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## Acetylation of barnyardgrass starch with acetic anhydride under iodine catalysis



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## ABSTRACT

Barnyardgrass (*Echinochloa crus-galli*) is an invasive plant that is difficult to control and is found in abundance as part of the waste of the paddy industry. In this study, barnyardgrass starch was extracted and studied to obtain a novel starch with potential food and non-food applications. We report some of the physicochemical, functional and morphological properties as well as the effect of modifying this starch with acetic anhydride by catalysis with 1, 5 or 10 mM of iodine. The extent of the introduction of acetyl groups increased with increasing iodine levels as catalyst. The shape of the granules remained unaltered, but there were low levels of surface corrosion and the overall relative crystallinity decreased. The pasting temperature, enthalpy and other gelatinisation temperatures were reduced by the modification. There was an increase in the viscosity of the pastes, except for the peak viscosity, which was strongly reduced in 10 mM iodine.

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### 1. Introduction

*Echinochloa crus-galli* (L.) Beauv., which originates from eastern Asia and is predominant in temperate regions, is a type of wild grass that produces very small grains and is commonly known as barnyardgrass (Holm, Plucknett, Pancho, & Herberger, 1991). This rice grass is a major weed of importance in global agriculture because it has been responsible for significant losses in the productivity of various crops, especially rice (Altup & Mennan, 2011).

Crop management, including intense chemical control, is employed to reduce the infestation of *E. crus-galli* in rice production systems throughout many countries (Gibson, Fischer, Foin, & Hill, 2002). However, the extensive use of herbicides has led to the selection of resistant biotypes of this plant (Tang, Xie, Chen, & Yu, 2009). Additionally, the morphological similarities of barnyardgrass to rice plants during the seedling stage complicates the implementation of alternative control methods. Unfortunately, when barnyardgrass growth reaches a point where it can be identified and removed, the yield loss of the crop is generally unavoidable (Holm et al., 1991).

Although the global interest in the species from the genus *Echinochloa* is focused on of the losses of agriculture products, African and Asian countries commonly use the grain as a food source

(Veena, Chimmad, Naik, & Shanthakumar, 2004). According to Veena et al. (2004) the carbohydrate content of the grains ranges from 52.9% to 66.2%, with the starch constituting the major fraction; however, the properties of this starch are still unknown. Novel unconventional sources of starch have been studied to meet the demand for starches in the food and non-food industries (Adebowale, Henle, Schwarzenbolz, & Doert, 2009; Colussi et al., 2014; Sánchez-Rivera, Flores-Ramírez, Zamudio-Flores, González-Soto, & Rodríguez-Ambríz, 2010). Due to the expressive availability of barnyardgrass grain as a byproduct of the industrialisation of rice, the extraction and characterisation of the properties of this starch could add value and promote the use of this unconventional source of starch.

The limitations inherent to the use of starch in its native form have forced researchers to explore different methods of modification that can broaden the spectrum of starch use. In this sense, acetylation is one of the most well studied and implemented chemical modifications at the industrial level and is based on the esterification of starch with acetyl groups ( $-\text{COCH}_3$ ) to form starch acetates (Singh, Kaur, & McCarthy, 2007).

The insertion of acetyl groups promotes the reduction in the interactions between the outer chains of amylopectin and amylose chains, conferring new features to the polymer. Acetic anhydride is commonly used as an acylating agent, and the reaction is activated in the presence of an alkaline catalyst. However, according to Sánchez-Rivera et al. (2010), the reaction times are long, and the

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modification efficiency is low due to hydrolysis of the acetic anhydride by water and alkali present in the solution.

Recently, studies have shown that iodine is an excellent acylating agent in the acetylation of polysaccharides (Biswas, Shogren, & Willett, 2005; Diop, Li, Xie, & Shi, 2011; Ramírez-Arreola, Robledo-Ortiz, Arellano, González-Núñez, & Rodrigue, 2009). Unlike the methods that require an alkaline pH to facilitate the displacement reaction to promote substitution, iodine acts as a Lewis acid catalyst by activating the carbonyl group of the acylating reagent, making it more reactive during synthesis. Furthermore, Biswas et al. (2005) emphasised the ability of iodine to form a complex with amylose and amylopectin, which may contribute to the solubilisation of starch in acetic anhydride. The reaction takes up to 10 min at a temperature of 100 °C, and the stoichiometric product is easily reduced with sodium thiosulfate to form iodine.

However, only studies by Sánchez-Rivera et al. (2010) with corn and banana starches, and by Diop et al. (2011) with corn starch, provided information about the physical and chemical properties obtained by acetylation catalysed by iodine. Thus, the influence of the catalysis of iodine on the structural aspects, including the substitution pattern as well as physicochemical, rheological and functional characteristics, of these and other starch sources have not yet been elucidated.

The objective of this study was to explore the potential of the weed barnyardgrass as an unconventional source of starch and the use of iodine as catalyst in the acetylation of starches. Furthermore, the effect of different iodine concentrations in catalysis the acetylation of barnyardgrass starch on the physico-chemical, morphological, thermal and pasting properties of the starches was investigated.

## 2. Materials and methods

### 2.1. Materials

Barnyardgrass grains (*E. crus galli* Beauv.) were sourced from irrigated rice crops located in southern Brazil. The grains of the barnyardgrass (*E. crus galli* Beauv.) were threshed, dried until the moisture content was less than 12%, and sieved to remove any foreign matter. The resulting material was stripped in rice mills (Kepler Weber S. A., Porto Alegre, RS, Brazil) and purified in an air machine.

### 2.2. Barnyardgrass starch isolation

Native starch was isolated from barnyardgrass grains as previously described by Bello-Pérez, Agama-Acevedo, Zamudio-Flores, Mendez-Montealvo, and Rodriguez-Ambriz (2010) with some modifications. The grains were submerged in a 0.02 M (pH 6.5) sodium acetate buffer solution and stirred for 1 h. The liquid was then drained, and the softened pellets were washed with distilled water and ground in a domestic blender for 1 min.

The crushed and moistened material was sieved successively in sieves of 200 mesh and 270 mesh. The residues were each sieved again, subjected to grinding in water (1:2 v/v) and sieved. The process was repeated until the whitish colour of the sieved liquid, indicating the presence of starch in the residue, was no longer observed.

The suspension was centrifuged at 2000g for 10 min, and the greyish top layer was removed with a spatula. The resulting material was immersed in an aqueous solution of 0.2 M NaCl and toluene (7:1 ratio) and stirred vigorously overnight. Afterwards, the material was centrifuged at 2000g for 15 min, allowing for the separation of the material into different fractions. The supernatant was discarded, and the dark top layer was carefully removed. The

remaining layer (starch) was washed in water and centrifuged again to remove any residual impurities. This process was performed three times. The starch obtained was dried in an oven at 40 °C overnight with continuous air circulation containing up to 11% moisture.

### 2.3. Chemical composition

Quantitative estimation of the moisture, ash, nitrogen, lipids of the barnyardgrass starch was performed using standard AOAC methods (AOAC – Association of Official Analytical Chemists., 2007) and the results were expressed on a dry basis. The amylose content was determined by the colorimetric method with iodine as described McGrance, Cornell, and Rix (1998) with modifications suggested by Hoover and Ratnayake (2001).

### 2.4. Preparation of acetylated starches under iodine catalysis

The acetylation reaction was conducted under the reaction conditions described by Biswas et al. (2005) with some modifications. A sample of the starch (80 g, 11% moisture) was previously dried to less than 5.0% moisture in an oven at 70 °C with continuous air circulation and placed in a round bottom flask equipped with reflux condenser, thermometer and stirring system, upon which 140 mL of acetic anhydride was added. The suspension was stirred continuously at room temperature for 5 min, at which point metallic iodine was added (previously dissolved in 10 mL of acetic anhydride) at final concentrations of 1, 5 and 10 mM (0.13, 0.63 and 1.27 g.) The temperature was raised to 100 °C for 10 min, and the reaction was halted by the addition of a saturated sodium thiosulfate solution (50 mL). The starch acetate obtained was washed with 70% ethanol (150 mL) and concentrated methyl alcohol (100 mL) to remove any remaining acetic anhydride and dried at 40 °C until reaching approximately 11% moisture.

### 2.5. Degree of substitution of acetylated starches

The percentage of acetyl groups (% Ac) (Eq. (1)) for determining the degree of substitution (DS) (Eq. (2)) was determined by titration as proposed by Würzburg (1986). Approximately 1 g of each sample was added to a glass jar with a lid along with 50 mL of 75% ethyl alcohol. The samples were heated in a water bath at 50 °C for 30 min and, after cooling, 40 mL of 0.5 N KOH was added; the mixture was maintained under agitation at 200 rpm for 72 h. Excess alkali was titrated out with 0.5 N HCl using phenolphthalein as an indicator. The neutralised solution was stirred for 2 h and excess alkali (which may have leached from the sample) was titrated. The average values for the titration of the native starch were used as a blank reaction.

$$\%Ac = \frac{[(\text{mL blank} - \text{mL sample}) \times \text{MHCl} \times 0.043 \times 100]}{\text{sample weight(g)}} \quad (1)$$

DS is defined as the average number of sites per glucose unit that receives a substituent group.

$$DS = \frac{(162 \times \%Ac)}{[4300 - (42 \times \%Ac)]} \quad (2)$$

### 2.6. Fourier Transform infrared (FT-IR) spectra

The sample preparation and analysis parameters were performed according to the recommendations of Diop et al. (2011). Tablets were prepared from a mixture of the sample with KBr at a ratio of 1:100 (starch:KBr). The infrared spectra of the native and acetylated barnyardgrass starch samples were obtained using

a Fourier transform spectrometer (IR Prestige-21, Shimadzu Corp. Japan) in the range of 4000–400  $\text{cm}^{-1}$ . Ten readings were collected at a resolution of 4  $\text{cm}^{-1}$ .

### 2.7. X-ray diffraction

The X-ray diffraction patterns of the starches were obtained with an X-ray diffractometer (X'pert PRO Multi-Purpose, PanAnalytical) in the region of diffraction scan ranging from 5° to 30°, with a target voltage of 30 kV, current of 30 mA and scanning speed of 1° $\text{min}^{-1}$ . The relative crystallinity (RC) of the starch granules was calculated as described by Rabek (1980), with the  $\text{RC} (\%) = (Ac / (Ac + Aa)) * 100$ , where *Ac* is the crystalline area and *Aa* is the amorphous area in the X-ray diffraction pattern.

### 2.8. Scanning electron microscopy (SEM)

The morphological features of the starch granules were examined by scanning electron microscopy (Shimadzu, SSX-550). Starch samples were initially suspended in acetone to obtain a 1% (w/v) suspension and samples were maintained in an ultrasound for 15 min. A small quantity of each sample was spread directly on the surface of the stub and dried in an oven at 32 °C for 1 h. All the samples were subsequently coated with gold and examined under a scanning electron microscope at an acceleration voltage of 15 kV at 1000× magnification.

### 2.9. Thermal properties

The thermal properties of the starches were determined by differential scanning calorimetry (DSC model 2100, TA Instruments, New Castle, USA). Starch samples (approximately 2.5 mg, dry basis) were weighed directly in an aluminium pan, and distilled water was added to obtain a starch–water ratio of 1:3 (w/w). The pan was hermetically sealed and allowed to equilibrate for 1 h before analysis. Sample pans were then heated from 30 to 120 °C at a rate of 10 °C/min.

### 2.10. Swelling power

The swelling power was evaluated according to the method described by Leach, McCowen, and Schoch (1959) at temperature of 90 °C. Approximately 1 g of starch was added 50 mL of water, homogenized and heated at indicated temperatures for 30 min. After cooling, the material was centrifuged at 1000g for 20 min and the supernatant discarded. The swollen starch was weighed and the swelling power obtained by the ratio of the swollen mass end of the initial mass of starch, expressed as g/g.

### 2.11. Amylose leaching

A suspension of 20 mg of starch in 10 mL of water was homogenised and heated at 90 °C for 30 min; after cooling, the suspension was centrifuged at 2000g for 10 min as described by Varatharajan, Hoover, and Seetharaman (2010). An aliquot of 1 mL of the supernatant was removed and the amylose content determined by colorimetric reaction as specified in item 2.3. The percentage of leached amylose was calculated from the leached amylose (mg) about 100 g of dry starch.

### 2.12. Pasting properties

The pasting properties of the starch were determined using a Rapid Visco Analyser (RVA-4, Newport Scientific, Australia) with a Standard Analysis 1 profile. The viscosity was expressed in rapid visco units (RVUs). A starch sample of 3.0 g (14 g/100 g wet basis)

was weighted directly on an RVA canister, and 25 mL of distilled water was then added.

### 2.13. Statistical analysis

Analytical determinations for the samples were performed in triplicate and standard deviations were reported for all of the data collected except for the X-ray diffractograms and thermal analysis. Means were compared by Tukey's test at a 5% significance level using analysis of variance (ANOVA).

## 3. Results and discussion

### 3.1. Barnyardgrass starch isolation and chemical composition

The extraction yields of barnyardgrass starch based on the mass of hulled grain (d.b.) and available starch content in the grains were 43.7% and 65.8%, respectively. Similar results of alkaline extraction yield for medium amylose rice starch (51.9%) were reported by Zavareze, Storck, Castro, Schirmer, and Dias (2010), based on the percentage of the rice mass.

The barnyardgrass starch showed high purity (98.5%), with low residual protein (0.2%), lipid (0.6%), fibre (0.4%) and ash (0.3%) levels. The use of toluene extraction on the liberated starch constituent caused the formation of aggregates, resulting in an emulsion in the supernatant composed of proteins and lipids and starch with high purity. The amylose content in barnyardgrass starch was 32.6%, which is higher than that commonly found in cereal starches such as corn and rice (Choi, Kim, Park, Kim, & Maik, 2009; Zavareze et al., 2010) but close to that reported in rice starch with high-amylose content (Zavareze et al., 2010).

### 3.2. Degree of substitution of acetylated starches

The different concentrations of iodine as a catalyst (1, 5 and 10 mM of iodine) interfered with the introduction of acetyl groups to the starch. In all of the modifications, the starches' DS increased significantly with increasing catalyst concentrations (Table 1). Depending on the values of DS, modified starches in the presence of 1 and 5 mM of iodine were classified as low DS (<0.1), whereas treatment with 10 mM of iodine resulted in an acetylated starch with medium DS (0.1–1.0). However, it should be noted that despite the increasing DS upon increasing concentrations of iodine, the observed increase was not proportional to the concentration of iodine used. For example, 5 mM of iodine only doubled the substitution than compared to acetate obtained with 1 mM of iodine (as opposed to increasing the substitution 5-fold), and the 10 mM iodine treatment only tripled this increase.

The use of iodine in starch acetylation is a recent development, and information on the influence of this catalyst in the reaction process is still scarce. However, regardless of the catalyst used, acetic anhydride is considered an acetylating agent that has limited granular penetration (Huang, Schols, Jin, Silmann, & Voragen, 2007). According to Chen, Schols, and Voragen (2004), acetylation using acetic anhydride occurs predominantly in the amorphous region of the granules. These authors observed that the DS was considerably higher in fractions of amylose compared to amylopectin fractions of starch from potatoes and sweet potatoes acetylated with acetic anhydride under alkaline catalysis.

Sánchez-Rivera et al. (2010) reported DS values of 0.025 and 0.077 for banana and corn starches, respectively, under similar reaction conditions and iodine catalysis. In the barnyardgrass starch produced in this study, it was possible to obtain starch acetate with a DS of 0.098 under the same molar amount of iodine (5 mM) per anhydroglucose unit under similar reaction conditions,

**Table 1**

Degree of substitution, swelling power, amylose leaching, intensity of the primary peaks of the X-ray diffractograms and relative crystallinity of barnyardgrass starches.

Starches	Degree substitution (DS) <sup>a</sup>	Swelling power (%) <sup>a</sup>	Amylose leaching (%) <sup>a</sup>	Intensity (CPS) <sup>c</sup>					CR <sup>d</sup> (%)
				15°	17°	17.8°	19°	23°	
Native	–	12.1 ± 0.1c	9.9 ± 0.5c	988	1133	1142	785	997	44.8
1 mM <sup>b</sup>	0.047 ± 0.003c	17.6 ± 0.2a	13.9 ± 0.1b	989	1128	1180	831	1040	42.8
5 mM	0.098 ± 0.002b	17.2 ± 0.2a	17.9 ± 0.4a	973	1104	1087	813	939	42.4
10mM	0.125 ± 0.009a	14.1 ± 0.4b	18.7 ± 0.3a	912	1049	1103	824	987	41.9

<sup>a</sup> Different letters in the same column differ statistically ( $p < 0.05$ ).<sup>b</sup> Acetylation (amounts of iodine used as catalyst).<sup>c</sup> CPS: counts per second.<sup>d</sup> CR: relative crystallinity.

indicating a greater susceptibility of barnyardgrass starch to accept acetyl groups with respect to banana and corn starches.

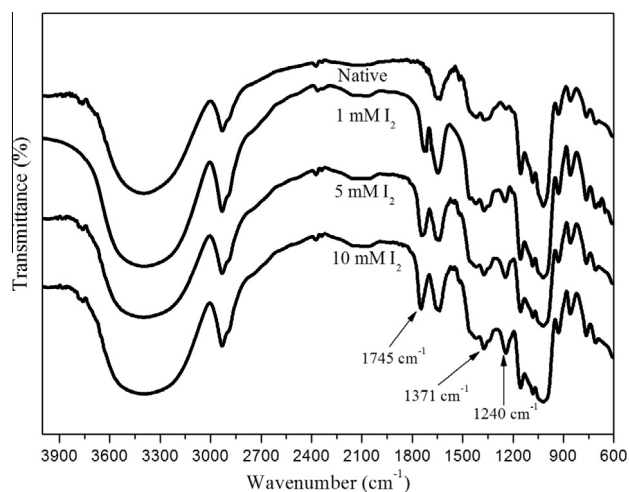
### 3.3. FTIR analysis

Fig. 1 shows the structural features of native and modified barnyardgrass starch analysed by FTIR. The presence of polysaccharides is described by the occurrence of absorption bands in the region known as the “fingerprint region” ranging from 1000 to 1220  $\text{cm}^{-1}$ . The spectrum of the native barnyardgrass starch shows intense peaks at wave numbers of approximately 1018  $\text{cm}^{-1}$ , 1080  $\text{cm}^{-1}$  and 1157  $\text{cm}^{-1}$ , which can be assigned to the vibration of the connecting (C–O) glycosidic units. An intense peak at 1641  $\text{cm}^{-1}$  relative to the tightly bound water was also recorded, and a wide band at 3450  $\text{cm}^{-1}$  resulting from the vibration of the hydrophilic hydroxyl groups (OH) was observed along with a peak at 2960  $\text{cm}^{-1}$ , which is derived from the stretching vibration of the CH groups on the glucose molecules (Diop et al., 2011).

Confirmation of the acetylation as well the extent of the incorporation of the acetyl groups to the starch molecule was observed by the appearance of absorption peaks in the region of 1745  $\text{cm}^{-1}$  and 1240  $\text{cm}^{-1}$  related to the axial deformation of C=O and C(=O)–O from acetyl groups introduced, respectively. Both bands were characterised by strong peaks, which became more intense with an increase in the DS in the starches. The band in the region of 1371  $\text{cm}^{-1}$  of the acetylated starches, which is related to the stretching of the CH acetyl group, also differs with the increase of acetyl groups bound to the starch molecules.

### 3.4. X-ray diffraction

The native barnyardgrass starch showed a crystalline pattern of the A-type, which is similar to those observed in cereal starches

**Fig. 1.** FTIR spectra of native and modified barnyardgrass starches.

such as rice (Zavareze et al., 2010) and corn (Chung, Liu, & Hoover, 2009). There were strong peaks of reflection at  $2\theta$  angles of 15° and 23°, one unresolved doublet at 17° and 17.8° and a peak of lower intensity at 19°.

There were no observed changes in the X-ray diffraction patterns between the native and modified starches, but there was a reduction of the overall relative crystallinity of the modified starches compared to the native starch (Table 1). The low reduction in the relative crystallinity of the acetylated starches is in agreement with literature data, where little or no change was reported in acetylated starches with low DS (Chen et al., 2004; Huang et al., 2007). As highlighted by Lawal (2004), when hydroxyl groups are partially replaced by acetyl groups, the hydroxyl groups that are still remaining are adjusted to form hydrogen bonds. Therefore, the characteristic crystalline peaks remain, but the intensities are slightly reduced.

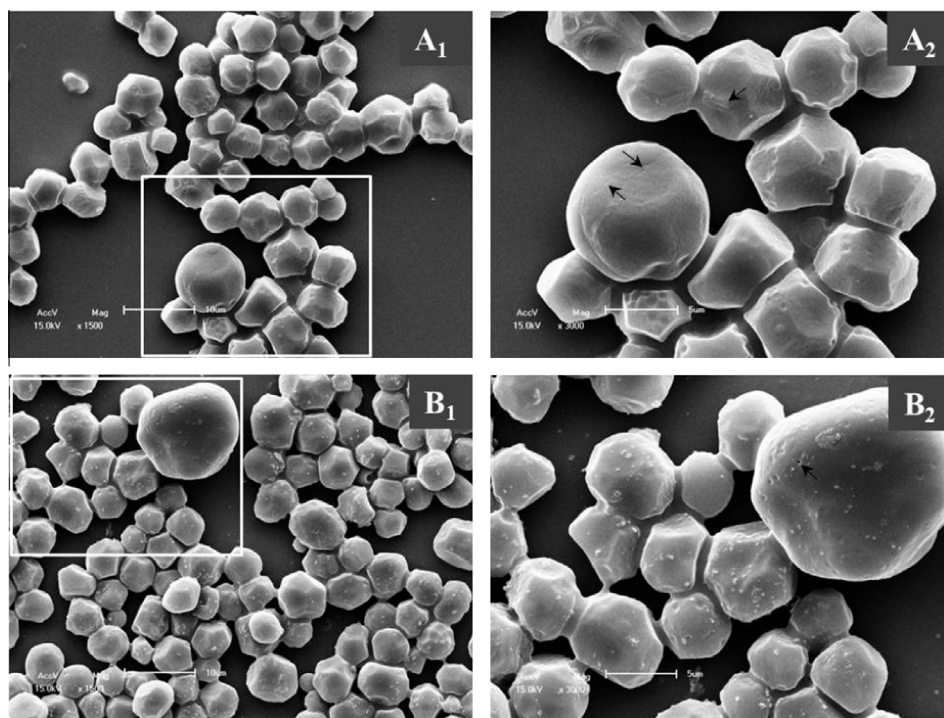
According Huang et al. (2007), the minimal crystallinity changes in the acetylated starches with low DS suggests that the acetyl groups are concentrated primarily in the amorphous region of the granules. There is significant evidence that amylopectin is the primary contributor of crystalline order in starch granules (Pérez & Bertoft, 2010). A concentration of acetyl groups in the amorphous region could explain the absence of major changes in the granular crystalline structure of starches with few substituted acetyl groups.

A relative increase in the intensity of the peak at 19° is attributed to the formation of a V-type complex. Acetylated starches in this formation are presumably a consequence of the association of amylose with the acetyl groups. As noted by Pérez and Bertoft (2010), the formation of the dry V-type structure is generally favoured when a bulky group is grafted into starch chains.

### 3.5. Morphology of starch granules

Scanning electron micrographs (SEM) of native and acetylated starch granules are shown in Fig. 2 at 1500× and 3000× magnifications, respectively. Barnyardgrass starch granules showed irregular forms (predominantly rounded and polygonal shapes) of varying sizes (1–33  $\mu\text{m}$ ) but were characterised by the predominance of small granules (4–8  $\mu\text{m}$ ). In some native granules, the presence of small surface pores was clearly noticeable, especially in the images under high magnification (A<sub>2</sub> and B<sub>2</sub>). These surface pores were also reported in corn and sorghum starch, and it is suggested that these pores provide channels to allow access to the interior of the granules (Huber & BeMiller, 2000). These structural characteristics may affect the reaction patterns by facilitating the flow of reagents within the matrix of the starch granules during modification reaction. In addition to the presence of pores, the small size of the barnyardgrass starch granules could also potentially contribute to the chemical substitution due to increased surface contact area.

After acetylation, the starch granules still remained intact, but the surface of the granules transitioned from smooth to slightly



**Fig. 2.** Scanning electron micrographs of native starch granules ( $A_1$  and  $A_2$ ) and granules acetylated under 10 mM of iodine ( $B_1$  and  $B_2$ ) at magnifications of 1500 $\times$  and 3000 $\times$ , respectively.

wrinkled and rough with saliences on their surface, suggesting the occurrence of slight corrosion. Little change in the morphology is presumed because the low DS of the starches and this is consistent with the observations of other authors with regard to acetylated starches under alkaline conditions and similar DS (Colussi et al., 2014; Sodhi & Singh, 2005). Furthermore, the amount of iodine used does not appear to affect the size and shape of the granules. Sánchez-Rivera et al. (2010) used iodine in the acetylation of banana and maize starches and observed differences in the morphological changes between the two sources. They also observed that corn starch was more susceptible to morphological changes after acetylation and showed signs of surface corrosion in the granules, which was not visualised in the acetylated banana starch.

### 3.6. Swelling power (SP) and amylose leaching (AL)

The swelling power and levels of leached amylose from native and modified starches at 90 °C are shown in Table 1. Acetylation promoted increases in granular swelling in all of the modified starches; however, the increased granular swelling after acetylation was not proportional to the insertion of acetyl groups, which were more significant in starches with a low DS (0.047 and 0.098) and less significant on the starch with higher DS (0.125). According to Singh, Kaur, and Singh (2004), the introduction of acetyl groups to starch molecules can promote a reduction in the interaction between the chains and result in partial disruption of the intragranular structure due to steric effects and disruption of hydrogen bonds, facilitating the penetration of water to the interior of the granules.

The levels of amylose leaching increased after acetylation and were more severe when higher concentrations of iodine were used (Table 1) to obtain higher DS. The increased amylose leaching observed in the acetylated starches suggests that the introduction of acetyl groups disrupts the hydrogen bonds between amylose chains. In accordance with Gunaratne and Corke (2007), the

introduction of bulky groups can promote discontinuity in the hydrogen bonding of adjacent starch chains and thus facilitate the dissociation of amylose chains out of the granules.

### 3.7. Pasting properties

The pasting properties of barnyardgrass starch showed variability after chemical modification (Table 2). Acetylation with different concentrations of iodine resulted in intense changes in the viscoamylograph of the starches' profiles (Fig. 3). Under 1 and 5 mM of iodine, either maintenance or an increase in the viscosities was observed compared to the native starch as well as a significant decrease in the pasting temperature. However, although the acetylation with the lowest tested concentration of iodine (1 mM) did not induce a difference in the viscosity peak value relative to the native starch, the RVA curves show that there is a distinct behaviour between them. With 1 mM of iodine, the RVA curves show two peaks with similar heights but recorded at different times. The major peak (right) was recorded at approximately 95 °C (near the peak temperature obtained for the native starch) and another peak (left) was recorded at approximately 85 °C (peak temperature similar to the other acetylated starches). This observation indicates that under these reaction conditions, the amount of catalyst used did not allow for homogenous acetylation in the barnyardgrass starch granules. The new pasting properties conferred on the starch are shown by the peak to the left of the primary peak, whereas the peak on the right confirms the maintenance of the characteristics of the native starch. Therefore, a change in the viscosity from the peak to a higher value (as obtained for the acetylated starch at 5 mM iodine) was not observed.

In studies by Sodhi and Singh (2005), an increase in viscosity of rice starch paste after acetylation was observed regardless of the amylose content. The introduction of bulky groups on the starch chains may increase the ability of the granule to retain water

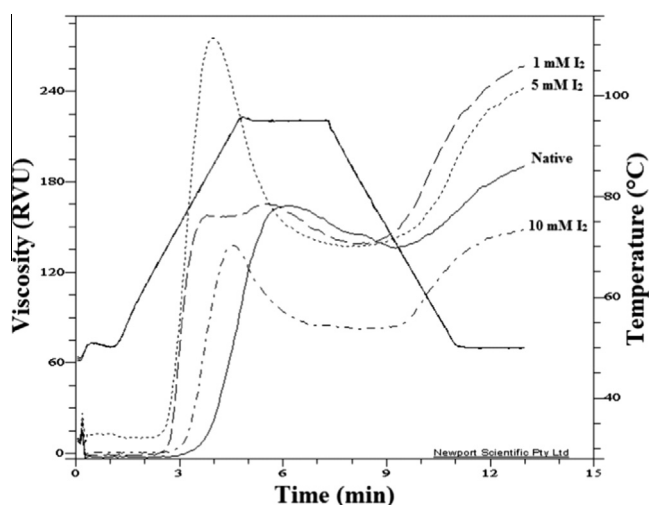
**Table 2**  
Pasting properties of native and acetylated barnyardgrass starches.

Starches <sup>a</sup>	Pasting temperature (°C)	Peak viscosity (RVU) <sup>c</sup>	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)
Native	80.5 ± 1.1a	165.0 ± 1.4b	28.8 ± 0.8c	194.3 ± 3.9c	56.2 ± 1.5d
1 mM <sup>b</sup>	68.6 ± 0.1c	166.5 ± 2.1b	26.5 ± 0.4c	256.8 ± 3.6a	114.5 ± 0.7a
5 mM	68.1 ± 0.7c	274.2 ± 1.3a	137.3 ± 1.4a	240.2 ± 2.7b	103.3 ± 2.8b
10 mM	75.0 ± 0.1b	139.0 ± 3.2c	55.1 ± 2.4b	147.7 ± 2.1d	66.9 ± 2.2c

<sup>a</sup> Different letters in the same column differ statistically ( $p < 0.05$ ).

<sup>b</sup> Acetylation (amounts of iodine used as catalyst).

<sup>c</sup> RVU: rapid visco unit.



**Fig. 3.** RVA curves of native and acetylated barnyardgrass starches.

(Sánchez-Rivera et al., 2010), thereby providing greater resistance to deformation and, consequently, a higher viscosity.

The acetylated starch under the highest concentration of iodine (10 mM) and higher DS (0.125) showed a decrease in the pasting temperature, peak viscosity and final viscosity, whereas the breakdown and setback values were superior to the native starch but lower than those values observed in other acetylated starches with a lower DS. A decrease in the viscosity was also reported by Sánchez-Rivera et al. (2010) in banana and corn starches that were acetylated under iodine catalysis (equivalent to the 5 and 10 mM treatments used in this study). The authors attributed the lowest swelling capacity in these starches to a possible partial disruption of the granules that occurred due to the introduction of bulky acetyl groups, which may confer a greater proportion of hydrophobicity and decrease the ability of the starch to absorb and retain water. Similar results were reported by Lawal (2004) in cocoyam starch with DS of 0.30 by Bello-Pérez et al. (2010) in barley starch with DS of 0.9 to 2.7 and by Choi et al. (2009) in corn starch, with DS values ranging from 0.046 to 0.089.

It is unlikely that a low DS ( $\leq 0.1$ ) is sufficient to impart hydrophobicity to starches. In fact, Xu, Miladinov, and Hanna (2004) showed that the hydrophobicity of acetylated starches increases when the DS value are greater than 0.5. The decrease in the peak viscosity observed with 10 mM of iodine can be related to the depolymerisation of the starch chains due to the occurrence of secondary hydrolysis during the reaction process. In this sense, Diop et al. (2011) speculated that iodine can also act as an oxidising agent on starch, thus intensifying the process. Erosion of the amorphous region can result in weaker granules that are more easily deformed under stirring, which can reduce the viscosity of the pastes (Gunaratne & Corke, 2007).

Acetylation increased retrogradation compared to the native starch, especially at concentrations of 1 and 5 mM of iodine.

**Table 3**  
Thermal characteristics of native and acetylated barnyardgrass starches.

Starches	To <sup>b</sup> (°C)	Tp (°C)	Tc (°C)	Tc – To (°C)	$\Delta H$ (J g <sup>-1</sup> )
Native	65.13	71.04	76.86	11.73	8.05
1 mM <sup>a</sup>	56.48	65.44	72.93	16.45	5.46
5 mM	59.13	64.85	72.60	14.47	4.81
10 mM	59.55	67.43	74.00	14.45	6.77

<sup>a</sup> Acetylation (amounts of iodine used as catalyst).

<sup>b</sup> To: onset temperature; Tp: peak temperature; Tc: conclusion temperature;  $\Delta H$ : enthalpy of gelatinization.

Sodhi and Singh (2005) stated that the introduction of acetyl groups should prevent the interaction between parallel amylose chains and thus provide a reduction in retrogradation. However, increasing the setback values after acetylation is also reported in rice starch (Sodhi & Singh, 2005), corn starch (Liu, Ramsden, & Corke, 1997) and yellow pea starch (Huang et al., 2007). Hoover and Ratnayake (2001) suggested that increased leaching of amylose (as noted in all acetylated starches) and the shearing action of the blades during RVA analysis can “nullify” any steric effects that arise upon the introduction of the substituent groups to reduce retrogradation. In this study, the decline was greater after acetylation with 5 mM iodine, indicating a major weakness of the acetylated granules.

Huang et al. (2007) suggest that the modification of the pasting properties of acetylated starch is strongly influenced by the starch source used and not only by the acetylation level. These authors observed variation in the setback values of different sources of starch acetylated with acetic anhydride and vinyl acetate. The setback values increased substantially for yellow pea starch regardless of acylating agent used, while acetylation reduced these values for cowpea starch, and for chickpea starch caused only slight change. Moreover, the effects of using iodine as catalyst starches are not very clear and it may have contributed for the changes in paste properties observed.

The peak viscosity of the native starch occurred at an RVA temperature of 95 °C, whereas for acetylated starches, the peak viscosity was slightly lower (approximately 85–90 °C). This peak viscosity is in agreement with the decrease of pasting temperature and the increased hydration of the acetylated granules as observed in the swelling experiment.

### 3.8. Thermal properties

Modified barnyardgrass starches showed marked differences in their thermal behaviour, both in relation to each other as well as to the native starch (Table 3). After acetylation, the To, Tp and Tc and  $\Delta H$  values were reduced, whereas the gelatinisation temperature range (Tc – To) was increased. The decrease in the gelatinisation temperatures after acetylation has been reported in previous studies (Choi et al., 2009; Sodhi & Singh, 2005; Sánchez-Rivera et al., 2010) and represents the weakening of the granules after

the introduction of acetyl groups, which leads to premature rupture of double helices of amylopectin during heating (Lawal, 2004).

The more substituted starch (DS 0.125) had the smallest decreases in all of the thermal parameters compared to the native starch. This behaviour can be attributed to the additional interactions between the polymer chains and the inserted acetyl groups, as evidenced by the low levels of amylose leaching.

All of the modified starches were enlarged within the gelatinisation temperature range. ( $T_c - T_o$ ) compared to the native starch. With 1 mM of iodine, this difference was more significant than that observed for the acetylated starches with a higher DS obtained from reactions using 5 and 10 mM iodine. This observed behaviour in the thermal properties of the acetylated starch with 1 mM iodine with regard to the viscoamylographic properties (where the starch had two peaks of viscosity) reinforces the notion that part of the granules remained unchanged during acetylation with 1 mM iodine, whereas the majority of the other granules were modified.

#### 4. Conclusion

Barnyardgrass starch was extracted with a good yield and purity, generating sufficient product to characterise its physicochemical properties and modify samples by acetylation catalysed by iodine. The degree of acetylation increased with increasing amounts of iodine used as a catalyst, which is confirmed by the changes observed in the FTIR bands and peaks. However, the ability of acetyl groups to substitute was not linearly induced by the increasing iodine concentrations. Barnyardgrass starch exhibited small granule size and the existence of small pores on the surface, which may be beneficial to chemical substitution. After acetylation, the granules remained intact but showed slight signs of surface corrosion and a small reduction in the relative crystallinity.

Additional studies regarding the acetylation under iodine catalysis, particularly at different concentrations of catalyst, as well using other starch sources with varying granule sizes and properties, could help to elucidate the potential of this procedure in the modification of starches. Following this work, the behaviour of barnyardgrass starch acetates under different pH conditions, its susceptibility to hydrolysis by  $\alpha$ -amylase and properties obtained by combining other modification methods, will be investigated.

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