FUNCTIONAL PROPERTIES OF ACETYLATED STARCHES OF TWO VARIETIES OF LOCAL COWPEAS

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ABSTRACT

This work studied the functional properties of native and acetylated carton brown and horse red cowpea starches as potential substitutes for conventional starches. Starch acetates were prepared by reacting previously isolated native cowpea starches with acetic anhydride using established method. Functional properties studied included the effects of temperature and pH on swelling power and solubility, water and oil absorption capacities, gelation properties, light transmittance and pasting properties. The starch acetates prepared had higher swelling and water absorption capacities than the native starches. Acetylation improved the gelling property of acetylated horse red starch. Light transmittance of all starches decreased with increase in the period of storage. Pasting properties of modified starches significantly reduced (p<0.05). Functional properties of native and acetylated cowpea starches showed that the local cowpeas are potential industrial raw materials.

1.0 INTRODUCTION

Starch, a naturally abundant polymer of plant origin, is an important raw material with versatile industrial applications. It exhibits a wide range of functional properties and it is probably the most commonly used hydrocolloid. Its use in its native form however, has limitations (Chiu & Solarek, 2009) such as insolubility in cold water, loss of viscosity and thickening power after cooking, retrogradation which results in syneresis (Lawal & Adebowale, 2005); thermal decomposition and low shear resistance (Xie & Liu, 2004) among others. Overcoming these limitations is usually achieved by modifications which introduce desirable alterations in the starch structure with a resultant ability to predict and control starch behaviour (Kaur, Singh & Singh, 2004).

Starch modifications are achieved by physical, chemical and biotechnological means. Derivatives obtained have varied uses in pharmaceutical/biomedical, food and non-food industries. They are used in pharmaceuticals as binders, disintegrants, fillers, glidants, lubricants (Olu-Owolabi, Afolabi & Adebowale, 2010), in body creams/lotions, blood plasma expanders, cryoprotective agents for erythrocytes, etc. In the food industry, they find applications as fat replacers, beverage concentrate flavour stabilisers, thickeners, gelling agents, salad dressing and mayonnaise etc. And in non-food applications they find use in water purification as biodegradable ion-exchange materials, metal scavengers and flocculants, binding agents in paper, sizing agents and improvers of printing dyes in textile industry, sanitary product manufacture, thermoset applications such as fibreglass insulation binders, plywood adhesive resins, fluid loss control in subterranean drilling (Chiu & Solarek, 2009).

The diverse use of starch derivatives and the attendant over-dependence on conventional sources such as corn, wheat, rice, sorghum, potato, tapioca, etc., which serve as major staple foods in developing countries, necessitated the search for alternative sources of starch to meet the ever-increasing demands of the insatiable starch industry. These resulted in researches into new sources of starch from unconventional/under-utilised sources such as mucuna beans (Adebowale & Lawal, 2003), jack bean (Lawal & Adebowale, 2005), bambara groundnut (Adebowale, Afolabi & Lawal, 2002), etc.

Cowpea is an important crop in Africa (Uwaegbute, Iroegbu, & Eke, 2000) containing about 25 % protein and 64 % carbohydrate with starch constituting about 52 g/100 g of the total carbohydrate (Ashogbon & Akintayo, 2013). The annual worldwide production of cowpea is over 5.4 million tons with Africa producing about 5.2 million and Nigeria accounts for 61% of cowpea production in Africa and by implication 58 % worldwide (Hamid, Muzzafar, Wani, & Masoodi, 2015). With the increasing need for starch as an industrial food raw material, various sources and modifications have been attempted in the recent past. Literatures however, are missing on the modification of starches of local varieties of cowpea such as carton brown and horse red (“ewa ibeji”) which are abundant with restricted use to cultural practices. This necessitated the need for this work as an attempt at unravelling the industrial potentials of the selected local cowpeas. The objectives of this work were to isolate, modify and determine the functional properties of acetate starches of two local cowpeas.

2.0 MATERIALS AND METHODS

2.1. Sample collection

Horse red and Carton brown cowpea seeds were purchased from the main market in Oja-Odan, Ogun State, Nigeria. The seeds were cleaned of foreign materials and damaged ones; they were separately milled into fine flours. All reagents used were of analytical grade.

2.2. Starch isolation

The flours were defatted for 72 hrs by cold extraction using n-hexane. The extract was decanted and the defatted flours were air-dried and stored for the isolation of starches. Isolation of starch was carried out by the method of Lawal and Adebowale (2005). 1 kg of defatted sample flour was soaked in distilled water and the pH was adjusted to between 8.0 – 9.0 using NaOH solution (0.2% $^{w}/_{w}$) at room temperature for 4 hours with continuous stirring. The suspension was centrifuged at 4600 rpm for 15 minutes. The residue obtained was suspended in distilled water (1:10) and screened using muslin cloth followed by centrifugation for 30 minutes at 4600 rpm. The starch obtained was washed twice with distilled water before drying in the air for 48 hours at 30 ± 20 °C.

2.3. Preparation of acetate starch

Acetylation was carried-out as described by Lawal (2004) with slight modifications. 100 g of starch was dispersed in 500 mL of distilled water; the mixture was stirred for 20 min. The pH of the slurry obtained was adjusted to 8.0 using 1 M NaOH. Acetic anhydride (10.2 g) was added over a period of 1 hour, while maintaining a pH range 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 four times with distilled water and air-dried at 30±2 °C for

48 hours. The starch acetate was ground into fine powder, labelled and stored in air-tight polythene bags until required as native horse red starch, NHS; acetate horse red starch, CHS; native carton brown starch, NCS; acetate carton brown starch, CCS.

2.4. Functional properties

2.4.1. Water and oil absorption capacity

The method of Beuchat (1977) was used to determine oil and water absorption capacity of the starch.

2.4.2. Effect of temperature on swelling power and solubility

Effect of temperature on swelling power and solubility determinations were carried out in the temperature range of 55–95 ^{o}C, using the method of Lawal (2004).

2.4.3. Effects of pH on swelling power and solubility

The effect of pH on solubility and swelling was studied using the method of Lawal (2004).

2.4.4. Gelation studies

Gelation studies was done using the method of Lawal (2004).

2.4.5. Pasting properties

Pasting properties of the cowpea starches were evaluated by the method of Sun, Zhang, and Ma (2016) using a Rapid Visco Analyzer (Newport scientific, RVA Super 3, Switzerland). Starch suspension (9 %, w/w; dry starch basis, 10 g total weight) were equilibrated at 30 ^{o}C for 1 min, heated at 95 ^{o}C for 5.5 min, at a rate of 6 ^{o}C/ min, held at 95 ^{o}C for 5.5 min, cooled down to 50 ^{o}C for 2 min. A programmed heating and cooling cycle was used. Parameter recorded were Pasting Temperature (PT), Peak Viscosity (PV), Trough Viscosity (i.e. minimum viscosity MV), Final Viscosity (FV), Peak Time (PT), Breakdown Viscosity (BV) was calculated as the difference between PV minus MV, while Total Setback Viscosity (SV) was determined as FV minus MV.

2.4.6. Light transmittance

Paste clarity was studied using the method of Bhandari and Singhal (2002).

2.5 Statistical analysis

All analyses were done in triplicate. Means and standard deviations of triplicate determinations were calculated. Analysis of variance was performed to calculate significant differences in treatment means and least significant difference ($p < 0.05$) was used to separate means using SigmaPlot 12 (Systat Sftware Inc).

3.0 RESULTS AND DISCUSSIONS

3.1 Functional properties

The results of the functional properties of native and acetate carton brown and horse red cowpea starches are presented in the Tables 1 – 3 and Figures 1 – 5.

Water and oil absorption capacities of native and acetate starches (Figures 1 and 2) revealed that modified starches had better water absorption capacities than the native starches. However, modification significantly decreased oil absorption capacity of horse red and carton brown starches than the native starches. Similar results have been reported that water absorption capacity of modified *Icacina* starch (83.25±0.2) was quite higher than that of the native (75.0±0.1) starch (Omojola, Orishadipe, Afolayan, Adedayo, & Adebiyi, 2012). Similar report was given by Adebiyi, Omolaja, Orishadipe, Afolayan and Olalekan (2011) that the water absorption capacity of modified Tacca starches were higher than the native Tacca starch. The water absorption capacity of the modified starches obtained were higher than the 1.60 and 1.94 g/g reported by for some cowpea varieties in Nigeria by Appiah, Asibuo, and Kumah (2011). The ability of acetate starches to bind water is indicative of its water absorption capacity. The observed variations in water absorption among cowpea starches may be due to different protein concentration, their degree of interaction with water and their conformational characteristics (Appiah, Asibuo, and Kumah 2011).

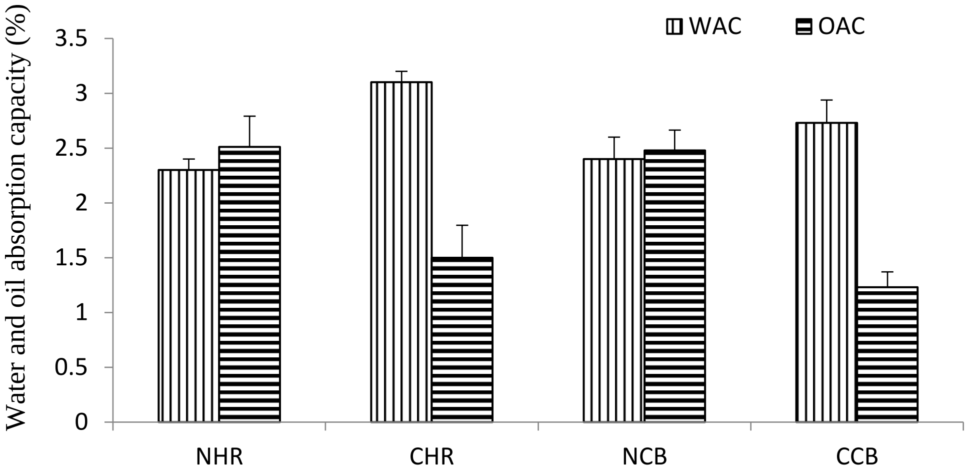

Fig. 1. Water and oil absorption capacities of native and citrate(modified) carton brown and horse red cowpea starches. Error bars: standard deviations; results are means of duplicate determinations.

The oil absorption capacities of cowpea starches ranged from 2.51 to 1.23 g/g (Figure 1). NHR and NCB had higher oil absorption capacities than their corresponding modified starches. Similar higher OAC of native starches over modified starches have been reported in literature for jack bean by Lawal and Adebowale (2005). The oil absorption capacities of the modified starches studied were higher than the 0.39 to 0.53 g/g reported for some Nigerian cowpeas (Ayana, Estefanos, Ashenafi, & Abubeker, 2013). The ability of these native starches to bind oil makes them useful in food systems where oil imbibition is desired. The starches could, therefore, have functional uses in foods such as sausage production. The high oil absorption capacity also makes the starches suitable in facilitating enhancement in flavour and mouth feel when used in food preparations. Native horse red and carton brown starches could, therefore, be superior as flavour retainer since they had significantly higher oil absorption capacities than the modified starches.

The swelling and solubility profile of native carton brown and horse red cowpea starches compared with that of acetate (modified) carton brown and horse red starch over temperature range of 55 – 95 ^{0}C are shown in Figures 2 and 3, respectively. The swelling and solubility profile showed a general trend of increase with increase in temperature which is more uniform in the modified starches than in the unmodified starches. Similarly, Icacina starch citrate had a significantly higher swelling capacity than acetylated and oxidized icacina starch as reported in an earlier work on modification of icacina starch (Omojola, Orishadipe, Afolayan, Adedayo, & Adebiyi, 2012). Increase in swelling power is indicative of suitability of a starch being used as disintegrant in the pharmaceutical industry. Also, high swelling power results into high digestibility and ability to use starch in solution suggesting improved dietary properties and the use of starch in a range of dietary applications (Omojola, Orishadipe, Afolayan, Adedayo, & Adebiyi, 2012). Adebiyi, Omolaja, Orishadipe, Afolayan and Olalekan (2011) reported that the swelling capacity of tacca starch increased with increase in temperature. Kunle, Ibrahim, Emeje, Shaba and Kunle (2003) also reported that the yellow tacca starch acetate had relatively higher swelling power and solubility than the white tacca starch acetate. Increased swelling power is indicative of suitability of starch acetate for use as a disintegrant in pharmaceutical industry, hence carton brown and horse red starch acetates might be better choices as disintegrants in the formulation of tablets.

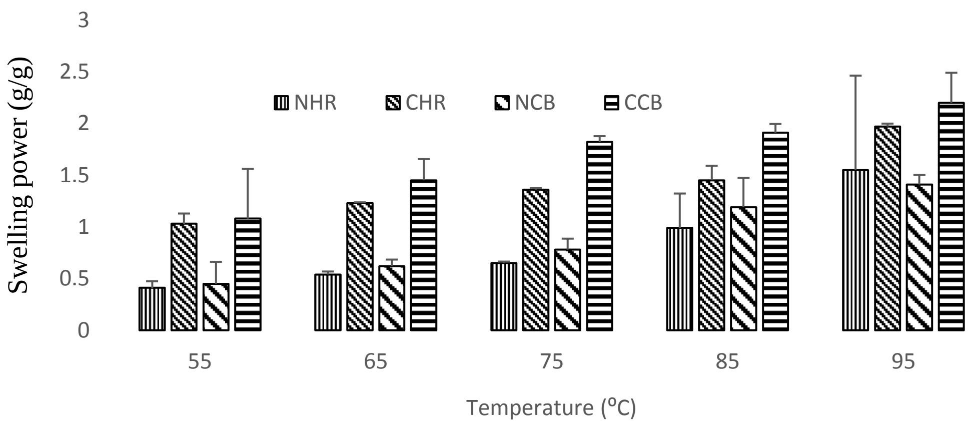

Fig. 2. Effect of temperature on swelling power of native and citrate(modified) carton brown and horse red cowpea starches. Error bars: standard deviations; results are means of duplicate determinations.

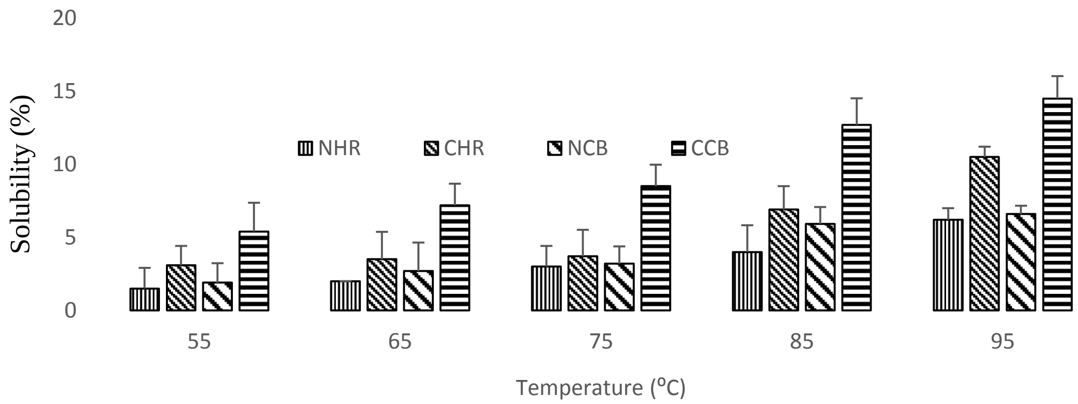

Fig. 3. Effect of temperature on solubility of native and citrate(modified) carton brown and horse red cowpea starches. Error bars: standard deviations; results are means of duplicate determinations.

Figures. 4 and 5 present the effect of pH on swelling power and solubility of starches, respectively. The results indicate that both swelling power and solubility were pH dependent. It was observed that swelling power and solubility of all the starches increased as pH increased. The effect of pH on swelling power and solubility of starches was however significant (P<0.05) with modification. At all pH values, both horse red and carton brown acetate starches had significant increase in the swelling capacity and solubility compared with native starches. Within the pH range 2 – 6, native carton brown had higher swelling capacity compared to the native horse red while the reverse was observed for the pH range 10 – 12. At pH 8, both native starches had the same swelling capacities.

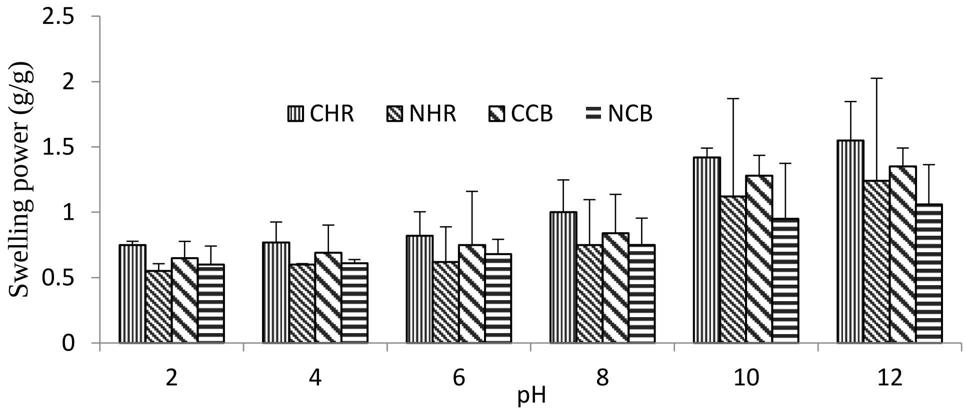

Fig. 4. Effect of pH on swelling power of native and citrate(modified) carton brown and horse red cowpea starches. Error bars: standard deviations; results are means of duplicate determinations

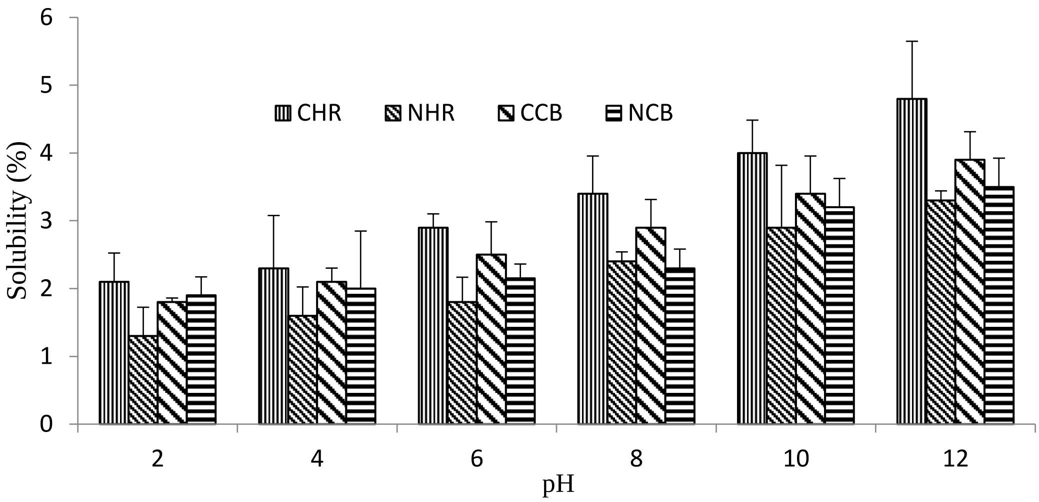

Fig. 5. Effect of pH on solubility of native and citrate(modified) carton brown and horse red cowpea starches. Error bars: standard deviations; results are means of duplicate determinations.

Gelation properties of the starches are presented in Table 1. The least gelation concentration (LGC) was used as the index of gelation. LGC reduced in both acetate starches after acetylation. CHR showed a significant ($p < 0.05$) reduction in LGC. Starch gelation is the process whereby starches undergo an irreversible change under heat and absorb water with swelling thereby making the granules swell more and become a paste rather than dispersion which it forms in cold water. The temperature or amount of heat energy required for the gelling properties of starch granules is lower. The more amylose a starch contains, the higher the gelling temperature. This is because amylose molecules as a result of their linearity line up more readily and have more extensive hydrogen bonding and consequently it requires more energy to break these bonds and gelatinize starch (Omojola, 2013). Starches with high amylose content also make a stronger, firmer gel because the linear amylose can move out of the granule into the water easily, align with one another and associate through hydrogen bonding; the freed amylose thickens and stabilize the water around it and thus making the mixture thick and viscous (Omojola, 2013). Good gelling ability is required in thickeners.

Table 1. Gelation properties of native and acetate carton brown and horse red cowpea starches.

Concentration (%w/v)	Starch sample			
	CHR	NHR	CCB	NCB
2	-Liquid	-Liquid	-Liquid	-Liquid
4	-Viscous	-Liquid	-Liquid	-Liquid
6	-Viscous	-Viscous	-Viscous	-Viscous
8	+Gel	-Very Viscous	-Very Viscous	-Very Viscous
10	+Firm gel	+Gel	-Very Viscous	-Very Viscous
12	+Firm gel	+Firm gel	+Gel	+Gel
14	+ Firm gel	+ Firm gel	+Firm gel	+Firm gel
LGC[a]	8	10	12	12

CHR, Acetate Horse Red; NHR, Native Horse Red; CCB, Acetate Carton Brown; NCB, Native Carton Brown; LGC[a], Least Gelation Concentration.

Pasting clarity and starch viscosity are important parameters in determining the stability of starch in many foods processing. Rapid Visco Analyzer (RVA) results (Table 2) of the acetate horse red and carton brown starches confirmed a low Peak Viscosity (PV) ranging from -13.0 to 19.0 RVU compared to native starches. CCB presented a viscosity profile higher than CHR, although substantially lower than the native starch. The lower PV of the modified starches could be due to considerable breakdown of amorphous regions and production of dextrins (Ayenampudi, Ramanathan, and Shalini, 2015). Trough viscosity (TV) and Break down (BD) values of the modified samples also displayed similar trend. CHR and CCB displayed a lower Final viscosity (FV) ranging between -6.0 and 49.0 RVU against the native starches. Setback (SB) is a measure of recrystallization of gelatinized starch. CCB shows a higher setback than CHR starch thus indicating that it has a higher retrogradation tendency than CHR. However, significantly higher SB were observed in native starches and thus higher tendencies for retrogradation. The low SB of the modified starches was likely due to insufficient time for the starch molecules to rearrange themselves during the stipulated period (Ayenampudi, Ramanathan, & Shalini, 2015). Native starch took more time to reach its PV than acetate starches. A similar trend was observed upon acid treatment of sorghum starch (Ayenampudi, Ramanathan, & Shalini, 2015).

Table 2. Pasting characteristics of native and acetate (modified) starches of carton brown and horse red cowpeas starches

Sample	Parameter						
	Peak Viscosity	Trough	Break Down	Final Viscosity	Set Back	Peak Time	Pasting Temp
NHR	3491.00[a]	2441.00[a]	1050.00[a]	4304.00[a]	1863.00[a]	12.05[a]	80.80[a]
NCB	3669.00[b]	2716.00[b]	953.00[b]	4239.00[a]	1523.00[b]	10.20[b]	81.50[a]
CHR	-13.00[c]	-15.00[c]	2.00[c]	-6.00[b]	9.00[c]	5.63[c]	61.50[b]
CCB	19.00[d]	16.00[d]	3.00[c]	49.00[c]	33.00[d]	6.93[c]	63.00[b]

Values with different alphabets in the same column are significantly different ($p < 0.05$). NHR: Native horse red; NCB: Native carton brown; CHR: Acetate horse red; CCB: Acetate carton brown.

Light transmittance of the native carton brown and horse red starches are higher than those of the modified (acetate) starches. Transmittance generally reduced as the storage days increased from 1st day to the 10th day (Table 3). Similar time-dependent reduction in transmittance (%) has been reported for banana starch (Lawal, Adebowale, Ogunsanwo, Barba, & Ilo, 2005). A significant ($p < 0.05$) reduction in light transmittance was observed in both native and acetate starches of carton brown derivatives. It is also noteworthy that there was an increase ($p < 0.05$) in percentage light transmittance even at the 1st day in native horse red and carton brown starch derivatives (NHR: 1.70%) and (NCB: 1.30%) compared with acetate starch derivatives (CHR: 0.40%) and (CCB: 0.55%). This development is associated with the electrostatic repulsion resulting from the functional group introduced on acetate starch, which effect lesser paste clarity. Louis, Chandra and William (2011) reported that yellow and white tacca starch acetate had minimum clarity at starch concentration of 1.5 % with light transmittance increasing as starch concentration increased.

Table 3. Influence of storage days on paste clarity of native and acetate (modified) carton brown and horse red starches

Starch	Transmittance (%)					
Sample	1st Day	2nd Day	4th Day	6th Day	8th Day	10th Day
NHR	1.70±0.13^{aA}	1.45±0.15^{aA}	1.55±0.17^{aA}	0.90±0.41^{aA}	1.65±0.36^{aA}	1.40±0.46^{aA}
CHR	0.40±0.08^{bB}	0.65±0.06^{bB}	0.45±0.13^{bB}	0.50±0.16^{bB}	0.30±0.14^{bB}	0.35±0.10^{bB}
NCB	1.30±0.06^{aA}	1.35±0.13^{aA}	1.60±0.08^{aA}	1.55±0.08^{aA}	1.40±0.22^{aA}	1.45±0.08^{aA}
CCB	0.55±0.06^{bB}	0.55±0.13^{bB}	0.40±0.10^{bB}	0.45±0.08^{bB}	0.40±0.06^{bB}	0.45±0.05^{bB}

Values are means ±standard deviation. Means in each row and column followed by different superscripts are significantly different at $P < 0.050$; capital (A, B) and small (a, b) show statistical differences for data in rows and columns, respectively.

CONCLUSION

Functional properties of native and acetate derivative of local cowpea starches were investigated. Both swelling power and solubility increased with increase in temperature. Modification decreased swelling power and solubility of cowpea starches. At pH (2-12), acetylation increased the swelling capacity of the native starches. Modification reduced water absorption capacity of the native starch but increased oil absorption capacity of the native starches. Least gelation reduced in acetate starch. Modification reduced the pasting temperature and retrogradation tendencies of the native starches. The results, though preliminary, showed the local cowpeas as a potential industrial raw material.

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