

## Characteristics of starch isolated from maize as a function of grain storage temperature



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### ABSTRACT

Considering the importance of maize starch and the lack of knowledge about the effects of storage temperature on the isolated starch properties; maize grains were stored during 12 months at different temperatures (5, 15, 25 and 35 °C). The extraction yield and the physicochemical, thermal, pasting, crystallinity and morphological properties of starches were determined. The starch isolated from grains stored at 35 °C was yellowish and showed a 22.1% decrease in starch extraction yield compared to freshly harvested maize grains. At 35 °C, a reduction in crystallinity was observed by the end of 12 months, despite a parallel rearrangement of the starch chains which resulted in an increase in X-ray peak intensities, gelatinisation temperatures and enthalpy. The starch isolated from maize grains stored at 35 °C appears to have smaller granules, which presents some points in their surface, potentially attributed to the protein matrix compressing the granules within maize grains.

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### 1. Introduction

Starch is widely used in the food industries, especially in the preparation of soups, sauces, baked goods, dairy, confectionery, snacks, pasta, coatings and products made with meat (Davies, 1995). The ability of starch to form a viscous paste when heated in water followed by the cooling property makes starch suitable for various uses in the food and non-food industries (Nguyen, Jensen, & Kristensen, 1998). The main botanical source used for extraction of starch is maize, accounting for about 80% of the world market (Jobling, 2004). Among all kinds of starches, maize starch is an important ingredient in the production of foodstuffs, and has been widely used as a thickener, stabiliser, colloidal gelling agent, water retention and as an adhesive (Singh, Singh, Kaur, Sodhi, & Gill, 2003). Starch is the main constituent of maize kernels, about 72–73% of the total weight (Sandhu, Singh, & Lim, 2007).

After harvested, the maize grains are subjected to various post-harvest steps, such as cleaning, drying and storage. Several studies have elucidated the effects of drying temperature on the properties

of isolated starches (Altay & Gunasekaran, 2006; Eckhoff & Watson, 2009; Haros, Tolaba, & Suarez, 2003; Lasseran, 1973; Malumba et al., 2010; Malumba, Massaux, Deroanne, Masimango, & Béra, 2009; Setiawan, Widjaja, Rakphongphairoj, & Jane, 2010). According to Malumba et al. (2009), drying temperatures of maize grains up to 100 °C cause changes in the pasting and texture properties of the starch gel, and reduce the extraction yield as well as purity of starch.

The storage results in reduced solubility and digestibility of grain proteins (Rehman, Habib, & Zafar, 2002), increased free fatty acids (Park, Kim, Park, & Kim, 2012), and these may form complexes with amylose or amylopectin short chains, altering the nutritional properties and the physical characteristics of the final products (Hasjim et al., 2010; Salman & Les, 2007). Long periods of storage reduce the yield of cassava starch extraction during wet-milling, as a result of starch degradation and the interactions between starch and other constituents (Abera & Sudip, 2004). Setiawan et al. (2010) stored maize grains at 27 °C and around 85–90% of relative humidity for 6 months, reporting changes in the pasting, thermal, morphological and crystallinity properties of starch; without considering the effects of storage temperature on the properties of isolated starch. Yousif et al. (2003) reported an increase in gelatinisation temperature of adzuki bean (*Vigna angularis*) starch with increasing storage temperature. Rupollo et al. (2011) evaluated the effects of storage

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conditions of common bean (*Phaseolus vulgaris* L.) grains on physicochemical, pasting, crystallinity and morphological properties of isolated starch, observing changes in the thermal properties and crystallinity of starch isolated from grains stored at 25 °C during 360 days.

Considering the importance of maize starch in the world market and the lack of knowledge about the effects of temperature during maize grains storage on the isolated starch properties, the aim of this study was to evaluate the physicochemical, pasting, thermal, morphological and crystallinity properties of starches isolated from maize grains stored for 12 months at different temperatures.

## 2. Materials and methods

### 2.1. Storage of grains

Maize grains produced in the 2012 growing season at Santo Augusto (27°53'18" S, 53°47'20" W, 489 m) in the State of Rio Grande do Sul, Brazil, were used. The grains were placed into raffia bags after harvested and immediately transported to the Postharvest, Industrialisation and Quality of Grains Laboratory of DCTA-FAEM-UFPel, where the experiments were carried out. The grains were harvested mechanically, subjected to artificial drying with air temperature of 35 °C until 14% of moisture was achieved, and subsequently purged using aluminium phosphide to prevent the interference of insects in the experiment. The maize grains were stored in polyethylene bags of 0.2 mm thick plastic film with a capacity of 0.9 kg at temperatures of 5, 15, 25 and 35 °C for 12 months, in triplicate. The grains were maintained covered from the light by an aluminium foil.

### 2.2. Starch isolation

The isolation was performed according to the method described by Sandhu, Singh, and Malhi (2005), with some modifications. Maize grains (200 g) were added to 500-ml of 0.1% sodium bisulfite (NaHSO<sub>3</sub>) in distilled water, and maintained for 20 h at 50 °C. After this period, the water was drained and the grains were crushed in a grinder (Electronic Filter 600 W, Britânia, São Paulo, Brazil) until the smallest possible fraction (wet milling) was achieved. The crushed samples were double filtered through 100 and 270-mesh sieves. The protein-starch filtrates were decanted for 4 h. The supernatant was removed and the sedimented protein-starch layer was resuspended in distilled water to be centrifuged at 5000 × g for 20 min. The resulting protein rich supernatant was removed and the remaining starch slurry was resuspended once again in distilled water before further centrifugation to completely remove any remaining protein content. The collected starch was dried at 40 °C for 12 h in an oven until 11% of moisture was achieved. Once dry, the starch was placed in a laboratory mill (Perten 3100, Perten Instruments, Huddinge, Sweden) with 70-mesh sieve for attaining uniform particle size distribution. A total of 100 g kernels were used to determine the percentage extraction yield by weighing the starch obtained after drying. The starch was isolated from freshly harvested maize grains, before storage, and considered as the initial treatment. Then, the starch was isolated from maize grains and stored under time-temperature conditions mentioned above.

### 2.3. Colour parameters

The colour of the isolated starches was determined using a colorimeter (Minolta, CR-310, Osaka, Japan). The colour parameters used were *L*\* (100 = white and 0 = black) and *b*\* (positive = yellow and negative = blue).

### 2.4. Protein and fat contents

The nitrogen content was determined using the AACC method 46-13 (AACC, 1995), and the protein content was obtained using a conversion factor of nitrogen to protein of 6.25. The fat content was determined in accordance with the AACC method 30-20 (AACC, 1995).

### 2.5. Swelling power and solubility

The swelling power and solubility of the starches were determined as described by Leach, McCowen, and Schoch (1959). Samples (1.0 g) were mixed with 50 ml of distilled water in centrifuge tubes. The suspensions were heated at 90 °C for 30 min. The gelatinised samples were then cooled to room temperature and centrifuged at 1000 × g for 20 min. The supernatants were dried at 110 °C until a constant weight was achieved so that the soluble fraction could be quantified. Solubility was expressed as the percentage of the dried solid weight based on the dry sample weight. Swelling power was represented as the ratio of wet sediment weight to initial dry sample weight (deducting the amount of soluble starch).

### 2.6. Pasting properties

The pasting properties of the maize starches (3.0 g, 14% wet basis) were determined with a Rapid Visco Analyser (RVA-4; Newport Scientific, Warriewood, Australia) and profile Standard Analysis 1. The viscosity was expressed in rapid visco units (RVU). The samples were held at 50 °C for 1 min, heated to 95 °C at 3.5 min and held at 95 °C for 2.5 min. The samples were then cooled to 50 °C in 4 min and held at 50 °C for 2 min. The rotating speed was held at 960 rpm for 10 s and then maintained at 160 rpm during the process. Parameters including pasting temperature, peak viscosity, breakdown, final viscosity and setback were recorded.

### 2.7. Differential scanning calorimetry (DSC)

Gelatinisation characteristics of the maize starches were determined using differential scanning calorimetry (TA-60WS, Shimadzu, Kyoto, Japan). Starch samples (approximately 2.5 mg on a dry basis) were weighed directly in an aluminium pan (Mettler, ME-27331), and distilled water was added to obtain an aqueous suspension containing 75% water. The pan was hermetically sealed and allowed to equilibrate for 1 h before analysis. An empty pan was used as a reference. The sample pans were then heated from 40 to 140 °C at the rate of 10 °C min<sup>-1</sup>. The onset temperature of gelatinisation (*T*<sub>o</sub>), peak temperature (*T*<sub>p</sub>), conclusion temperature (*T*<sub>c</sub>) and gelatinisation enthalpy ( $\Delta H$ ) were determined. The range of gelatinisation was calculated by subtracting *T*<sub>o</sub> from *T*<sub>c</sub>.

### 2.8. Crystallinity

The crystallinity of starches was determined with an X-ray diffractometer (XRD-6000, Shimadzu, Brazil). The scanning region of the diffraction ranged from 5° to 30° with a target voltage of 30 kV, current of 30 mA and scan speed of 1° min<sup>-1</sup>. The relative crystallinity (RC) of the starch granules was calculated as described by Rabek (1980) using following the equation:

$$RC(\%) = \frac{A_c}{A_c + A_a} \times 100$$

where *A*<sub>c</sub> is the crystalline area; and *A*<sub>a</sub> is the amorphous area on the X-ray diffractograms.

**Table 1**

Extraction yield, colour parameters and chemical composition of starch isolated from freshly harvested (initial treatment) and stored maize grains at different temperatures for 12 months.

Storage <sup>a</sup> (°C)	Extraction yield (%)	Colour parameters <sup>b</sup>		Chemical composition	
		Value <i>b</i> *	Value <i>L</i> *	Protein (%)	Fat (%)
Initial	59.07 ± 0.31 <sup>a</sup>	6.27 ± 1.46 <sup>b</sup>	96.26 ± 0.49 <sup>b</sup>	0.23 ± 0.03 <sup>b</sup>	0.61 ± 0.08 <sup>a</sup>
5	62.88 ± 1.25 <sup>a</sup>	6.55 ± 0.40 <sup>b</sup>	97.47 ± 0.52 <sup>a</sup>	0.27 ± 0.08 <sup>b</sup>	0.63 ± 0.03 <sup>a</sup>
15	66.94 ± 0.71 <sup>a</sup>	5.98 ± 0.69 <sup>b</sup>	97.34 ± 0.90 <sup>a</sup>	0.32 ± 0.00 <sup>b</sup>	0.62 ± 0.04 <sup>a</sup>
25	63.36 ± 2.32 <sup>a</sup>	6.00 ± 0.44 <sup>b</sup>	96.82 ± 0.25 <sup>ab</sup>	0.29 ± 0.06 <sup>b</sup>	0.60 ± 0.04 <sup>a</sup>
35	45.99 ± 6.58 <sup>b</sup>	10.67 ± 0.87 <sup>a</sup>	92.44 ± 0.27 <sup>c</sup>	0.74 ± 0.01 <sup>a</sup>	0.40 ± 0.04 <sup>b</sup>

<sup>a</sup> Results are the means of three repetitions ± standard deviation. Values followed by different letter in the same column are significantly different ( $p \leq 0.05$ ).

<sup>b</sup> *L*\* (100 = white; and 0 = black), and *b*\* (positive = yellow; and negative = blue).

## 2.9. Scanning electron microscopy (SEM)

The morphology of the starch granules was examined using a scanning electron microscope (Shimadzu, SSX-550). Starch samples were initially suspended in acetone to obtain a 1% (w/v) suspension, and the samples were maintained in an ultrasound for 15 min. A small quantity of each sample was spread directly onto the surface of the stub and dried in an oven at 32 °C for 1 h. Subsequently, all of the samples were sputter coated with gold and examined an acceleration voltage of 15 kV and magnifications of 1500× and 3000×.

## 2.10. Statistical analysis

Analytical determinations for the samples were performed in triplicate, and standard deviations were reported, except for DSC analysis and X-ray diffractograms, which were performed in duplicate. A comparison of the means was ascertained by Tukey's test to a 5% level of significance using an analysis of the variance (ANOVA).

## 3. Results and discussion

### 3.1. Extraction yield, colour parameters and chemical composition

The extraction yield, colour parameters, protein and fat contents of maize starch isolated from grains post storage treatment are presented in Table 1. The lowest extraction yield was observed in the starch isolated from grains stored at the highest temperature (35 °C). No statistical differences occurred in starch isolated from grains stored at 5, 15 and 25 °C compared to starch isolated from freshly harvested grains (initial treatment). The extraction yields of starch are similar to those reported by Malumba et al. (2009), who obtained extraction yields between 43.3% and 64.4% when evaluating the extraction of maize grains subjected to drying air temperatures between 80 and 130 °C.

The greatest starch colour change occurred in maize grains stored at 35 °C, which presented a 3.97% decreased in *L*\* value and a 41.24% increase in *b*\* value compared to the starch isolated from grains before storage (Table 1). In summary, starch isolated from grains stored at 35 °C is yellowish, while the others are clear. The increase in the *L*\* value and the decrease in *b*\* value can be attributed to their higher residual content of proteins. The protein content of starch isolated from freshly harvested (initial treatment) maize grains was 0.23% and reached up to 0.74% in the grains stored at 35 °C for 12 months. This increase is due to the interactions of the starch chains with proteins, resulting from the strengthening of disulfide bonds during storage (Martin & Fitzgerald, 2002; Park et al., 2012; Zhou, Robards, Helliwell, & Blanchard, 2003) which hinder the separation of starch and protein during the wet-milling process. No differences were observed in the protein content of starch isolated from grains stored at 5, 15 and 25 °C compared to

starch isolated from freshly harvested maize grains (initial treatment). The residual levels of proteins are in agreement with those reported by Malumba et al. (2009), who observed values lower than 1.5% in starches isolated from maize grains as a function of drying temperature (between 80 and 120 °C).

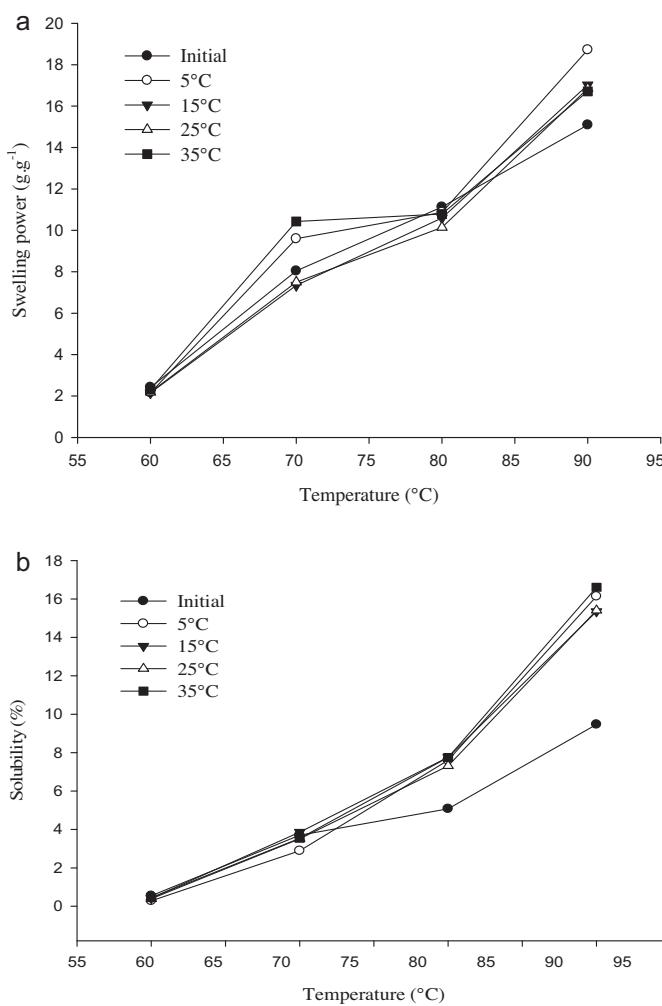
The fat content of starch isolated from maize grains stored at 35 °C was lower than the other treatments (Table 1). There was no difference between the fat content of starch isolated from freshly harvested grains (initial treatment) and grains stored at 5, 15 and 25 °C. According to Debet and Gidley (2006), the residual presence of lipids and proteins in the starch granule may cause restriction of the swelling power of the starch during the gelling. Haros et al. (2003) and Altay and Gunasekaran (2006) stated that the proteins that remain in the maize starch may possibly reduce the entry of water into the granules during gelatinisation, limiting the interactions between the water and components, and causing an increase in starch gelatinisation temperatures.

### 3.2. Swelling power and solubility

The swelling power and solubility of starches isolated from maize grains stored under different temperatures are presented in Fig. 1a and b, respectively. In general, there was an increase in the swelling power and solubility with increasing temperature from 60 to 90 °C, as expected. The starch isolated from maize grains stored at 5 °C showed the highest swelling power at 90 °C ( $p < 0.05$ ). The results are consistent with those described by Sandhu and Singh (2007), which reported swelling power at 90 °C between 13.0 and 20.7 g of water per gram of dry starch in nine maize varieties in the Iowa State (USA). According to Leach et al. (1959) the internal bond strength of starch granules influence the swelling power, being a highly complexed starch, it should be relatively resistant to swelling, consequently, should have lower swelling power. The starch solubility at 80 and 90 °C (Fig. 1b) increased for all treatments at the end of 12 months of storage compared to freshly harvested grains (initial treatment) ( $p < 0.05$ ). Major changes in solubility were observed at 80 and 90 °C, as a result of amylose leaching from the starch granule and diffusion during the swelling. The highest solubility can be attributed to a less rigid structure of the starch granules obtained from stored grains, allowing the leaching of amylose during heating.

### 3.3. Pasting properties

The pasting properties of maize starches verified in the RVA are shown in Table 2. The highest pasting temperature was verified in starch isolated from grains stored at 35 °C. There was no difference between the initial treatment and the other storage temperatures. According to Sandhu and Singh (2007), the pasting temperature is the temperature that the starch viscosity starts to increase. The increase in the pasting temperature from 70.50 to 76.30 °C after 12 months of storage at 35 °C can be attributed to the greater presence



**Fig. 1.** Swelling power (a) and solubility (b) of maize starches isolated from freshly harvested (initial) and stored maize grains at different temperatures for 12 months.

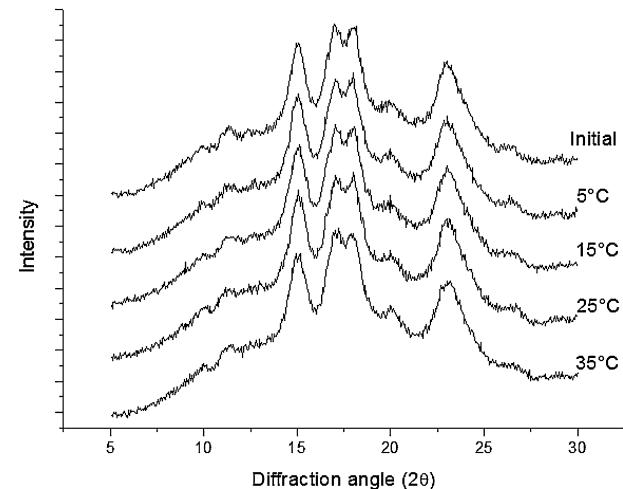
of residual proteins in starch, which hinders the swelling of the granules during the hydration process and eventually elevate the temperature to which starch gelatinisation occurs. Similar results were found by Setiawan et al. (2010).

The peak and final viscosities increased in the starches isolated from grains stored at 5, 15 and 25 °C compared to starch isolated from freshly harvested maize (initial treatment). On the other hand, the lowest peak and final viscosities were observed in starch isolated from grains stored at 35 °C (Table 2). According to Singh et al. (2003), the reduction in viscosity reflects the lower ability of starch granules to freely swell before their physical collapse. The breakdown was higher in the starch isolated from maize grains stored at 5 °C compared to starch isolated from freshly harvested grains (initial treatment), while the breakdown of starches isolated from grains stored at 15, 25 and 35 °C was lower than the breakdown of starch isolated from freshly harvested grains (initial treatment)

**Table 3**  
Thermal properties of maize starches.

Storage ( $^{\circ}\text{C}$ )	Gelatinisation temperatures <sup>a</sup>			$\Delta T(T_c - T_o)$	$\Delta H(\text{J g}^{-1})$
	$T_o$ ( $^{\circ}\text{C}$ )	$T_p$ ( $^{\circ}\text{C}$ )	$T_c$ ( $^{\circ}\text{C}$ )		
Initial	69.16	73.25	76.98	7.82	22.41
5	69.94	73.43	79.05	9.11	37.81
15	71.01	74.45	78.91	7.90	25.54
25	70.76	74.29	79.16	8.40	28.87
35	70.04	73.70	78.42	8.38	31.67

<sup>a</sup>  $T_o$  = onset temperature,  $T_p$  = peak temperature,  $T_c$  = conclusion temperature,  $\Delta T$  = gelatinisation temperature range, and  $\Delta H$  = gelatinisation enthalpy.



**Fig. 2.** X-ray diffraction patterns of starches isolated from maize grains stored for 12 months at different temperatures.

(Table 2). The decrease in breakdown indicates a higher rigidity of starch granules after being stored at those temperatures, making the granule resistant to disrupt and collapse while heating and shearing. The highest setback was also presented by starch isolated from grains stored at 5 °C (Table 2). According to Hughes et al. (2009), the greater breakdown and setback reflect the higher swelling power of the starch granules and rapid aggregation of leached amylose chains, respectively. This statement is in accordance with the results of swelling power presented in Fig. 1a, where the highest swelling power at 90 °C was observed in starch isolated from maize grains stored at 5 °C.

### 3.4. Differential scanning calorimetry (DSC)

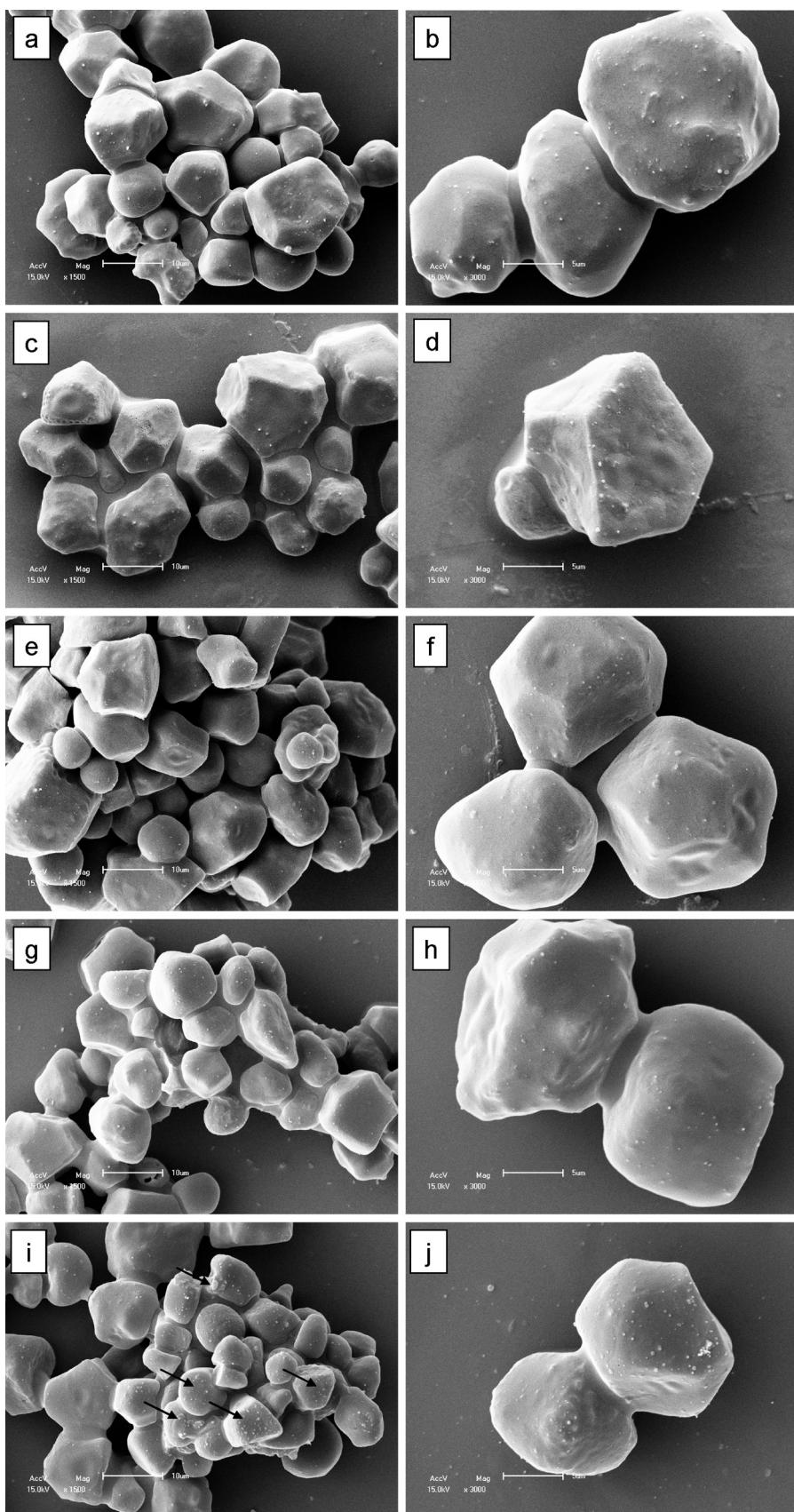
The gelatinisation temperatures, the temperature range of gelatinisation ( $T_c - T_o$ ) and the gelatinisation enthalpy ( $\Delta H$ ) of starches isolated from maize grains stored at different temperatures are presented in Table 3. There was a small increase in the onset temperature of gelatinisation ( $T_o$ ), peak temperature of gelatinisation ( $T_p$ ) and conclusion temperature of gelatinisation ( $T_c$ ) of starch isolated from stored maize grains compared to starch isolated from freshly harvested grains (initial treatment) (Table 3).

**Table 2**

Pasting properties of starches isolated from freshly harvested (initial treatment) and stored maize grains at different temperatures for 12 months.

Storage <sup>a</sup> ( $^{\circ}\text{C}$ )	Pasting temperature ( $^{\circ}\text{C}$ )	Peak viscosity (RVU)	Breakdown (RVU)	Setback (RVU)	Final viscosity (RVU)
Initial	$70.50 \pm 0.52^b$	$312.00 \pm 5.75^c$	$115.60 \pm 6.07^b$	$114.74 \pm 4.84^b$	$311.06 \pm 3.66^c$
5	$70.60 \pm 0.40^b$	$352.33 \pm 2.00^a$	$143.80 \pm 3.25^a$	$134.63 \pm 0.70^a$	$343.21 \pm 0.54^a$
15	$71.40 \pm 0.35^b$	$317.79 \pm 2.79^b$	$102.70 \pm 0.96^c$	$119.00 \pm 0.58^b$	$334.08 \pm 1.25^b$
25	$71.00 \pm 0.45^b$	$318.84 \pm 0.41^b$	$107.50 \pm 0.46^c$	$119.80 \pm 3.63^b$	$331.08 \pm 2.75^b$
35	$76.30 \pm 0.40^a$	$284.12 \pm 0.20^d$	$105.30 \pm 0.42^c$	$115.04 \pm 0.29^b$	$293.92 \pm 0.34^d$

<sup>a</sup> Results are the means of three repetitions  $\pm$  standard deviation. Values followed by different letter in the same column are significantly different ( $p \leq 0.05$ ).



**Fig. 3.** Scanning electron micrographs (SEM) of starches isolated from maize grains stored for 12 months at different temperatures: initial (a and b), 5 °C (c and d), 15 °C (e and f), 25 °C (g and h) and 35 °C (i and j) at low and high magnifications, respectively.

**Table 4**

Intensity of the main peaks of the X-ray diffractograms and relative crystallinity of maize starches.

Storage (°C)	Intensity (CPS <sup>a</sup> )					Relative crystallinity (%)
	15	17	18	20	23	
Initial	3216	3529	3499	2299	2934	30.54
5	3228	3494	3622	2401	2928	28.96
15	3291	3616	3576	2470	2940	28.34
25	3446	3700	3742	2477	2993	27.08
35	3337	3721	3670	2506	2904	26.26

<sup>a</sup> Counts per second.

The storage resulted in an increase in the enthalpy of gelatinisation ( $\Delta H$ ) of 3.13 to 15.40 J g<sup>-1</sup> above the value observed in the starch isolated from freshly harvested (initial treatment) maize grains. The largest increases were observed in the starches isolated from grains stored at 5 and 35 °C. The highest  $\Delta H$  presented by starch isolated from grains stored at 5 °C indicates a high level of starch chain intramolecular bonds, since the protein and fat contents (Table 1) were similar between initial, 5 °C, 15 °C and 25 °C treatments. This phenomenon is probably why the starch isolated from grains stored at 5 °C presented the highest swelling power at 90 °C (Fig. 1) and peak viscosity (Table 2). On the other hand, the increase in the  $\Delta H$  of starch isolated from grains stored at 35 °C may be due to the lower purity of starch, as reported in Table 1. According to Chung, Liu, Pauls, Fan, and Yada (2008) and Piecyk, Drużyńska, Worobiej, Wołosiak, and Ostrowska-Ligęza (2013), the increase in  $\Delta H$  may be influenced by the residual levels of proteins and lipids, impairing the starch gelatinisation. This increase can also be attributed to the increased rigidity of the granules at the end of storage, which increases the energy required to disrupt the structure of the starch granules, due to the complexation that occurs in the grain constituents. In a study conducted to evaluate the thermal properties of rice under different conditions, Zhou, Robards, Helliwell, and Blanchard (2010) reported that the enthalpy of gelatinisation and the gelatinisation temperatures are affected by both temperature and storage time.

### 3.5. Crystallinity

The X-ray diffractograms of starch isolated from freshly harvested (initial treatment) maize grains and from maize grains stored at different temperatures is presented in Fig. 2. The maize starches showed a typical A-type diffraction pattern, with main 2θ peaks at 15°, 17°, 18°, 20° and 23° (Zobel, 1964). There was a decrease in starch crystallinity at the end of 12 months of storage (Table 4). Higher storage temperatures provided higher decreases in starch crystallinity. The largest reduction was observed at 35 °C of storage, where the crystallinity reduced from 30.54% (initial treatment) to 26.26%. Although there was a reduction in crystallinity, there was an increase in the intensity of the peaks 15°, 17°, 18° and 20° of the starch isolated from grains stored at 35 °C compared to starch isolated from freshly harvested grains (initial treatment) (Table 4). The highest peak intensity indicates that even though there is less crystalline area in the starch granule; there was a rearrangement that left the crystals in a more parallel array. This finding is in agreement with the results observed in RVA (Table 2) and DSC (Table 3) analyses. Our results of crystallinity differ from those reported by Setiawan et al. (2010), who found increased relative crystallinity of starch isolated from maize grains after six months of storage. According to Chrastil (1990), storage can alter the activity and properties of the endogenous enzymes present in the kernels, such as amylase, protease, and phosphatase. Dhaliwal, Sekhon, and Nagi (1991) and Awazuhara et al. (2000) attributed changes in the chain length of the branched

amylopectin to enzymatic hydrolysis, where the α-amylase attacks the amorphous region of amylopectin, particularly the long chains, reducing the molecular weight of amylopectin.

### 3.6. Scanning electron microscopy (SEM)

The scanning electron micrographs of maize starch granules are shown in Fig. 3. The starch granules presented shapes varying from spherical to polyhedral, which are typical of maize starch (Fig. 3a and b). Although no changes in the granules shape was perceived as a function of storage temperature, the starch granules isolated from maize grains stored at 35 °C showed a greater appearance of sub-micron particulates on the surface of the granules in comparison to other treatments (Fig. 3i, indicated by arrows). Further examination via X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) could perhaps shed light on the nature of these particulates. The starch granules isolated from maize grains stored at 35 °C appear to have lower size than the granules from other treatments, which has possibly occurred due to the presence of a strong protein matrix compressing the starch granules within maize grains. The strength of the interactions of starch with proteins and lipids resulted in a greater residual content of protein (Table 1) and can be associated with the observed reduction in peak viscosity (Table 2). Setiawan et al. (2010) evaluated the effects of 6-months of maize grains storage after the grains being dried under different conditions and found an increase in the number of damaged starch granules. The damage of starch granules was attributed to starch-hydrolyzing enzyme activities. Similar observation was not verified in our work.

## 4. Conclusions

This was the first study to evaluate effects of storage temperature on the physicochemical, pasting, thermal, crystallinity and morphological properties of maize starch isolated from maize grains stored for 12 months. The storage of maize grains at 35 °C caused a reduction of 22.1% in the extraction yield of starch and provides a yellowish colour to starch, which makes it less attractive for applications where paste clarity is important. The starch isolated from grains stored during 12 months showed lower crystallinity than starch isolated from freshly harvested grains. However, this has probably resulted in a more organised rearrangement of the starch chains within the granule and promoted interactions with other constituents, mainly in starch isolated from maize grains stored at 35 °C. This produced higher gelatinisation temperatures and higher enthalpy of gelatinisation as observed by DSC analysis, and lower peak and final viscosities verified in RVA. The SEM of starch isolated from maize grains stored at 35 °C showed the presence of some points in the granule surface and the granules also appear to be smaller than those from other treatments, probably as a function of a stronger protein matrix around starch granules within the grains. Further studies should be conducted in order to evaluate effects of the grain storage temperature during short-time storage on the properties of isolated starch. Studies about effects of the moisture content of the grains in short- and long-time storage on the properties of isolated starch are also necessary.

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