitating access of water and oil into the starch matrices. However, increased in starch crystallinity as earlier suggested, probably restricted access of oil and water into the granules of the acid thinned starch derivative.

3.6. Gelation properties

Gelation properties of native, oxidised, acetylated and acid thinned starches of jack bean are presented in Table 2. The least gelation concentration (LGC) was used as the index of gelation. The lower the LGC, the better the gelating property of the starch. The result obtained indicates that LGC increased following oxidation but reduced after acid thinning. Both acetylated starch and native starch had LGC value of 8 but at 6 %w/v, native starch appeared viscous while acetylated starch remained a liquid. In addition, a firm gel was formed in native starch at 12 %w/v concentration. These observations suggest that native starch could have better gelating properties than the acetylated starch. Among the starches, Atjs had the least LGC. During starch gelation process, gelatinization, swelling and absorption of water to build a three-dimensional network takes place. The building of the structural network involves as well, the bridging of the intergranular binding forces among the starch molecules, which largely involves hydrogen bonding. Following oxidation, introduction of carbonyl and carboxyl groups probably limited this interaction and caused electrostatic repulsion among the starch molecules, thus increasing LGC values. Contrarily, acid thinning improved gelation property. Similar results have been obtained on improvement of gelation capacity of corn starch, potato starch and rice starch following acid thinning (Wang & Wang, 2001).

3.7. Pasting properties

Pasting temperature (T_p) of the native starch reduced following acetylation and oxidation but increased after acid thinning (Table 3). Peak viscosity (Pv), hot paste viscosity (Hv), hot paste viscosity after 30 min holding (Hv_{30}) and cold paste viscosity (Cv) also reduced after modifications. The reduction in pasting temperature lends credence to the notion that modification processes vitiate the strength of intragranular binding forces, thus making the starch respond to heat and loose birefiringence at lower temperature compared to the unmodified starch. Set back value, a measure of starch retrogradation increased in Atjs compared with native starch. However, both oxidation and acetylation reduced the starch's tendency to retrograde as indicated by the reduction in setback value from 875 BU in native starch to 805 and 780 BU in Ojs

Table 2
Gelation properties native, oxidised, acetylated and acid thinned Jack bean starch

Concentration (% w/v)	Starch sample			
	Native	Ojs	Ajs	Atjs
2	−Liquid	−Liquid	−Liquid	−Liquid
4	−Liquid	−Liquid	−Liquid	+Gel
6	−Viscous	−Liquid	−Liquid	+Gel
8	+Gel	−Liquid	+Gel	+Firm gel
10	+Gel	+Gel	+Gel	+Firm gel
12	+Firm gel	+Gel	+Gel	+Very firm gel
14	+Very firm gel	+Firm gel	Firm gel	+Very firm gel
LGC[a]	8	10	8	4

Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch.
[a] Least gelation concentration.

Table 3
Pasting Characteristics of native, oxidised, acetylated and acid thinned starches of Jack bean

Sample	Parameter						
	T_p (°C)	Pv (BU)	Hv (BU)	HV_{30} (BU)	Cv (BU)	SB (BU)	BD (BU)
Native	84	645	615	550	1520	875	95
Ojs	82	545	530	405	1350	805	140
Ajs	83	560	540	440	1340	780	120
Atjs	86	440	415	325	1450	1010	115

Values are means of triplicate determinations. T_p, initial pasting temperature; Pv: peak viscosity during heating; Hv: hot paste viscosity (at 95 °C); Hv_{30} viscosity after 30 min holding at 95 °C; Cv: cold paste viscosity (at 50 °C); SB, setback value = Cv − Pv; BD, breakdown = Pv − Hv_{30}; BU, Brabender unit. Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs: Acetylated jack bean starch.

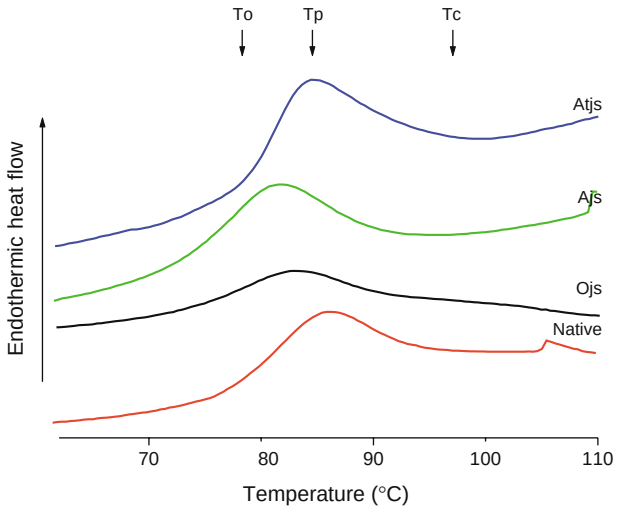

Fig. 9. DSC gelatinization thermograms of native, oxidised, acetylated and acid thinned jack bean starch. Ojs, oxidised jack bean starch; Ajs, acetylated jack bean starch; Atjs, acid thinned jack bean starch. T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature.

and Ajs respectively. Similar result has been obtained in modified cocoyam starch (Lawal, 2004) and cornstarch (Pomeranz, 1991). Following oxidation and acetylation, electrostatic repulsion among the starch molecules led to a reduction in reassociative tendency in starch gels and these resulted in a reduction in starch viscosity. As observed in this work, breakdown value (BD) defined as the difference between the peak viscosity and the viscosity after holding for 30 min at 95 °C (Muhammad, Hussin, Man, Ghazali, & Kennedy, 2000) and a measure of fragility of the starch (Thayumanavan & Kumari, 1998) increased following modifications. This is probably as a result degradation that occurred in modified starches during the processes of modifications.

3.8. Gelatinization and retrogradation studies

Gelatinization endotherms of native and modified starches are presented in Fig. 9. The corresponding enthalpies associated with gelatinization are also presented in Table 4, while values for onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) and gelatinisation temperature range are presented in Table 5. The results indicate that gelatinization enthalpy reduced from 0.75 J/g in native starch to 0.54 and 0.56 J/g in Ojs and Ajs, respectively. However, enthalpy of gelatinization increased in acid thinned starch compared with native starch. Onset temperature (T_o) shifted to lower values in Ajs and Ojs compared to native starch but increased following acid thinning. When starch pastes were stored for seven days to monitor retrogradation, regelatinization enthalpies reduced compared with gelatinization enthalpy obtained for each starch on the first day. It was also observed that enthalpy of regelatinization as well as percentage retrogradation (%R) increased as storage days increased. Regelatinization endotherms as depicted in Figs. 10 and 11 show that onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) shifted to lower values compared to gelatinization endotherm of the first scan. It has been reported that starch molecules recrystallization occurs in less ordered manner in stored starch gels than in native starches (Morikawa & Nishinari, 2000). This accounts for reductions observed in regelatinization enthalpies compared with gelatinization enthalpy. Increases observed in regelatinization enthalpies and *%R* with storage days confirms that retrogradation is a time dependent process. In this sense, starch recrystallization increased with time. *%R* values were lower in Ajs and Ojs compared with native starch, these observations could be attributed to substitution of hydroxyl groups on the starch molecules with acetyl groups in Ajs and carbonyl and carboxyl groups in Ojs. Since starch retrogradation involves reaggregation of starch molecules in stored gels, the process uses hydrogen bonding as a vital tool for recystallisation, hydrogen bonding was restricted in Ojs and Ajs as result of inter and intramolecular electrostatic repulsion in the starch molecules.

Table 4
Enthalpies associated with gelatinization and retrogradation of native, oxidised, acetylated and acid thinned Jack bean starch

Starch sample	Enthalpy (ΔH J/g).			Percentage retrogradation	
	1st day scan	2nd day scan	7th day scan	$\%R_2$	$\%R_7$
Native	0.75	0.19	0.35	14	47
Ojs	0.54	0.09	0.20	17	37
Ajs	0.56	0.11	0.23	20	41
Atjs	0.81	0.24	0.41	30	51

Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs: Acetylated jack bean starch $\%R_1$, percentage retrogradation after 2nd day $\%R_2$, percentage retrogradation after 7th day.

Table 5
Temperature and temperature range associated with gelatinization and retrogradation of native, oxidised, acetylated and acid thinned Jack bean starch

Starch sample	1st day scan				2nd day scan				7th day scan			
	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)
Native	76	86	95	19	47	52	55	8	58	67	74	16
Ojs	74	83	89	15	42	47	53	11	53	63	77	24
Atjs	80	86	97	17	45	51	55	10	56	67	75	19
Ajs	72	82	93	21	43	52	57	14	55	64	69	14

Ojs, oxidised jack bean starch; Atjs, acid thinned jack bean starch; Ajs, acetylated jack bean starch.

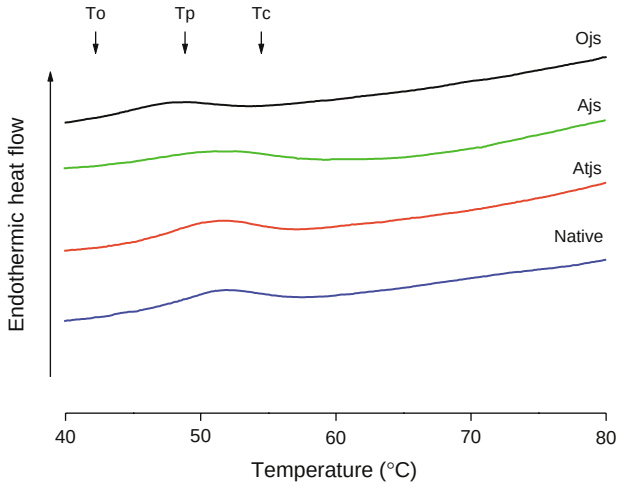

Fig.10. DSC regelatinization thermograms of native, oxidised, acetylated and acid thinned jack bean starch after storage for two days. Ojs, oxidised jack bean starch; Ajs, acetylated jack bean starch; Atjs, acid thinned jack bean starch. T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature.

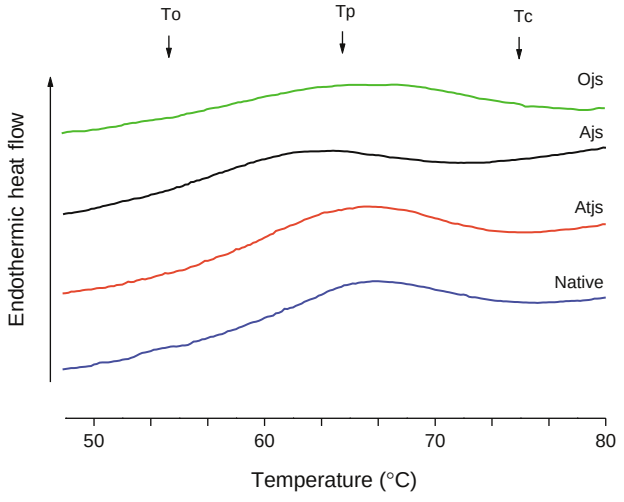

Fig. 11. DSC regelatinization thermograms of native, oxidised, acetylate-dand acid thinned jack bean starch after storage for seven days. Ojs, oxidised jack bean starch; Ajs, acetylated jack bean starch; Atjs, acid thinned jack bean starch. T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature.

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