RESEARCH ARTICLE

Hydroxypropyl derivatives of legume starches: Functional, rheological and thermal properties

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Hydroxypropyl derivatives were prepared from the native starches isolated from lima bean and jack bean. Both native and hydroxypropylated starches were evaluated for functional, rheological and thermal properties. Molar substitution (MS) value ranged from 0.24 to 0.37 while degree of substitution (DS) from 0.02 to 0.04. The values of swelling power, solubility and pasting parameters of the native starches were significantly different from the hydroxypropyl derivatives with deviations of insignificant difference obtained for lima bean starches. Non-Newtonian shear-thinning behaviour was exhibited by all the samples. Their viscosity consistency, K, deduced from Herschel–Bulkley model, increased progressively with MS. Starches at low-substitution possessed high shear viscosities compared to the native and high-substituted starches. Increase in yield stress (σ_o) was observed at low-substitution level and decrease at high-substitution level of hydroxypropylation. Upon hydroxypropylation, enthalpies of gelatinisation (ΔH_{gel}) increased whereas the transition gelatinisation temperatures (T_o, T_p and T_c) decreased. Comparatively, lower values of transition temperature and enthalpy were obtained for retrograded starch gels than the gelatinised gels. Upon hydroxypropylation, native starches experienced increase in MW and polydispersity index (PDI). All the native starches exhibited type-A XRD patterns, which remained unchanged after hydroxypropylation.

1 Introduction

Starch is a versatile biopolymer that has found numerous applications in many industries due to its environmental friendliness and availability. Such applications range from food to non-food industries. In its native form, starch suffers limitations under rigorous industrial processing. Such limitations can be eliminated or reduced significantly through modification. There are several known modification processes such as hydroxypropylation, oxidation, crosslinking, annealing, HMT, pregelatinisation and enzymatic (or acid) hydrolysis of starch. Unlike physical modification processes, chemical modification causes structural alterations, and it introduces new functional groups, which, consequently alter the physicochemical properties of the starch and making it appropriate for various industrial uses [1].

Hydroxypropylated starches are a popular type of chemically modified starch, which are important in food applications due to their relatively low pasting temperature, high paste clarity and desirable low-temperature storage stability [1]. Hydroxypropylation of starch is achieved by etherification process with propylene oxide as the etherifying reagent, which causes the introduction of hydroxypropyl groups onto the polymeric chain of starch [2]. Incorporation of hydroxypropyl groups in the starch chains has been reported to cause reduction of retrogradation, gelatinisation temperature, paste viscosity and clarity [3–5]. The alteration of starch properties by hydroxypropylation does not only dependent on the molar substitution (MS), but on the botanical source as well [6]. Hydroxypropylation of starches

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Abbreviations: DS, degree of substitution; **LS,** level of substitution; **MS,** molar substitution; **PDI,** polydispersity index; **SP,** swelling power

Colour online: See the article online to view Fig. 1 in colour.

has been reported for corn [1], rice [4, 7], maize [8, 9], potato [10, 11], wheat [3], amaranth [6], mung bean [1, 12] and saba banana [13].

In spite of previous studies on hydroxypropylation of starches from major sources, especially corn, potato and wheat, the quest for starch and its derivatives is ever-increasing in the global market. Recently, exploring underutilised crops as possible alternative to the commercial starches has been reported for pigeon pea by Lawal [2]. There are several of such underutilised crops that can be investigated, which are, hitherto, considered wild or abandoned due to laborious processing. Sourcing for alternative starches in addition to the highly commercial maize, potato and cassava starches is imperative in order to meet the high demand for starch for both food and non-food applications.

It is in this light this study came up. In this present study, lima bean (*Phaseolus lunatus*) and jack bean (*Canavalia ensiformis*) seeds were investigated with the view to isolating their native starches, preparing their hydroxypropyl derivatives at low and high substitution, and evaluating the native and modified samples for functional, rheological and thermal properties. Unveiling the latent potentials of the native and hydroxypropyl derivatives of these starches would, in no doubt, be a relief to competitiveness of starch and serve as means of wealth and job creation, especially in the developing countries.

2 Materials and methods

2.1 Materials

The seeds of lima bean (*P. lunatus*) were purchased at Jattu Market, Edo State, Nigeria while seeds of jack beans (*C. ensiformis*) were harvested from the residential orchard of one of the authors (Abraham Olasupo Oladebeye), Auchi, Nigeria. All the reagents used were analytical grades.

2.2 Isolation and purification of native starch

Starches from the legumes were isolated following the method described by Galvez and Resurreccion [12]. Figure 1 shows the general scheme used for the extraction.

2.3 Preparation of hydroxypropyl derivatives

The method described by Choi and Kerr [11] was used to prepare the hydroxypropyl derivatives of the native starches with some modifications. Fifty grams of dry starch was dispersed in 110 mL distilled water containing 10 g Na_2SO_4 in a 250 mL screw-cap jar. The jar was screwed with cap and the slurry stirred at 150 rpm at 35°C in an orbital incubator shaker (SI-600R, JEIO Tech, Seoul, Korea) for 40 min. pH of the slurry was brought to pH 11 by adding few drops of 1.0 M NaOH. Propylene oxide (10 and 20%, db) was added and the jar was immediately capped, shaken vigorously at 150 rpm and incubated at 35°C for 24 h to prevent sedimentation to obtain a suspension, which was brought to pH 5.5 with 1.0 M HCl. The resulting starch slurry was rinsed with distilled water, centrifuged several times for 15 min at 2300*g*. The starch cake was isolated and oven-dried at 40°C for 2–3 days and mill-ground and sieved to the size of 250 μm.

2.4 Determination of molar substitution and degree of substitution

One hundred milligrams of dry hydroxypropylated starch was weighed into a 100 mL volumetric flask and 25 mL of 0.5 M H_2SO_4 was added. A sample of the native starch was prepared in the same manner. The flasks were placed in a boiling water bath and heated until clear solutions were obtained. The resulting clear solutions were cooled and made up to 100 mL with distilled water. One millilitre of each of the solutions was pipetted into 25 mL graduated test tube with glass stopper. The tubes were immersed in cold water and 8 mL concentrated H_2SO_4 was added dropwise to each tube. After thorough shaking, the tubes were placed in boiling water-bath, one after the other, for exactly 3 min. Immediately, the tubes were transferred to an ice-bath until the solutions were chilled. Ninhydrin reagent (0.6 mL) was carefully added by allowing the reagent to run down the walls of the test tubes. Immediately, the test tubes were shaken well and placed in a water-bath for 100 min at 25°C. The volume of the solution in each tube was adjusted to 25 mL with concentrated H_2SO_4, followed by mixing by inverting the tubes several times. The tubes were not shaken. Immediately, portions of the solutions were transferred to 1-cm cells designed for a Beckman Model B Spectrophotometer, and after 10 min, the absorbance was taken at 590 nm, using the starch blank as the reference. A calibration curve was prepared with 1-mL aliquots of standard aqueous solutions containing 10, 20, 30, 40 and 50 μg of propylene glycol per mL [14].

$$\text{Hydroxypropyl group, } HP(\%) = \frac{C \times 0.7763 \times 10 \times F}{w} \quad (1)$$

where c is amount of propylene glycol in the sample read from the calibration curve; F is dilution factor (if a further dilution has been necessary); w is weight of the sample.

MS was deduced using the equation,

$$MS = \frac{162W}{100M - (M-1)W} \quad (2)$$

where W is equivalent hydroxypropyl group in 100 mg of starch and M is molar mass of propylene glycol (C_3H_6O).

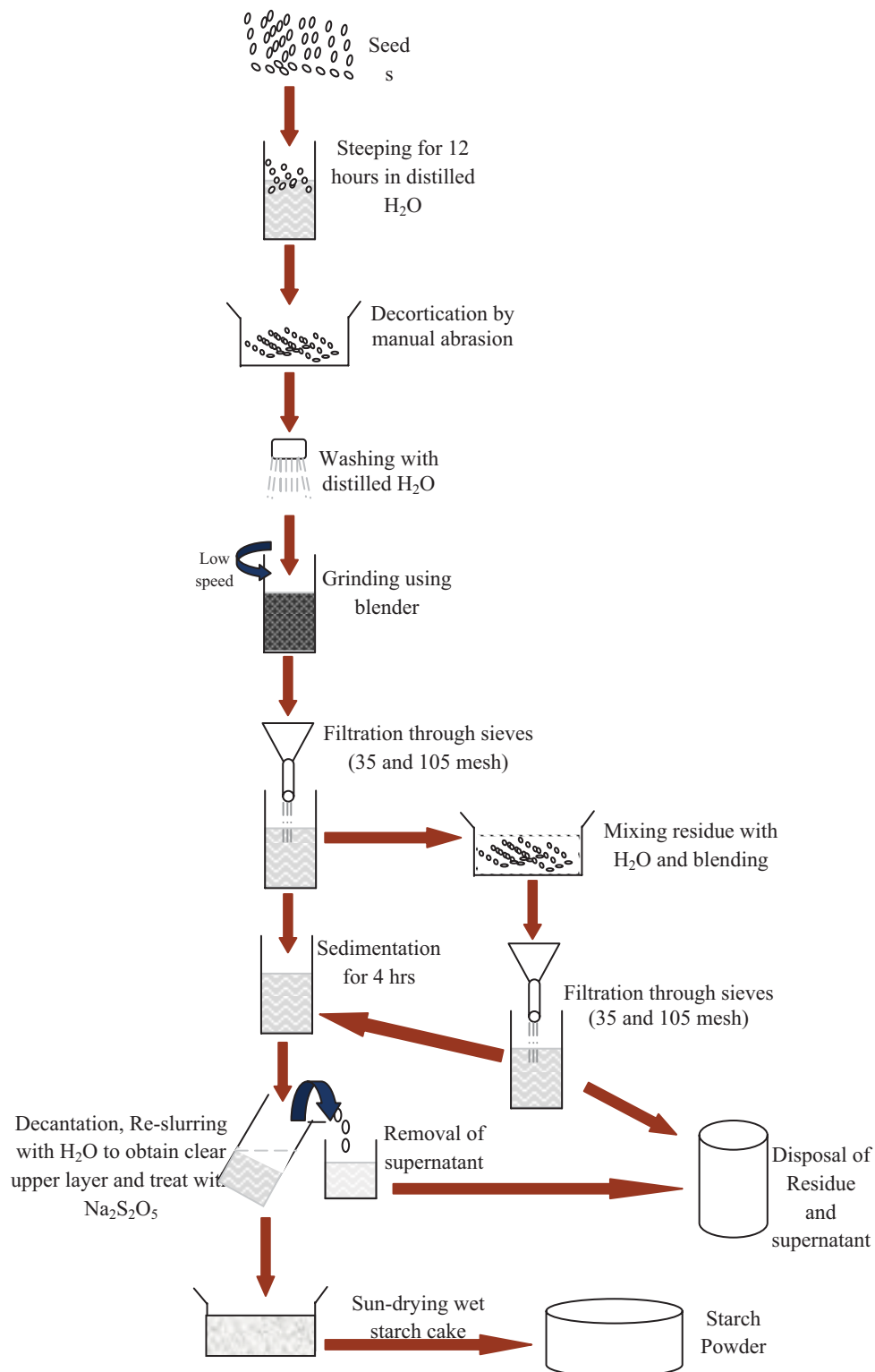The degrees of substitution, DS of the starches were estimated from their hydroxypropyl contents, using

Figure 1. Schematic diagram of isolation and purification of native from legumes.

Wurzburg's equation [15]:

$$DS = \frac{162 \times \left(\frac{\%HP}{58}\right)}{100 - \left[\frac{57}{58} \times \%HP\right]} \quad (3)$$

2.5 Functional properties

The chemical method described by Schoch [16] was adopted for the determination of both swelling power and solubility of starch samples. Swelling power was expressed as the ratio of the weight of the difference between the weight of swollen sediment and dry test tube to the initial weight of dry starch. The solubility was expressed as the ratio of dried supernatant weight initial dry starch weight.

The pasting profiles (pasting temperature, peak viscosity, hot paste viscosity, breakdown, cold paste viscosity, setback and peak time) of the starches were determined using the Rapid Visco Analyzer (model RVA Series 4, Newport Scientific Pvt. Ltd, Warriewood, Australia) following the method described by Karim et al. [1] with some modifications. The samples (8% w/w, db) were equilibrated at 50°C for 1 min and then raised to 95°C in 3.75 min, held for 2.5 min, cooled to 50°C in 3.75 min, and held for 5 min. The paddle speed was set at 960 rpm for the first 10 s to evenly disperse the starch slurry and reduced to 160 rpm throughout the remainder of the experiment. The units of viscosity were expressed as rapid visco units (RVUs).

2.6 Molecular mass distribution

The standard method described by Politz et al. [17] was adopted for the gel permeation chromatographic analysis of the starch samples with some modifications. Forty-five milligrams of the starch sample were added to 5 mL of dimethylacetamide (DMAC) in 10 mL Reacta-Vials (Pierce, Rockford, IL) in a heating block. The temperature was raised to 150°C for 1 h 15 min, cooled to 100°C and dried LiC1 (to 8% w/v) was added. Further temperature lowering to 50°C was ensured with continuous stirring overnight before incubating until clear solutions were obtained. The resulting solutions were quantitatively diluted to 50 mL with DMAC. Prior to injection, solutions were filtered in vacuo through Teflon solvent-resistant disposable filters (Millex SR, 0.5 pm, Millipore) using 4 mL glass vials (WISP, Waters) in a Baker 10 extraction apparatus fitted with glass syringes (10 cm^3). The mobile-phase solvent for GPC was DMAC containing 5% LiCl. The GPC system consisted of an automatic sampler (Waters WISP) with an HPLC pump (Waters Model 590), detected by multi-angle laser light scattering (MALLS) (DAWN EOS, Wyatt Technology Corp, Santa Barbara, CA) and differential RI (Waters Corp., Milford, MA) detectors. The RI calibration constant was measured with a series of NaCl standards. The 90° photodiode detector of MALLS was calibrated using toluene (HPLC grade). This system was equipped with four columns

(Ultra-styragel 10^3, 10^4, and 10^5 (Baxter, Muskegon, MI) and 10^6 (Phenomenex, Torrance, CA)) connected in series and preceded by a guard column (Phenogel, linear, Phenomenex). The system was maintained at 50°C. Standard injection volume was 40 μL and the mobile phase was pumped at a rate of 1.0 mL min^{-1}. Run times were 60 min. The software package Unical based upon ASYST (Unical, Version 3.02, Viscotek) was used for data acquisition and analysis. The system was calibrated with the polystyrene standards. Data were obtained from two dissolutions per sample with two GPC runs per dissolution.

2.7 Flow behaviour measurement

The flow behaviours of the starches were measured using CSL^{2}100 Carri-Med Rheometer (AR 1000, TA Instruments Ltd, New Castle, DE, USA) with plate–plate geometry (40 mm diameter) at a gap of 55 μm by adopting the method of Lee et al. [18] with some modifications. Starch sample (5% w/w) was weighed into a 100 mL screw-cap conical flask and topped with deionised water to a total of 30 g. The mixture was heated on a hot plate while stirring with a magnetic bar at 160 rpm until the suspension turned from opaque to translucent solution. The flask was immediately transferred into a water-bath preset at 95°C and held for 15 min. The flask was subsequently transferred to another water-bath preset at 80°C for another 10 min for full viscosity development. The sample was held at 80°C throughout the experiment to prevent gelatinisation. Continuous shear test was carried out by monitoring the shear rate from 0 to 900 s^{-1} in 180 s and subsequently return to the initial rate. With this, a plot of shear stress (or viscosity) and shear rate was obtained. Torque was fixed at logarithmic ramp to minimise the inertia effect of the rheometer. Herschel–Bulkley rheological model, $\sigma = \sigma_o + K\gamma^n$, was used to express the flow behaviour parameters. Each sample was freshly prepared and all measurements were carried out at 25°C and in triplicates.

2.8 Thermal properties

The gelatinisation and retrogradation characteristics of the native starches and their hydroxypropyl derivatives were studied using a Differential Scanning Calorimeter (DSC-Q100, TA Instruments Ltd) by adopting the method of Chan et al. [19]. To study the gelatinisation profiles of the samples, starch slurries were prepared at 1:3 dry starch/water ratios, hermetically sealed using a DuPont encapsulation press (DuPont Co., DE, USA), and reweighed. Samples were heated at a rate of 5°C/min from 20 to 100°C. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinisation (ΔH_{gel}) were calculated. Enthalpies were calculated on a dry starch basis. Thereafter, the gelatinised starch samples (in the original sealed pan) were stored at 4°C for 7 days for retrogradation

studies. After the storage period, the samples were removed and allowed to equilibrate at room temperature for 1 h before being rescanned using the DSC with the same heating programme. After 7 days, samples were removed and allowed to equilibrate at room temperature for 1 h before being rescanned using the DSC with the same heating programme. Likewise, Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of retrogradation (ΔH_{ret}) were evaluated automatically and percentage of retrogradation (%R) was calculated as:

$$\%R = \frac{\text{Enthalpy of retrogradation}}{\text{Enthalpy of gelatinization}} \times 100 \qquad (4)$$

2.9 X-ray diffraction patterns

The X-ray patterns of the native and hydroxypropylated starches were studied using the method described by Jayakody et al. [20] with some modifications. The starch samples were equilibrated above distilled water in a desiccator for 3 days. The hydrated starch powders were packed in tightly sealed glass dishes and patterns were obtained with Ni-filtered Cu Kα radiation of wavelength 0.1542 nm using a D-5000 Siemens Diffractometer (Madison, WI, USA) by exposing the samples to the X-ray beam from an X-ray generator running at 40 kV and 60 mA. The scanning regions of the diffraction angle 2θ were 1.5–60° at 0.05 step size with a count time of 3 s. From the resulting X-ray patterns, peak positions were identified using the instrument's software and these peak positions were used to determine the crystalline nature.

2.10 Statistical analysis

Means were compared using Duncan's least significant test at the 5% significance level. SPSS 12.0 software (SPSS, Inc., Chicago, IL) was used to analysis the data for Pearson Correlation coefficients.

3 Results and discussion

3.1 Molar substitution (MS) and degree of substitution (DS)

MS, the measure of the average number of hydroxyl unit that are derivatised by substituent groups [2], was elevated as the level of substitution increased, ranging from 0.24 to 0.37 (Table 1). The peak MS value was obtained for high-substituted lima bean starch and the lowest for low-substituted jack bean starch. These differences may be adduced to different access of propylene oxide into the interior or subsurface of the starch granules, which is a function of varying degrees of inter- and intra-molecular forces of the molecules. The range of MS for the starches was within the permissible limit of

Table 1. Molar substitution, degree of substitution, swelling power and solubility of starch samples[a),b)]

Starch	LS	MS	DS	SP (g/g)	Solubility (%)
Lima Bean	Native	–	–	11.59 ± 0.03[a]	4.78 ± 0.02[a]
	Low	0.26	0.02	11.69 ± 0.08[a]	6.20 ± 0.07[b]
	High	0.37	0.03	11.69 ± 0.15[a]	11.65 ± 0.08[c]
Jack Bean	Native	–	–	10.69 ± 0.07[c]	6.17 ± 0.07[b]
	Low	0.24	0.02	9.80 ± 0.11[a]	5.53 ± 0.04[a]
	High	0.30	0.03	10.29 ± 0.04[b]	12.57 ± 0.07[c]

a) LS – level of substitution, i.e. percentage of propylene oxide at dry basis: 10% db (low), 20% db (high); MS – molar substitution; DS – degree of substitution; SP – swelling power.
b) Results are the means of triplicate determinations ± SD ($n = 3$). Values in the same column with the same superscript letters are not significantly different ($p < 0.05$).

$\leq$7% hydroxypropyl group by US Food and Drug Administration for the use of hydroxypropylated starch in food application [21]. Likewise, the degrees of substitution (DS) for all the starches varied between 0.02 and 0.03, which is below the limit of DS for all commercial food grade starches stipulated at DS $\approx$ 0.1–0.3 [22]. Hydroxypropyl derivatives of these starches can be used as indirect food additives such as thickeners in fruit pie fillings, puddings and gravies.

3.2 Functional properties

There was insignificant difference in the swelling behaviour of the native starch from lima bean upon hydroxypropylation whereas the hydroxpropyl derivatives of jack bean behaved differently from the native starch (Table 1). The data obtained showed that the native starches possess higher swelling power than their hydroxypropyl derivatives of jack bean. The insignificant differences in swelling powers of the native and hydroxypropylated starches could be adduced to the resistance of the starch granules in compromising their structure in spite of the introduction of hydroxypropyl groups, which could have resulted in the disruption of the hydrogen bonds in the glucan chains. Thus, the matrix of the granule structure was preserved upon hydroxypropylation. Strong bonded micellar network, amylopectin molecular structure and amylose content have been reported as influence on swelling properties of starches [23, 24]. These observations compare favourably with the report by Singh et al. [25] that granular stability increases when long amylopectin chains recrystallise, thereby lowering the extent of granular swelling.

The solubility profiles of the native and their hydroxypropyl starches from lima and jack beans were significantly different (Table 1). Generally, the impact of hydroxypropylation was better in high-substituted starches than the low-substituted from both starch sources. The differences in solubility of the starches could largely be due to structural differences, differences in chain length distributions and granular size [26–28]. The decreased solubility could suggest

Table 2. RVA results of native and hydroxypropylated starch samples

Starch	LS	Peak viscosity (RVU)	Hot paste viscosity (RVU)	Breakdown (RVU)	Cold Paste Viscosity (RVU)	Setback (RVU)	Peak time (min)	Pasting temperature (°C)
Lima Bean	Native	84.67 ± 4.47[b]	63.63 ± 2.05[c]	21.03 ± 2.44[a]	74.28 ± 2.96[a,b]	10.64 ± 0.97[a]	5.01 ± 0.10[c]	86.54 ± 0.75[c]
	Low	105.88 ± 2.30[c]	57.71 ± 1.47[b]	48.17 ± 0.83[c]	95.54 ± 2.89[c]	37.83 ± 1.41[c]	4.15 ± 0.04[b]	82.35 ± 0.07[b]
	High	78.36 ± 2.25[a]	45.67 ± 1.34[a]	32.69 ± 0.92[b]	72.45 ± 2.71[a]	26.78 ± 1.38[b]	3.87 ± 0.04[a]	76.24 ± 0.41[a]
Jack Bean	Native	32.52 ± 1.99[a]	29.50 ± 1.80[b]	3.03 ± 0.65[a]	38.92 ± 2.30[a]	9.42 ± 1.82[a]	5.65 ± 0.88[c]	86.31 ± 1.96[c]
	Low	35.00 ± 0.50[b]	30.22 ± 0.51[c]	4.78 ± 0.13[b]	54.36 ± 1.29[b,c]	24.14 ± 0.79[b]	4.63 ± 0.08[b]	82.46 ± 0.10[b]
	High	37.80 ± 0.25[c]	28.83 ± 0.17[a]	8.97 ± 0.19[c]	54.72 ± 0.67[b]	25.89 ± 0.59[c]	3.94 ± 0.10[a]	74.59 ± 0.48[a]

Results are the means of triplicate determinations ± SD ($n = 3$). Values in the same column with the same superscript letters are not significantly different ($p < 0.05$).

an incomplete weakening of the granular structure of the starch despite the introduction of hydroxypropyl groups.

The pasting properties of the native starches and their hydroxypropyl derivatives are depicted in Table 2. The pasting temperatures of the native starches consistently and significantly reduced as MS varies. Notably, consistently progressive decrease in the pasting temperature was observed between the low- and high-substituted hydroxypropylated starches with respect to increase in MS. Decreased range from 4.19 to 10.30°C was observed for lima bean starch and 3.85 to 11.72°C was observed for jack bean starch. The decrease in pasting temperature of the starch after modification could be due to the weakening of the starch granules during the modification process. The lowering of pasting temperature of low-substituted hydroxypropylated starches (MS≤0.2) has also been reported for waxy and normal corn, amaranth and potato [6, 29, 30].

The peak viscosity of the native starches general increased upon hydroxypropylation with slight deviation in high-substituted lima bean starch. Chuenkamol et al. [31] have opined that the lowered pasting temperature and increase in peak viscosity are a reflection of decrease in the strength of the associative bonding forces within the starch granules and could be ascribed to the hydrophilic nature of the substitutes. Incorporated hydroxypropyl groups facilitate the penetration and absorption of water into the starch granules and increase the initial rate of plasticisation of the amorphous regions of modified granules [3].

Hot paste viscosity reduced upon modification for the native starches with slight deviation observed at low-substitution level for jack bean starch. Significantly, cold paste viscosity, also known as final viscosity increased in jack bean starch upon modification whereas lima bean starch showed mixed trend of both elevation and lowering of cold paste viscosity at low and high substitution levels respectively.

Breakdown is a measurement of the starch granule stability during the pasting process. Extensive breakdown occurs when the starch granule swells to maximum volume but lacks the ability to retain its structure and subsequently collapses. From Table 2, breakdown viscosity was on increase

among all the native starches after hydroxypropylation. It has been suggested that introduction of hydroxypropyl groups reduces associative forces within the starch granule [32]. This reduction in bond strength affects the hydroxypropyl starch, which cannot withstand heating and shear strain conditions. The observations obtained in this present study translated to reduced ability of hydroxypropyl starch to retain its swollen structure during the pasting process, resulting in more breakdown.

Setback, a measure of retrogradation, progressively increased after hydroxypropylation for native starches isolated from lima bean and jack bean. Increased setback viscosity could be ascribed to increased granule fragments or remnants. These fragments would be embedded in the matrix of the associated polymer network, thus enhancing the viscosity of the system [2].

The peak times for the hydroxypropyl starches obtained in this present work are lower than the corresponding native starches. The peak time implies a measure of rate of attainment of equilibrium point between swelling and polymer leaching, and rupture and polymer alignment. These observations were in compliance with the observations expressed for pasting temperature of these starches. The ranges of peak time with increasing MS for the hydroxypropylated starches were 3.87–4.15 and 3.94–4.63 min for lima bean and jack bean starches respectively. The differences in peak time of the starches could indicate differences in intrinsic behaviours and responses of the starch granules to paste formation and botanical variations.

From the foregoing, therefore, it is postulated that the starch granules of the low- and high-substituted starches of both sources seemed to respond differently to pasting processes under the same alkaline treatment of hydroxypropylation. Different botanical sources of the starches used in this study could contribute to these differences.

3.3 Molecular mass distribution

The MW, that is, average weight, M_w and number average, M_n, weight DP = DP_w, number average molecular = DP_n

and PDI = polydispersity index (M_w/M_n) of the native and hydroxypropylated starches are presented in Table 3. Low- and high-substituted lima bean and jack bean starches were respectively selected for analysis to explore more information on the mixed on integrity of the starch pastes as earlier discussed for data obtained in paste viscosity analysis. The values of M_w of the native starches significantly increased after hydroxypropylation while M_n respectively increased and decreased for lima bean and jack bean starches upon hydroxypropylation. The M_w increased relative to the native starches by 16.67% (lima bean) and 85.42% (jack bean) whereas M_n increased and decreased relative to the native starches by 8.74% (lima bean) and 43.52% (jack bean) respectively. The increase in M_w upon hydroxypropylation could suggest the reinforcement of the granule structure rather than the expected depolymerisation of the amylopectin chains in the starch granules. This structural reinforcement instead of depolymerisation could be responsible for the increased values of peak viscosity, cold paste viscosity, breakdown and setback for the lima bean and jack bean starches at low and high substitution levels respectively. Chen et al. [33] had reported that MW appeared to be an important factor affecting the pasting properties. In the same vein, DP_w increased for the two native starches after hydroxypropylation whereas DP_n increased and decreased for lima bean and jack bean starches respectively. PDI increased upon hydroxypropylation by 8.08 and 226.96% for lima bean and jack bean respectively relative to their native forms. This arbitrarily high increment could be due to the reorganisation and reinforcement of the fragments of the starch granules, which resulted in high MW and PDI.

3.4 Flow behaviours

Table 4 depicts the flow parameters, the consistency coefficients (K), flow behaviour indices (n) and yield stresses (σ_o) deduced from the flow curves by Herschel–Bulkley model for the native starches and their hydroxypropyl derivatives. The flow behaviours of starch pastes appear closely bound to the starch structure in the pastes. The starch paste is regarded as a blend of swollen granules, pieces of disrupted granules and starch molecules liberated from the granules and then solubilised; the ratio of these different fractions varied [18].

All the starches exhibited non-Newtonian behaviour, usually called pseudoplastic flow, because their viscosity values decreased with increase in shear rate (Fig. 2) and as evidenced by $n<1$ for all starches. Similar results had been reported for other starch pastes [18, 33–36]. The data obtained in this study showed that the hydroxypropylated starches had lower flow behaviour indices than their corresponding native starches with slight deviation for high-substituted lima bean starch. These implied that lower values of flow behaviour indices, n obtained for the hydroxypropylated starches made them exhibit more pronounced shear-thinning behaviours than the native, thereby confirming the data obtained for the starches in terms of peak viscosity (Table 2).

The consistency coefficients (K) of the native starches were favourably increased after hydroxypropylation. These could

Table 3. Molecular mass distribution of native and hydroxypropylated starches[a),b)]

Starch	LS	Weight average (M_w) $\times 10^6$	Number average (M_n) $\times 10^6$	[a]$DP_w \times 10^4$	[b]$DP_n \times 10^3$	PDI
Lima Bean	Native	3.06 ± 0.01	1.03 ± 0.01	1.89 ± 0.01	6.36 ± 0.01	2.97 ± 0.05
	Low	3.57 ± 0.03	1.12 ± 0.01	2.20 ± 0.01	6.91 ± 0.01	3.21 ± 0.01
Jack Bean	Native	3.43 ± 0.01	1.08 ± 0.01	2.11 ± 0.01	6.67 ± 0.01	3.19 ± 0.03
	High	6.36 ± 0.01	0.61 ± 0.01	3.93 ± 0.01	3.77 ± 0.01	10.43 ± 0.37

a) M_w = weight average; M_n = number average; DP_w = weight DP (calculated by dividing M_w by 162, i.e. the MW of anhydrous glucose); DP_n = number DP (calculated by dividing M_w by 162, i.e. the MW of anhydrous glucose); PDI = polydispersity index (M_w/M_n).
b) Results are expressed as means ± SD ($n = 2$).

Table 4. Herschel–Bulkley parameters for native and hydroxypropylated starch gels at 25°C

Starch	LS	σ_0 (Pa)	K (Pa s^n)	n
Lima Bean	Native	$2.86 \pm 0.08^{a,b}$	0.83 ± 0.03^a	0.62 ± 0.00^a
	Low	6.46 ± 6.00^a	1.97 ± 0.72^b	0.60 ± 0.02^a
	High	1.20 ± 0.05^a	1.13 ± 0.11^a	0.65 ± 0.01^b
Jack Bean	Native	0.64 ± 0.13^a	0.08 ± 0.02^a	0.76 ± 0.02^c
	Low	9.25 ± 0.84^b	0.86 ± 0.16^b	0.65 ± 0.00^b
	High	1.89 ± 0.23^b	1.42 ± 0.12^c	0.62 ± 0.01^a

Results are expressed as means ± SDs ($n = 3$). Values in the same column with the same superscript letters are not significantly different ($p<0.05$).
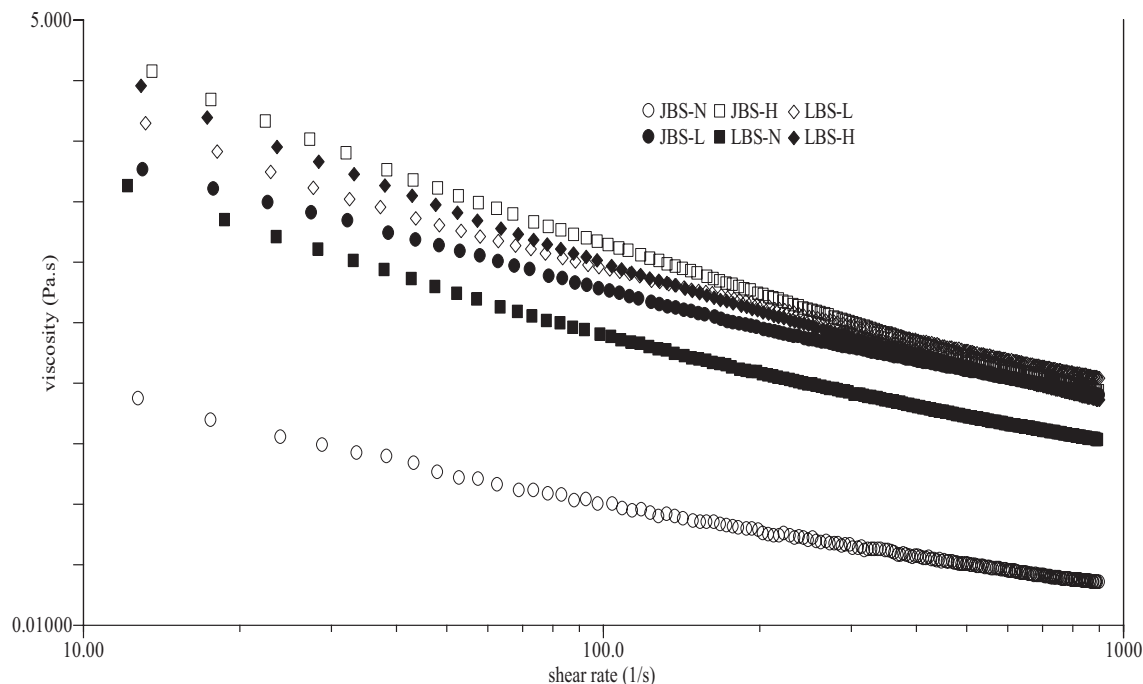
Figure 2. Effect of shear rate on shear viscosity of native and hydroxypropylated starches at 25°C (JBS-N – native jack bean; JBS-L – low substituted jack bean; JBS-H – high substituted jack bean; LBS-N – native lima bean; LBS-L – low substituted lima bean and LBS-H – high substituted lima bean).

be an indication that hydroxypropylation seemed to enhance the viscous properties of the native starches isolated from lima bean and jack bean. The yield stresses (σ_o) of the native starches experienced avalanche increase at low substitution level and abrupt drop at high substitution level. Miller et al. [37] had reported that high K and σ_o values are mainly due to the existence of interactions between swollen granules and/or between swollen granules and an extra granular network of exudates. This in turn means that the structural elements formed by molecular interactions that resist the flow are more when the viscosity of one starch pastes is high [38].

Shear viscosities (0–900 s^{-1}) of native and hydroxypropy-lated starches at 25°C are presented in Fig. 3. All the low-substituted starches showed significant rise in viscosity compared to the both the native and high-substituted starches. The increase in viscosity had earlier been adduced to increase in MW of the hydroxypropylated starches as a result of possible reorganisation and reinforcement of the gluten chains and its fragment instead of the expected depolymer-isation of the amylopectin chains (Table 3). A study of the rheological properties of the starch pastes also enables the engineering scale up in production [39], quality control as well as improvement of final products [40].

3.5 Thermal properties

The results of gelatinisation and retrogradation (after 7 days) studies of the natives starches and their derivatives are pre-

sented in Table 5. The gelatinisation temperatures, that is, onset, T_o; peak, T_p; and conclusion, T_c were significantly different among the starches and consistently decreased as level of substitution increased with slight deviation observed for lima bean at low level of substitution. The enthalpies of gelatinisation, ΔH_{gel} of the starches varied between 7.17 and 16.44 J/g. The low-substituted lima bean and high-substituted jack bean starches expressed the effect of hydroxypropylation on thermally treated starches. They showed appreciable rise in ΔH_{gel} compared to their corre-sponding native starches. The data obtained in this work partially agreed with the report of Gunaratne and Corke [41] for hydroxypropylated potato, true yam, gourd yam and taro starches. They reported that after hydroxypropylation, the decreased gelatinisation temperatures and decreased enthal-pies in all the starches were anticipated, because extensively hydrated granules require less driving force and energy to achieve gelatinisation. According to Seow and Thevamalar [4], hydroxypropylation facilitates water penetration and absorp-tion into the starch granules and increases the initial rate of plasticisation of the amorphous region, promoting gelatini-sation. In addition, Perera et al. [30] suggested that a possible disruption of double helices due to the rotation of the flexible hydroxypropyl groups within the amorphous region would leave fewer helices to melt during gelatinisation. The PHI, a measure of gelatinisation uniformity, consistently decreased among the starches as the level of substitution increased (Table 5). This observation was in opposite trend to the values
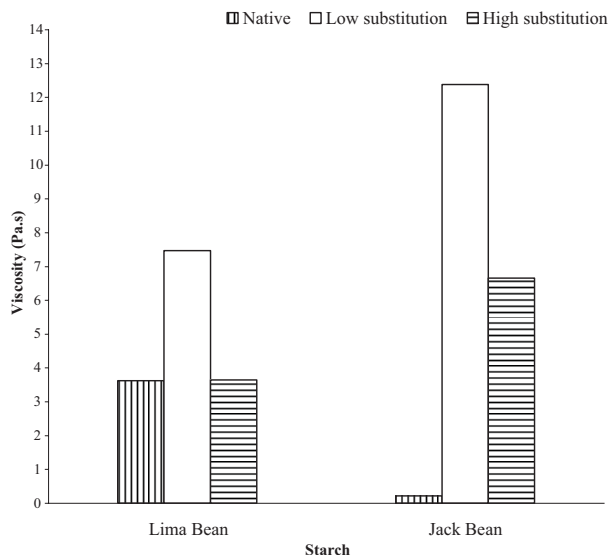
Figure 3. Viscosity native and hydroxypropylated (low- and high-substitution) lima bean and jack bean starches. Measurements were made at 25°C.

of temperature range, *R* of the starches. The high *R* values of the hydroxypropylated starches suggested the possible presence of crystallite of varying stabilities within the crystalline domains of the starch granules [42].

The results of retrogradation studies of the starches showed that the retrograded starch gels after 7 days of storage at 4°C showed insignificant differences in terms of T_p, T_c,

ΔH_{ret} and *R* for all the starches (Table 5). Onset temperature, T_o was significantly similar among the native and hydroxypropyl derivatives of jack bean starch, and significantly different among the native and hydroxypropyl derivatives of lima bean starch. Lima bean starch exhibited drop in %*R* at low substitution level of hydroxypropylation, but showed no significant difference at high substitution level. Likewise, jack bean starch exhibited elevation at high substitution level of hydroxypropylation, but showed no significant different at low substitution level. However, the transition temperatures, temperature ranges and enthalpies of transition of the retrograded starch gels after 7 days of storage at 4°C were lower in values than the gelatinised starch gels. This might be due to the fact that recrystallisation of amylopectin branched chains occurred in a less ordered manner in stored gels, as it is present in native form [41]. These observations compete favourably with the reports of Sandhu and Singh [43] for corn starches, and Lawal [2] for hydroxypropylated pigeon pea starch.

3.6 X-ray diffraction

The XRD patterns obtained for the native and hydroxypropylated starches are shown in Fig. 4. Low- and high-substituted lima bean and jack bean starches were respectively selected for XRD based on the earlier reasons adduced for the analysis of molecular mass distribution of the starches used in this study.

XRD peaks for the native starches appeared at 15.20°, 17.20° and 22.900° 2θ for lima bean, and 15.25°, 17.00°

Table 5. Gelatinisation and retrogradation profiles of native and hydroxypropylated starches[a),b)]

Starch	LS	Gelatinisation studies
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	R (°C)	PHI (J/g °C)
Lima Bean	Native	75.45 ± 0.77^c	79.89 ± 0.11^c	86.41 ± 0.68^b	8.34 ± 0.47^b	10.97 ± 1.45^a	1.89 ± 0.17^b
	Low	66.06 ± 0.13^b	75.43 ± 0.00^b	89.23 ± 0.21^c	16.44 ± 0.21^c	23.17 ± 0.08^b	1.76 ± 0.01^b
	High	59.36 ± 0.79^a	69.10 ± 0.71^a	83.17 ± 0.22^a	7.17 ± 0.23^a	23.81 ± 1.01^b	0.74 ± 0.03^a
Jack Bean	Native	77.54 ± 0.01^b	80.93 ± 0.15^b	88.99 ± 0.39^a	10.51 ± 0.71^a	11.45 ± 0.38^a	3.10 ± 0.07^c
	Low	72.00 ± 3.70^b	$76.28 \pm 4.45^{a,b}$	87.00 ± 1.99^a	11.16 ± 1.77^a	15.00 ± 1.75^a	2.61 ± 0.02^b
	High	62.22 ± 0.20^a	72.52 ± 0.42^a	86.32 ± 2.95^a	13.16 ± 2.20^a	24.10 ± 2.75^b	1.28 ± 0.18^a

Starch	LS	Retrogradation studies
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{ret} (J/g)	R (°C)	%R
Lima Bean	Native	43.01 ± 0.64^a	56.55 ± 0.00^a	71.67 ± 0.47^a	5.45 ± 0.13^a	5.44 ± 0.13^a	65.10 ± 2.40^b
	Low	$43.53 \pm 0.12^{a,b}$	59.48 ± 0.71^a	73.88 ± 1.56^a	6.23 ± 0.21^a	6.23 ± 0.21^a	37.89 ± 1.72^a
	High	45.09 ± 0.91^b	58.96 ± 5.23^a	72.77 ± 0.17^a	5.23 ± 1.08^a	5.22 ± 1.08^a	72.72 ± 12.75^b
Jack Bean	Native	44.62 ± 0.54^a	59.28 ± 0.13^a	71.53 ± 0.37^a	4.73 ± 0.37^a	4.73 ± 0.37^a	44.98 ± 0.47^a
	Low	45.17 ± 0.65^a	61.00 ± 0.00^c	72.18 ± 1.41^a	4.77 ± 0.56^a	4.77 ± 0.56^a	42.84 ± 1.78^a
	High	43.76 ± 0.33^a	60.62 ± 0.13^b	72.51 ± 1.32^a	6.88 ± 1.00^a	6.88 ± 1.01^a	52.40 ± 1.12^b

a) *R* – temperature range ($T_c - T_o$); PHI $\left(\frac{\Delta H}{T_p - T_o}\right)$; %*R* – percentage retrogradation.
b) Results are the means of triplicate determinations ± SD. Values in the same column with the same superscript letters are not significantly different (*p*<0.05).
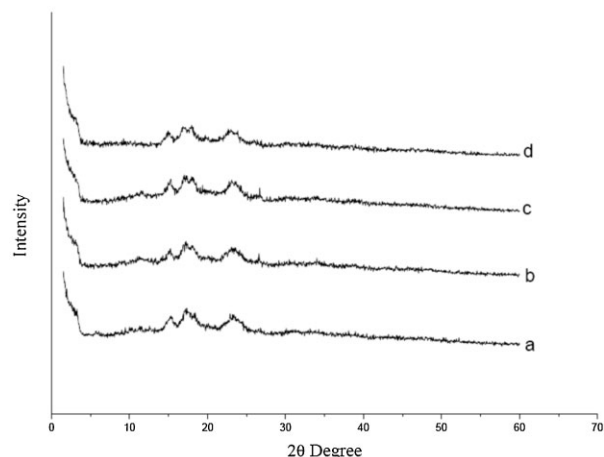
Figure 4. X-ray patterns of (a) native lima bean, (b) low-substituted lima bean, (c) native jack bean and (d) high-substituted jack bean starches.

and 22.75° 2θ for jack bean. These reflections indicated that the native starches were of A-type crystalline nature, which is typical of legumes. This was in agreement with the report of Lawal [2] for XRD patterns of the native pigeon pea starch, where a peak at 15°, a doublet at 17° and 18° and a single peak at 23° were obtained. It has been shown that type-A and type-B starches are based on the parallel stranded double helices in which the double helices are closely packed in type-A, but loosely packed in type-B, and that starches with short chain length (<20 residues) exhibit type-A crystallinity whereas those with longer average chain length of amylopectin exhibit type-B crystallinity [44, 45]. After hydroxypropylation, there were drops in the intensities for the starches, although the strongest and broadest peaks were singlet for lima bean at 17.25° 2θ and doublet for jack bean at 16.85° and 17.95° 2θ. These observations showed no pronounced pattern differences between the native and hydroxypropylated starches. Therefore, these observations concretised the fact that hydroxypropylation affects the amorphous component of the starch granules being more accessible to the reagents and consequently, the crystalline region is preserved [2].

4 Conclusions

Hydroxypropyl derivatives of the native starches extracted from lima bean and jack bean were prepared to unveil their latent potentials as alternative sources to versatile commercial starches for extensive uses in textile, pharmaceuticals, pulp and paper, paints, tissue engineering and other raw materials for polymer technologies in addition to the conventional food applications. To achieve these, comparative functional, rheological and thermal behaviours of both the native and hydroxypropylated starches were investigated. Low-substituted lima bean and high-substituted jack bean starches

exhibited pronounced effect of hydroxypropylation on the intrinsic behaviours of the starch granules. With the ever-increasing demand for starch in the global market, these underutilised starches in both their native and modified forms could serve as alternative sources of starch and add value to the socio-economic growth.

The authors have declared no conflict of interest.

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