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## Effects Of Cross-Linking And Acetylation On Some Physicochemical Properties Of Cowpea (*Vigna Unguiculata*) Starch



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### ABSTRACT

The effects of cross-linking with  $\text{POCl}_3$  and acetylation, singly and in combination on the physicochemical properties of cowpea starch were investigated. Cross-linking enhanced the solubility and swelling power but generally decreased paste clarity of cowpea starch whereas acetylation improved the paste clarity. Both acetylation and cross-linking caused a decrease in the initial pasting temperature. The paste consistency at  $95^\circ\text{C}$  was significantly increased by cross-linking but was decreased upon acetylation and double modification. Acetylation improved the freeze-thaw stability of cowpea starch gels but the improvement upon cross-linking appeared to depend on the degree of substitution and the number of freeze-thaw cycles. Cross-linking and acetylation resulted in significant reductions in the onset ( $T_o$ ) and completion ( $T_c$ ) temperatures, and gelatinization enthalpy ( $\Delta H$ ) of the starch. Acetylation resulted in significant reductions in maximum storage modulus ( $G_{\max}'$ ) and loss modulus ( $G_{\max}''$ ) whereas cross-linking resulted in significant reductions in  $G_{\max}''$ . Overall, acetylation was more effective than cross-linking in modifying the pasting, freeze-thaw stability, and dynamic mechanical properties of cowpea starch.

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### KEYWORDS

Cowpea;  
 Starch;  
 Cross-linking;  
 Acetylation;  
 Physicochemical properties.

## INTRODUCTION

Although the major component of pulses such as cowpea is starch, greater attention has been given to their proteins<sup>[1-4]</sup>. Legume starches are deemed to be of commercial interest by virtue of their unique properties that differ from those of classical starches.

Cowpea is one of the legumes grown extensively in Africa and South East Asia. Cowpea is a crop that is well adapted to the semi-arid zones because of its drought tolerance. Although cowpea starch is an abundant natural resource in Kenya, it has hitherto not been commercially exploited due to its unavailability and lack of knowledge about its functional properties.

It is an established fact that native starches, as extracted from their natural sources, are not suited for most applications in the food-processing sector. Some of the shortcomings of native starches include liquefaction and watery separation of ingredients during sterilization/canning operations, thinning under shear, retrogradation under frozen storage, and poor dispersibility even in hot water. In order to fulfil the various demands for functionality in different products, industrially processed starch is usually modified enzymatically, physically and chemically<sup>[5]</sup>. A broad range of modified starches is used in food and non-food sectors with empirically tailored application profiles<sup>[6]</sup>.

Various researchers have isolated starch from cowpea and reported on its physicochemical properties such as pasting profiles, gelatinization characteristics and mechanical properties of the starch gels<sup>[7-11]</sup>. However, there is a dearth of information on chemical modification of cowpea starch and how this affects its physicochemical properties. The extraction of starch from cowpea is viewed as one of the strategies aimed at expanding the utilization of this grain legume beyond the traditional utilization currently obtaining.

The present study aimed at investigating the effect of cross-linking and acetylation on the pasting, gelatinization and rheological properties, as well as the solubility, swelling, paste clarity and freeze-thaw stability of cowpea starch. It is hoped that the information garnered herein can provide the technical

basis for the establishment of an industry to commercially exploit cowpea starch.

## EXPERIMENTAL

### Isolation of cowpea starch

Cowpea starch was extracted using a wet-milling process described by Watson<sup>[12]</sup>, with modifications. The seeds were steeped in water (1:2 w/v seed:water) containing 0.20% sodium metabisulfite for 20h at 10°C. The swollen seeds were rinsed and then ground with water (1:3 w/v seed:water) in a Waring blender for 5min. The homogenate was passed successively through 105- $\mu$ m, 63- $\mu$ m, and 44- $\mu$ m sieves. The grinding and screening of the residue were repeated three more times. The combined filtrate was allowed to sediment for 3h at room temperature. The supernatant was discarded and the sediment was repeatedly suspended in distilled water, passed through a 44- $\mu$ m screen, let to sediment and finally washed until the surface was deemed to be free of pigmented impurities. The starch was then dried at 40°C for 48h. The isolated starch contained 7.19% moisture, 0.07% ash, 0.34% protein and 97.93% starch.

### Methods

#### 1. Cross-linking

Cross-linking using POCl<sub>3</sub> was performed using the method described by Zheng et al<sup>[13]</sup>. After adjusting pH to 10.5 with 0.1M NaOH, 0.10%, 0.15%, and 0.20% of the reagent (dry starch weight basis) was added. The reaction mixture was stirred at 40°C for 2h, then adjusted to pH 6.5 with 1N HCl. Modified starch was recovered by centrifugation and washing with distilled water. It was then dried at 40°C under vacuum. The phosphorus content of the modified starch, determined using the vanado-molybdate method of Takagi<sup>[14]</sup>, was used to calculate the degree of substitution (DS) as follows:

$$DS = 162P / (3100 - 124P)$$

$$P = \% \text{ phosphorus (dry basis)}$$

#### 2. Acetylation

Acetylation and determination of degrees of substitution were carried out according to the methods

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described by Wurzburg<sup>[15]</sup>. Acetic anhydride (0.10, 0.20 and 0.30 moles) was reacted with 150g (d.b.) of starch to give the desired degrees of substitution.

### 3. Double modification

Cross-linked starch (0.10% POCl<sub>3</sub>) was acetylated using 0.10 moles of acetic anhydride (Ac<sub>2</sub>O) in accordance with the methods described in 1 and 2.

### 4. Swelling power and solubility

Swelling power and solubility were determined using the method described by Leach et al<sup>[16]</sup> with some modifications. Starch (ca 1000mg d.b.) was weighed into a 50-ml centrifuge tube, 30ml of distilled water were added and the tube was shaken gently by hand. The tube was dipped in a water bath controlled at 80°C for 5min with occasional gentle shaking by hand to avoid the sedimentation of starch granules. The tube was filled up to 50ml with water and heating at 80°C was continued for 35 min with gentle shaking every 5min. The tube was then filled up to the mark and centrifuged at 3000rpm for 15 min. The volume of sediment (V<sub>1</sub> ml) was read immediately. A 5-ml aliquot of the supernatant was pipetted into a 25-ml graduated tube. The tube was heated in boiling water for 10 min. After cooling, 1ml of glucoamylase solution (ca 30 I.U) was added followed by incubation at 50°C for 60min. The tube was then heated for 10 min in boiling water, cooled and made up to the 25-ml mark. Glucose content was determined by the Somogyi-Nelson method<sup>[17]</sup>. The swelling power and solubility were then calculated.

### 5. Paste clarity

The clarity of starch pastes was determined by the method of Wu and Seib<sup>[18]</sup>. Starch suspension (1%) was heated in a boiling water bath for 30min and cooled to 25°C. The clarity was evaluated using percent transmittance (%T) at 650nm against a water blank in a Jasco V-530 Spectrophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan).

### 6. Brabender pasting characteristics

The pasting behavior of starch samples was examined in a Brabender Viscoamylograph (Brabender OHG, Duisburg, Germany) at 700cm-g torque and

75 rpm shear rate. The procedure described by Deffenbaugh and Walker<sup>[19]</sup> was adopted with some modifications. Starch (6% w/v, d.b.) was slurried in distilled water. The slurry was heated from 50°C to 95°C at the rate of 1.5°C/min and the paste held at 95°C for 15min, then cooled at 1.5°C/min to 50°C, and held at 50°C for 15min. The following measurements were taken from the amylograph curve: initial pasting temperature, consistency at 95°C, consistency after 15min at 95°C, consistency at 50°C, consistency 15min at 50°C. Consistency was recorded in Brabender units (BU). The starch paste was collected after the completion of the cycle and used for the determination of freeze-thaw stability.

### 7. Freeze-thaw stability

The stability of the starch paste to freeze-thaw treatment was determined by the method of Wu and Seib<sup>[18]</sup> using paste prepared during the amylograph test (6). Starch paste at 50°C paste was poured (20g) into polypropylene centrifuge tubes (50mL capacity) and the tubes tightly capped. After cooling to room temperature the tubes were weighed to determine the weight of the starch gel. The tubes were stored at 4°C for 24h before being subjected to freeze-thaw cycles. In each cycle the tubes were put into a freezer at -20°C for 22h and thawed at 30°C in a water bath for 2h. After every four cycles, triplicate tubes were centrifuged for 15 min at 1500xg and the amount of liquid separated determined by weight. The freezing and thawing process was repeated for a total of 16 cycles.

$\% \text{ Syneresis} = \frac{\text{Weight of liquid separated (g)} \times 100}{\text{total weight of gel (g)}}$

### 8. Differential scanning calorimetry

All samples were prepared by mixing starch with sufficient distilled water to give starch concentration of 30% (d.b.). Samples (1g) were heated at 1°C/min from 30°C to 100°C in a Setaram Micro DSC II (Setaram Co., Cedex, France). The reference pan contained 0.7ml of water. For each endotherm, onset (T<sub>o</sub>), peak (T<sub>p</sub>), termination (T<sub>t</sub>) temperatures and enthalpy of gelatinization (H) were computed using Setaram CS922-GII analysis software.

### 9. Dynamic mechanical properties

The dynamic mechanical properties of pastes and gels of the starches were determined according to the method described by Katsuta<sup>[20]</sup>. The starch was weighed into a 50-ml flask and degassed under vacuum for 15min and suspended in previously degassed distilled water to give 10% (d.b.) concentration. The suspension was stirred under vacuum for 90 min. The starch suspension was then heated for 5min at a temperature 10°C below its onset temperature ( $T_o$ ) as determined by DSC and immediately measured for dynamic viscoelasticity on a Rheometrics Dynamic Analyzer (RDA) II (Rheometrics Inc., Piscataway, NJ, USA). The temperature sweep of a starch suspension (10% w/v, d.b.) was conducted from 30 to 95°C at a heating rate of 1.5°C/min. The experimental conditions were as follows: dynamic mechanical analysis mode, time/cure sweep, cone cylinder geometry (cup radius 14.0mm, bob radius 12.5mm, bob length 32.0mm), auto strain control, and frequency 10 rad/sec. The following parameters were derived using the Rhios analysis software (Rheometrics Inc., Piscataway, NJ, USA): onset temperature of  $G'$  and  $G''$ , maximum  $G'$  and  $G''$ , temperature at maximum  $G'$  and  $G''$ , and  $\tan \delta$  at maximum  $G'$ .

### Statistical analysis

Statistical analysis, analysis of variance, regression and Duncan's multiple range test were computed using SPSS for Windows Release 6.1<sup>[21]</sup>.

**TABLE 1: Effect of cross-linking and acetylation on the solubility, swelling power and paste clarity of cowpea starch<sup>1</sup>.**

Modification	DS <sup>2</sup>	Solubility (%)	Swelling power (ml/g)	Paste clarity (%T <sub>650</sub> )
Native	-	1.63±0.01 <sup>f</sup>	10.22±0.03 <sup>e</sup>	12.70±0.30 <sup>d</sup>
Cross-linked (0.10% POCl <sub>3</sub> )	0.003	2.93±0.05 <sup>cd</sup>	16.56±0.45 <sup>c</sup>	10.10±0.30 <sup>f</sup>
Cross-linked (0.15% POCl <sub>3</sub> )	0.004	3.17±0.03 <sup>bc</sup>	19.31±0.10 <sup>a</sup>	12.95±0.15 <sup>d</sup>
Cross-linked (0.20% POCl <sub>3</sub> )	0.006	2.33±0.17 <sup>e</sup>	18.16±0.38 <sup>b</sup>	11.10±0.30 <sup>e</sup>
Acetylated (6.8% Ac <sub>2</sub> O)	0.030	1.11±0.09 <sup>g</sup>	7.93±0.28 <sup>f</sup>	18.40±0.14 <sup>c</sup>
Acetylated (13.6% Ac <sub>2</sub> O)	0.080	1.42±0.08 <sup>f</sup>	8.10±0.28 <sup>f</sup>	34.40±0.00 <sup>b</sup>
Acetylated (20.4% Ac <sub>2</sub> O)	0.190	3.52±0.10 <sup>ab</sup>	19.81±0.19 <sup>a</sup>	51.50±0.30 <sup>a</sup>
Cross-linked and acetylated <sup>3</sup>	-	3.26±0.06 <sup>b</sup>	12.96±0.14 <sup>d</sup>	6.70±0.14 <sup>g</sup>

<sup>1</sup> Means of duplicate analyses ± standard error. In each column, values followed by the same letter are not significantly different ( $p < 0.05$ ).

<sup>2</sup> DS is degree of substitution.

<sup>3</sup> Cross-linked with 0.10% POCl<sub>3</sub> followed by acetylation with 0.10mole (6.8%) acetic anhydride (Ac<sub>2</sub>O) per 150g of starch.

## RESULTS AND DISCUSSION

### Effect of modification on solubility, swelling power and paste clarity

The solubility, swelling power and paste clarity of cowpea starch were affected to varying extents by cross-linking and acetylation, singly or in combination as shown in TABLE 1. Cross-linking (POCl<sub>3</sub>) significantly ( $p < 0.05$ ) increased solubility compared to the native starch. Starch cross-linked to degree of substitution (DS) 0.004 exhibited higher solubility (3.17%) compared to those of DS 0.003 (2.93%) and DS 0.006 (2.33%). Acetylation to DS 0.030 significantly decreased solubility whereas acetylation to DS 0.080 and 0.190 gave solubility similar to, and higher than the native starch, respectively. The doubly modified (cross-linked and acetylated) starch exhibited significant improvement in solubility over the native starch. For acetylated starch solubility increased with increasing levels of DS. The results obtained for acetylated starch in the present study tended to suggest that a threshold level of substitution was necessary in order to effect a significant improvement in the solubility of the starch. The improvement in solubility upon acetylation is probably due to the weakening of the hydrogen bonds between starch chains caused by the acetyl groups.

The swelling power (SP) of the starch increased significantly upon cross-linking. The SP increased with increasing DS attaining a maximum at DS 0.004

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(19.31ml/g) followed by a slight decrease at DS 0.006 (18.16ml/g). The swelling power of starch is generally expected to decrease upon cross-linking since this modification is deemed to increase the degree of bonding between starch chains thereby inhibiting granule swelling. It is conceivable that the relatively low levels of substitution achieved in the present study were not sufficient to suppress granule swelling but the exposure of the starch to NaOH at pH 10 instead enhanced the process. Sitohy et al.<sup>[22]</sup> observed that the swelling power of corn, rice, and potato starches phosphorylated up to 0.5 molar ratio increased but decreased at substitution levels above this value. Increasing levels of acetylation resulted in increased swelling power, however the swelling power of cowpea starch acetylated to DS 0.030 and 0.080 was significantly lower than that of the native starch. The SP of starch acetylated to DS 0.190 (19.81ml/g) was almost double the value for native starch (10.22ml/g). The doubly modified starch exhibited moderate improvement in SP.

Cross-linking caused significant reduction or no change in the paste clarity relative to the native starch. Acetylation resulted in significant improvement in paste clarity and the increase was positively related to DS. The doubly modified starch presented clarity (6.70%) that was close to 50% the value for the native starch (12.70%).

The results obtained in the present study were at variance with those of previous studies that had in-

dicated that SP, solubility and paste clarity decreased with cross-linking for waxy barley<sup>[18]</sup>, waxy maize<sup>[23]</sup>, partially waxy wheat<sup>[24]</sup>, and waxy hull-less barley<sup>[13]</sup>. Liu et al.<sup>[25]</sup> reported a slight decrease in the SP of acetylated waxy rice and a considerable reduction on cross-linking but that it was unchanged in normal rice starch. The same authors also observed that acetylation profoundly increased the solubility of waxy rice starch.

### Effect of modification on the pasting properties

The effects of modification on the Brabender pasting profiles of cowpea starch are presented in figure 1 and summarized in TABLE 2. The initial pasting temperature was not affected by cross-linking at DS 0.003 but was significantly ( $p < 0.05$ ) reduced at DS 0.004 and 0.006. Acetylation resulted in considerable and significant reduction in the initial pasting temperature. Generally the pasting temperature decreased with increasing DS for both types of modification. The initial pasting temperature of the doubly modified starch was intermediate in value to those of acetylated and cross-linked starches. The cross-linked starch exhibited significantly higher consistency at 95°C and after 15min at 95°C compared to the native starch. Starch cross-linked to DS 0.004 exhibited significantly higher consistency at 50°C and after 15min at 50°C than the native and starches cross-linked to DS 0.003 and 0.006. Starch cross-linked to DS 0.006 and 0.004 exhibited the highest

**TABLE 2: Effect of cross-linking and acetylating on the Brabender pasting characteristics of cowpea starch<sup>1</sup>.**

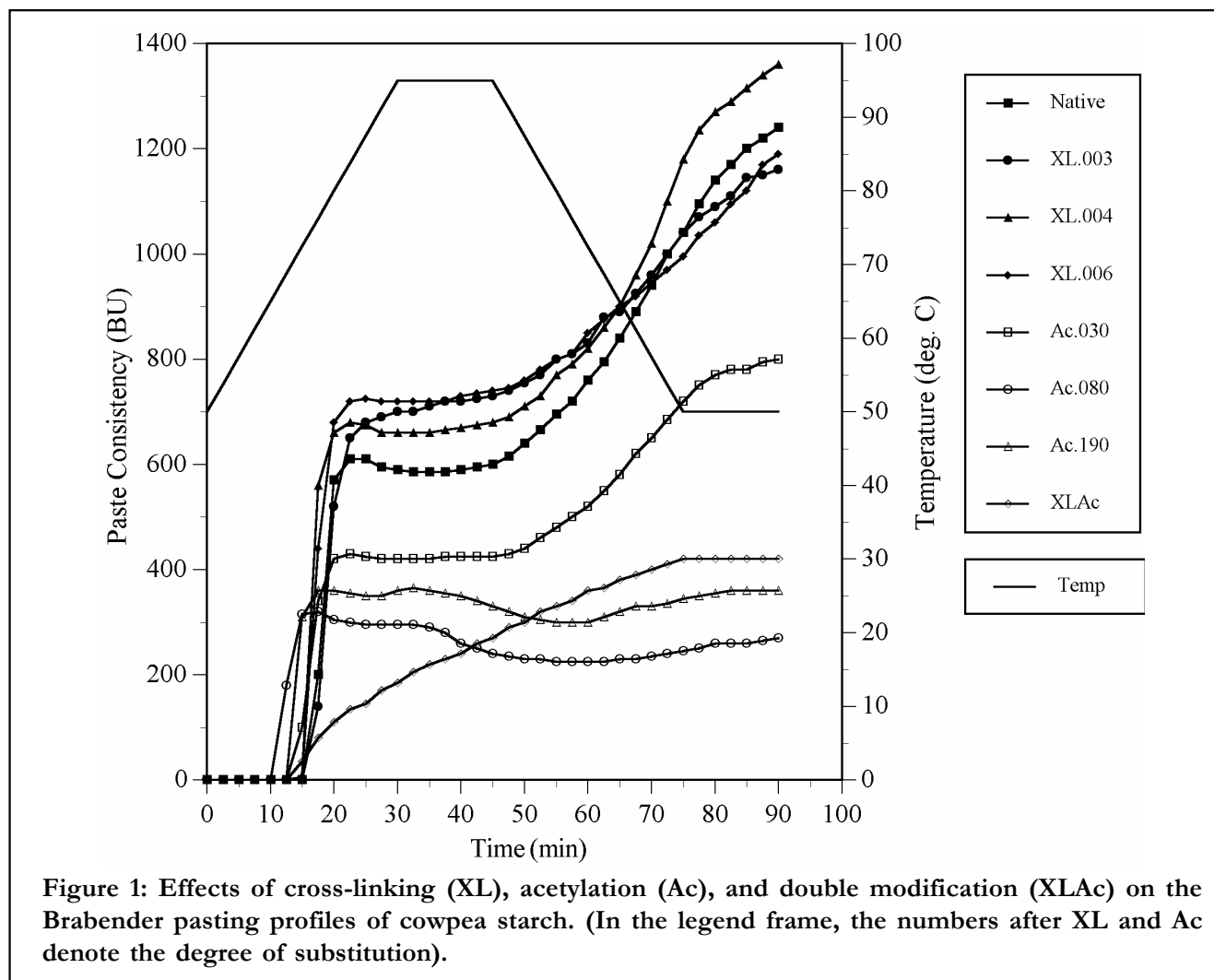
Modification	DS <sup>2</sup>	Initial pasting temperature (°C)	Consistency at 95°C (BU) <sup>3</sup>	Consistency after 15 min at 95°C (BU)	Consistency at 50°C (BU)	Consistency after 15 min at 50°C (BU)
Native	-	73.6±0.10 <sup>a</sup>	590.0±0 <sup>b</sup>	600±0 <sup>c</sup>	1057.5±8 <sup>b</sup>	1245.5±3 <sup>b</sup>
Cross-linked (0.10% POCl <sub>3</sub> )	0.003	73.4±0.10 <sup>a</sup>	675.0±25 <sup>a</sup>	710.0±20 <sup>ab</sup>	1020.0±25 <sup>b</sup>	1130.0±30 <sup>c</sup>
Cross-linked (0.15% POCl <sub>3</sub> )	0.004	71.9±0.15 <sup>b</sup>	645.0±15 <sup>a</sup>	665.0±15 <sup>b</sup>	1155.0±15 <sup>a</sup>	1355.0±5 <sup>a</sup>
Cross-linked (0.20% POCl <sub>3</sub> )	0.006	71.4±0.40 <sup>b</sup>	690.0±20 <sup>a</sup>	720.0±20 <sup>a</sup>	1050.0±30 <sup>b</sup>	1200.0±10 <sup>b</sup>
Acetylated (6.8% Ac <sub>2</sub> O)	0.030	69.9±0.40 <sup>c</sup>	422.5±3 <sup>c</sup>	430.0±0 <sup>d</sup>	720.0±0 <sup>c</sup>	795.0±5 <sup>d</sup>
Acetylated (13.6% Ac <sub>2</sub> O)	0.080	67.4±0.60 <sup>d</sup>	310.0±0 <sup>e</sup>	250.0±10 <sup>f</sup>	270.0±10 <sup>f</sup>	300.0±5 <sup>f</sup>
Acetylated (20.4% Ac <sub>2</sub> O)	0.190	67.2±0.20 <sup>d</sup>	375.0±15 <sup>d</sup>	340.0±20 <sup>e</sup>	335.0±10 <sup>e</sup>	345.0±15 <sup>f</sup>
Cross-linked and acetylated <sup>4</sup>	-	70.8±0.75 <sup>bc</sup>	175.0±10 <sup>f</sup>	255.0±15 <sup>f</sup>	395.0±15 <sup>d</sup>	402.5±18 <sup>e</sup>

<sup>1</sup> Means of duplicate analyses ± standard error. Within each column, values followed by the same letter are not significantly different ( $p < 0.05$ ).

<sup>2</sup> DS is degree of substitution.

<sup>3</sup> BU is Brabender units.

<sup>4</sup> Cross-linked with 0.10% POCl<sub>3</sub> followed by acetylating with 0.10moles (6.8%) acetic anhydride (Ac<sub>2</sub>O) per 150g of starch.



hot paste consistency (720BU) and cold paste consistency (1355BU), respectively. Acetylation resulted in significant reductions in consistency at all selected reference points on the amylogram. The doubly modified starch gave a paste consistency of 175BU at 95°C that was approximately 30% the value for native starch.

Acetylation has been reported to cause a reduction in the pasting temperature of waxy barley and waxy maize starches<sup>[18]</sup>, and waxy rice starch<sup>[25]</sup>. High paste consistency and shear stability, considered the most important characteristics of cross-linked starches used as thickeners, were obtained by cross-linking cowpea starch in the present study. The doubly modified cowpea starch may find ready application in processes requiring low paste viscosity, e.g. those that involve pumping and mixing due its low

consistency and good to stability shear. Although it is generally accepted that strengthening of the bond between starch chains by cross-linking will increase resistance to swelling leading to a lower paste consistency, this behavior was not observed in the cross-linked cowpea starch in the present study. This was probably due to the fact that cross-linking preferentially occurs to amylopectin and that some amylose is linked to amylopectin during the process<sup>[26]</sup>. This may have modified the solubility properties of the granule leading to enhanced leaching of the polysaccharide molecules. This may partly account for the higher paste consistencies at 95°C observed for the cross-linked cowpea starches compared to the native starch.

#### Effect of modification on freeze-thaw stability

As shown in TABLE 3 cross-linking and acety-

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TABLE 3: Effect of cross-linking and acetylating on the freeze-thaw stability of cowpea starch<sup>1</sup>.

Modification	DS <sup>2</sup>	% Syneresis after n cycles:			
		n=4	n=8	n=12	n=16
Native	-	10.68±0.25 <sup>fg</sup>	11.19±0.15 <sup>f</sup>	14.29±0.19 <sup>bc</sup>	15.21±0.24 <sup>a</sup>
Cross-linked (0.10% POCl <sub>3</sub> )	0.003	13.04±0.28 <sup>d</sup>	13.92±0.28 <sup>c</sup>	14.50±0.16 <sup>bc</sup>	15.32±0.12 <sup>a</sup>
Cross-linked (0.15% POCl <sub>3</sub> )	0.004	13.88±0.17 <sup>c</sup>	7.25±0.20 <sup>jk</sup>	6.39±0.21 <sup>l</sup>	6.49±6.49 <sup>l</sup>
Cross-linked (0.30% POCl <sub>3</sub> )	0.006	9.74±0.06 <sup>h</sup>	6.45±0.15 <sup>l</sup>	7.58±0.23 <sup>i</sup>	6.63±0.06 <sup>kl</sup>
Acetylated (6.8% Ac <sub>2</sub> O)	0.030	7.25±0.13 <sup>jk</sup>	3.65±0.17 <sup>m</sup>	2.38±0.27 <sup>n</sup>	3.90±0.08 <sup>m</sup>
Acetylated (13.6% Ac <sub>2</sub> O)	0.080	7.23±0.08 <sup>jk</sup>	7.39±0.27 <sup>i</sup>	5.41±0.23 <sup>m</sup>	6.56±0.22 <sup>l</sup>
Acetylated (20.4% Ac <sub>2</sub> O)	0.190	8.55±0.21 <sup>i</sup>	6.32±0.20 <sup>l</sup>	6.34±0.28 <sup>l</sup>	6.71±0.10 <sup>kl</sup>
Cross-linked and acetylated <sup>3</sup>	-	14.87±0.16 <sup>ab</sup>	10.25±0.36 <sup>gh</sup>	10.62±0.24 <sup>fg</sup>	11.78±0.16 <sup>e</sup>

<sup>1</sup> Means of triplicate analyses ± standard error. Values followed by the same letter are not significantly different ( $p < 0.05$ ).

<sup>2</sup> DS is degree of substitution

<sup>3</sup> Cross-linked with 0.10% POCl<sub>3</sub> followed by acetylating with 0.10mole (6.8%) acetic anhydride (Ac<sub>2</sub>O) per 150g of starch.

lation resulted in changes to the freeze-thaw stability of cowpea starch. After 4 freeze-thaw cycles, cross-linked (DS 0.003 and 0.004) and doubly modified starch gels exhibited significantly ( $p < 0.05$ ) higher % syneresis than those of the native starch. Acetylated and cross-linked (DS 0.006) starch gels exhibited % syneresis similar to or significantly lower than the native starch gel. Upon further cycling, starch gels from starch cross-linked to DS 0.003 exhibited % syneresis similar to, or significantly higher than the native starch gel but the remaining cross-linked samples gave lower values. Overall, acetylation was more effective than phosphorylation in suppressing syneresis over the 16 freeze-thaw cycles investigated in the present study. For the acetylated starch it was observed that syneresis decreased with the number of freeze-thaw cycles but increased with the level of substitution. Cross-linking to DS 0.004 and 0.006 improved the freeze-thaw stability of gels subjected to 8 or more cycles.

Cross-linking and acetylation have been reported to respectively decrease and increase the freeze-thaw stability of waxy barley starch<sup>[18]</sup>. The above observation is in agreement with the results obtained for acetylated and starch cross-linked to DS 0.003 in the present study. Cross-linking was observed to increase syneresis in waxy hull-less barley starch and increasing the dosage of cross-linking reagent resulted in higher levels of syneresis<sup>[13]</sup>. A reverse effect was observed for cowpea starch in the present study probably due to the differences in the amylose-amylopec-

tin ratios of the two starches. Both acetylation and cross-linking improved the freeze-thaw stability of normal rice starch but reduced in waxy starch gels<sup>[25]</sup>. The deterioration for waxy rice starch was attributed to the fact that modification possibly caused restrictions in the starch chains. This resulted in gels that were too rigid to accommodate ice crystal growth during freezing leading to bond breakage within the chains and this culminated in an increase in syneresis upon thawing. The above explanation may account for the observed reduction in freeze-thaw stability of gels made from cowpea starch cross-linked to DS 0.003 in the present study.

### Effect of modification on thermal properties

The effects of modification on the gelatinization characteristics (differential scanning calorimetry) of cowpea starch are shown in TABLE 4. Cross-linking significantly ( $p < 0.05$ ) decreased the onset ( $T_o$ ), slightly decreased completion ( $T_c$ ) but had no effect on peak ( $T_p$ ) temperatures compared to the native starch. The gelatinization enthalpies ( $\Delta H$ ) of the cross-linked starches were significantly lower than  $\Delta H$  of native starch. The DS appeared to exert no effect on these characteristics. Acetylation caused significant reductions in all the characteristic parameters of the thermogram. The reductions in  $T_o$ ,  $T_c$ ,  $T_p$  and  $\Delta H$  were directly related to the DS. The doubly modified starch exhibited lower  $T_o$ ,  $T_c$ ,  $T_p$  and  $\Delta H$  compared to the native and cross-linked starches.

It has been previously reported that cross-link-

**TABLE 4: Effect of phosphorylation and acetylation on the onset ( $T_o$ ), peak ( $T_p$ ), termination ( $T_c$ ) temperatures and gelatinization enthalpy ( $\Delta H$ ) of cowpea starch<sup>1</sup>.**

Modification	DS <sup>2</sup>	$T_o$ (°C)	$T_p$ (°C)	$T_c$ (°C)	$\Delta H$ (J/g)
Native	-	67.67±0.08 <sup>a</sup>	72.22±0.01 <sup>a</sup>	93.44±0.05 <sup>a</sup>	13.35±0.03 <sup>a</sup>
Cross-linked (0.10% POCl <sub>3</sub> )	0.003	67.00±0.03 <sup>b</sup>	72.03±0.11 <sup>a</sup>	92.20±0.78 <sup>ab</sup>	12.69±0.09 <sup>b</sup>
Cross-linked (0.15% POCl <sub>3</sub> )	0.004	66.88±0.02 <sup>b</sup>	71.91±0.06 <sup>a</sup>	92.65±0.50 <sup>b</sup>	12.67±0.00 <sup>b</sup>
Cross-linked (0.20% POCl <sub>3</sub> )	0.006	66.88±0.04 <sup>b</sup>	71.96±0.09 <sup>a</sup>	92.06±0.25 <sup>b</sup>	12.88±0.03 <sup>b</sup>
Acetylated (6.8% Ac <sub>2</sub> O)	0.030	64.02±0.14 <sup>c</sup>	69.59±0.18 <sup>b</sup>	91.45±0.44 <sup>c</sup>	12.18±0.07 <sup>c</sup>
Acetylated (13.6% Ac <sub>2</sub> O)	0.080	51.19±0.23 <sup>c</sup>	61.47±0.07 <sup>c</sup>	85.56±0.21 <sup>c</sup>	11.44±0.06 <sup>d</sup>
Acetylated (20.6% Ac <sub>2</sub> O)	0.190	50.39±0.08 <sup>f</sup>	62.15±0.11 <sup>d</sup>	88.09±0.28 <sup>f</sup>	11.38±0.10 <sup>d</sup>
Cross-linked and acetylated <sup>3</sup>	-	57.24±0.20 <sup>d</sup>	64.56±0.01 <sup>c</sup>	86.20±0.25 <sup>d</sup>	11.44±0.00 <sup>d</sup>

<sup>1</sup> Means of duplicate analyses ± standard error. In each column, values followed by the same letter are not significantly different ( $p < 0.05$ ).

<sup>2</sup> DS is degree of substitution

<sup>3</sup> Cross-linked with 0.10% POCl<sub>3</sub> followed by acetylating with 0.1 moles (6.8%) acetic anhydride (Ac<sub>2</sub>O) per 150g of starch.

ing resulted in higher gelatinization temperatures and enthalpies of waxy hull-less barley starch<sup>[13]</sup>, and slight decrease in  $T_o$  and significant decrease in  $\Delta H$  of waxy rice starch<sup>[27]</sup>. Liu et al.<sup>[25]</sup> reported that acetylation caused a large reduction in  $T_o$ ,  $T_c$ ,  $T_p$  and  $\Delta H$  in waxy rice starch; an observation that is consistent with the results obtained in the present study.

### Effect of modification on dynamic mechanical properties

This test basically involved the determination of the temperature dependence of storage modulus ( $G'$ ) and loss modulus ( $G''$ ). The loss factor ( $\tan \delta$ ) was measured as the ratio  $G''/G'$ . Typically the temperature dependence curves of  $G'$  and  $G''$  for cowpea starch began to increase rapidly at a characteristic temperature (onset temperature) until maxima were attained (peak temperature). After passing the peak temperature the values of  $G'$  and  $G''$  gradually decreased to a constant value. Cross-linking of cowpea starch resulted in a significant reduction ( $p < 0.05$ ) in the onset temperature of  $G'$  as shown in TABLE 1. The onset temperature of  $G'$  was not, however, significantly affected by the DS. Cross-linking to DS 0.003 and 0.006 caused a significant reduction in maximum storage modulus ( $G'_{max}$ ) but the starch cross-linked to DS 0.004 showed no significant change compared to the native starch. Acetylation and double modification caused significant reductions in the onset temperature of  $G'$ , and  $G'_{max}$ ; and both were inversely related to DS for the the acety-

lated samples. Cross-linking had no effect on the temperature at  $G'_{max}$  but acetylation and double modification caused significant reductions. Both cross-linking and acetylation resulted in significant reductions in the onset temperature of  $G''$  and maximum loss modulus ( $G''_{max}$ ) except starch acetylated to DS 0.190 that exhibited higher values than the native starch.  $\tan \delta$  values indicated that cross-linking has slight effects on the viscoelasticity of starch gels whereas acetylation imparted liquid-like properties to the gels. When  $\tan \delta$  is  $> 0.1$ - $0.2$  viscous forces add to elastic forces to give a composite force vector<sup>[24]</sup>.

Won et al.<sup>[28]</sup> reported that cowpea starch exhibited higher  $G'$  and  $G''$  values than corn and potato and that  $G'$  was positively related to gel hardness. Manual examination of the native cowpea starch gels in the present revealed the gels to be very brittle and rigid. This factor may impact negatively on the functional properties of some food products to which cowpea starch may be incorporated. This negative property can be ameliorated by acetylation or by a combination of cross-linking and acetylation as evidenced by the reduction of  $G'_{max}$  from 13100 Pa for native starch to 1275 Pa for starch acetylated to DS 0.190 (TABLE 5). Gels with reduced rigidity may be more suited for applications in such products as desserts, sauces and dressings that require some degree of flexibility. The higher  $\tan \delta$  values of acetylated and doubly modified cowpea starches compared to the native starch are further evidence of their paste-like properties.



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**TABLE 5: Effect of phosphation and acetylation on the dynamic mechanical properties of cowpea starch<sup>1</sup>.**

Modification	DS <sup>2</sup>	G' Onset temperature (°C)	G' max (Pa)	Temperature at G' max (°C)	G'' Onset temperature (°C)	G'' max (Pa)	Temperature at G'' max (°C)	Tanδ at G' max
Native	-	72.23±0.13 <sup>a</sup>	13100.0±300 <sup>a</sup>	79.62±0.22 <sup>a</sup>	72.78±0.18 <sup>a</sup>	5101.3±49 <sup>b</sup>	78.67±0.06 <sup>a</sup>	0.15±0.003 <sup>e</sup>
Cross-linked (0.10% POCl <sub>3</sub> )	0.003	70.32±0.64 <sup>b</sup>	6525.0±275 <sup>d</sup>	79.40±0.11 <sup>a</sup>	70.28±0.42 <sup>b</sup>	2227.0±150 <sup>c</sup>	77.87±0.12 <sup>b</sup>	0.21±0.003 <sup>d</sup>
Cross-linked (0.15% POCl <sub>3</sub> )	0.04	69.66±0.25 <sup>b</sup>	12450.0±150 <sup>ab</sup>	79.14±0.10 <sup>a</sup>	69.66±0.25 <sup>b</sup>	3361.8±77 <sup>d</sup>	77.35±0.21 <sup>c</sup>	0.15±0.002 <sup>e</sup>
Cross-linked (0.20% POCl <sub>3</sub> )	0.06	69.44±0.23 <sup>b</sup>	12000.0±700 <sup>b</sup>	79.25±0.16 <sup>a</sup>	68.20±0.08 <sup>c</sup>	4279.3±44 <sup>c</sup>	77.75±0.22 <sup>bc</sup>	0.16±0.004 <sup>e</sup>
Acetylated (6.8% Ac <sub>2</sub> O)	0.030	66.62±0.35 <sup>c</sup>	9680.0±20 <sup>e</sup>	77.21±0.33 <sup>b</sup>	66.49±0.22 <sup>d</sup>	4304.3±22 <sup>c</sup>	75.47±0.27 <sup>d</sup>	0.36±0.001 <sup>b</sup>
Acetylated (13.6% Ac <sub>2</sub> O)	0.080	55.16±0.25 <sup>e</sup>	5725.0±65 <sup>de</sup>	68.70±0.29 <sup>d</sup>	54.66±0.25 <sup>f</sup>	3455.1±43 <sup>d</sup>	66.58±0.13 <sup>f</sup>	0.45±0.008 <sup>a</sup>
Acetylated (20.4% Ac <sub>2</sub> O)	0.190	52.60±0.15 <sup>f</sup>	1275.0±25 <sup>f</sup>	67.81±0.05 <sup>e</sup>	51.99±0.52 <sup>g</sup>	5504.7±79 <sup>a</sup>	65.93±0.00 <sup>g</sup>	0.32±0.001 <sup>c</sup>
Cross-linked and acetylated <sup>3</sup>	-	61.18±0.04 <sup>d</sup>	4963.5±30 <sup>e</sup>	71.24±0.08 <sup>c</sup>	61.53±0.20 <sup>c</sup>	2093.4±2 <sup>c</sup>	69.33±0.04 <sup>c</sup>	0.33±0.004 <sup>c</sup>

<sup>1</sup>Means of duplicate analyses ± standard error. Within each column, values followed by the same letter are not significantly different ( $p < 0.05$ ).

<sup>2</sup>DS is degree of substitution.

<sup>3</sup>Cross-linked with 0.10% POCl<sub>3</sub> followed by acetylating with 0.10moles (6.8%) acetic anhydride (Ac<sub>2</sub>O) per 150g of starch.

**TABLE 6: Linear regression between Brabender, differential scanning calorimeter and rheometer parameters of native and modified cowpea starch.**

Dependent variable (X)	Independent variable (Y)	Regression equation	R <sup>2</sup>	Significant F
Brabender initial pasting temperature	Onset temperature (DSC)	Y=0.296X+52.528	0.80	0.0025
Brabender initial pasting temperature	G' Onset temperature (rheometer)	Y=0.307X+50.822	0.87	0.0007
Brabender initial pasting temperature	G'' Onset temperature (rheometer)	Y=0.300X+51.329	0.88	0.0005
DSC Onset temperature	G' Onset temperature (rheometer)	Y=0.988X-2.579	0.98	0.0000
DSC Onset temperature	G'' Onset temperature (rheometer)	Y=0.954X-0.097	0.97	0.0000

An attempt was made to relate the various physical properties that have a bearing on the gelation process of cowpea starch (amylograph, rheometer and DSC). As shown in TABLE 6, significant ( $p < 0.0025$ ) relationships were observed between the Brabender initial pasting temperature and DSC onset temperature ( $R^2=0.80$ ), DSC glass transition temperature ( $R^2=0.80$ ), rheometer G' onset temperature ( $R^2=0.87$ ), and G'' onset temperature ( $R^2=0.88$ ). Significant relationships were also observed between DSC onset temperature and DSC glass transition temperature ( $R^2=0.99$ ), G' onset temperature ( $R^2=0.98$ ) and G'' onset temperature ( $R^2=0.97$ ). The regression equations obtained could be useful in predicting the behavior of native and modified cowpea starches in the event one of the instruments is not available.

### CONCLUSION

Cross-linking and acetylation, singly or in combination of resulted in significant changes in the past-

ing, thermal and dynamic mechanical properties of cowpea starch. Acetylation caused a tremendous improvement in the freeze-thaw stability of cowpea starch gels. Overall, acetylation was more effective than cross-linking in altering the physicochemical properties of cowpea starch. Further investigations are indicated to assess the performance of modified cowpea starch in specific food products.

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