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Pasting, morphological, thermal and crystallinity properties of starch isolated from beans stored under different atmospheric conditions

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ABSTRACT

Few studies have elucidated the changes in starch properties during the long storage period, and none have explained the starch properties of bean grains stored under a nitrogen-modified atmosphere at a low-temperature. This study aims to evaluate the changes in pasting, morphological, thermal and crystallinity properties of starch isolated from carioca bean grains stored for 360 days under three different atmospheric conditions: hermetic storage at 5 $^{\circ}$ C, a nitrogen-modified atmosphere at 15 $^{\circ}$ C and a normal atmosphere at 25 $^{\circ}$ C. The starch isolated from grains stored under nitrogen at 15 $^{\circ}$ C did not differ in the solubility and pasting properties. However, they showed lower crystallinity, swelling power and heat that is necessary for gelatinisation compared to starch isolated from grains stored under a normal atmosphere at 25 $^{\circ}$ C. Therefore, the storage under nitrogen-modified atmosphere is an alternative for the preservation of the thermal and crystallinity properties of bean starch.

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1. Introduction

Bean consumption (P. vulgaris L.) is substantial in developing countries and has increased in the developed countries as an alternative to animal protein for the prevention of health problems related to meat consumption and due to the discovery of the benefits of legumes in diet and their protection against diseases of the colon (Champ, 2001; Hangen & Bennink, 2002; Lee, Prosky & DeVries, 1992; Mathres, 2002; Pujóla, Farreras & Casaños, 2007). In Brazil, the world's largest producer of beans (Food and Agriculture Organization, 2010), the carioca bean represents approximately 60% of the total beans produced and is naturally the most consumed. Carioca beans are cream-coloured, with tan stripes. To allow a plentiful supply of carioca beans throughout the entire year, they are mostly stored under adverse environmental conditions that favour the development of the hard-to-cook (HTC) defect. The HTC beans take longer to cook, do not thicken the sauce and do not become soft after cooking, and are thus devalued by consumers, who consider HTC beans a low-quality product as a result of its features. Kaur and Singh (2007) found that the properties of starch in the HTC beans are different than normal beans.

Carbohydrates are the main constituents of beans, and starch is the most abundant carbohydrate in the legume seed (22–45%) (Hoover & Sosulski, 1991). Legume starches are digested slowly, have a low glycemic index and are fermented in the large intestine to produce short-chain fatty acids (Sandhu & Lim. 2008) that are beneficial for colon health. Thus, due to the important role played by legume starches in human nutrition, there have been increased studies on legume starches. In particular, food processors and nutritionists have been looking for legume starches with unique functionalities to meet consumer demands (Hughes et al., 2009). As an example, bean paste has been used in several Oriental cereal products (Su, Lu & Chang, 1997). According to Krupa, Rosell, Sadowska, & Soral-Smietana (2010), the use of hydrothermally modified bean starch in gluten-free bread improves their chemical composition and the fresh bread quality. These authors reported that the addition of hydrothermally modified bean starch increased the resistant starch content of the bread. Starches with desirable functional properties can play a significant role in improving the quality of different food products and can replace chemicallymodified starches that are currently being used in a number of products (Singh, Singh, Kaur, Sodhi, & Gill, 2003). The structure and functionality of starch are important factors in determining the functional properties of products made from beans. Yousif, Batey, et al. (2003) found an increase in the gelatinisation temperature of starch from adzuki beans (Vigna angularis) that is positively correlated with the increase of the storage temperature.

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The moisture content, storage temperature and relative humidity are the factors that are most critical to the preservation of the post-harvest quality of beans (Aguilera & Stanley, 1985; Berrios, Swanson & Cheong, 1999). The storage of faba beans (*Vicia faba*) under a nitrogen-modified atmosphere is the best storage system to preserve the coat colour and content of phenolic compounds after 12 months of storage (Nasar-Abbas et al., 2008a). Changes in colour and phenolic content seem to occur in conjunction with the hardening of the grain, according to the research performed by Brackmann, Neuwald, Ribeiro, & Freitas (2002) and Nasar-Abbas et al. (2008b).

According to previous studies, the nitrogen-modified storage atmosphere (Brackmann et al., 2002; Nasar-Abbas et al., 2008a) under low temperatures (Coelho, Bellato, Santos, Ortega, & Tsai, 2007; Nasar-Abbas et al., 2008b, 2009; Yousif, Deeth & Caffin, 2002; Yousif, Batey, et al., 2003; Yousif, Kato & Deeth, 2003) appear to be more effective in preserving the physical, chemical and technological bean characteristics during storage. However, there have been few studies on the effects of different storage technologies on the properties of starch from the beans. In addition, few studies have evaluated the pasting, morphological, thermal and crystallinity properties of isolated starch after a long period of storage. Because the changes in starch respond in part by the appearance of the HTC defect and these changes affect the use of the products developed based on the starch, it is important to study their properties.

This study aims to evaluate the changes in the pasting, morphological, thermal and crystallinity properties of starch isolated from carioca beans stored for 360 days under hermetic conditions at 5 $^{\circ}$ C, nitrogen-modified atmosphere at 15 $^{\circ}$ C and normal atmosphere at 25 $^{\circ}$ C.

2. Materials and methods

2.1. Plant material

Carioca beans (*Phaseolus vulgaris* L.), cv. Pérola, were cultivated in an irrigation system during the 2008 growing season (July–October) at Primavera do Leste (54°09′28″W, 15°18′09″S) in the State of Mato Grosso, Brazil. Carioca beans were harvested when the moisture content was approximately 12.5% and were subsequently submitted to a cleaning process. The grains were placed into raffia bags and immediately transported to the Postharvest, Industrialisation and Quality of Grains Laboratory of DCTA-FAEM-UFPel, where the experiments were carried out. The initial cooking time of the grains was 16 min and the hardness was 54 N.

2.2. Storage conditions

The grains were stored under three different conditions for 360 days: hermetic conditions at 5 °C, nitrogen-modified atmosphere at 15 °C and normal atmosphere at 25 °C. For the hermetic storage, 5 kg of grains were stored in polyethylene bags of 0.20 mm thickness, sheltered from light by aluminium foil and placed in a cold room at 5 °C. The package was sealed using a Webomatic® machine. In the nitrogen-modified atmosphere at 15 °C, 5 kg of grains were placed into polyethylene bags of 0.20 mm thickness, sheltered from light by aluminium foil and placed in storage at $15\,^{\circ}$ C with $75\pm5\%$ relative humidity. To modify the atmosphere, O₂ was withdrawn from the package using a Webomatic[®] machine, and N₂ gas was added. The package was sealed immediately after changing the atmosphere. Another aliquot of 5 kg of bean grain were stored under normal atmosphere at 25 °C, and the grains were packaged in cotton cloth bags, sheltered from light and placed in a room at 25 °C with 75 \pm 5% relative humidity. The grains stored in this condition were purged every 60 days, using 3 g doses of aluminium phosphide per cubic meter as the principle active component, so as to avoid any interference in the quality of the stored grain due to insect attack. The grains were stored in triplicate for the three different atmospheric conditions. The cooking time of the beans and hardness of each sample after 360 days of storage were 18 min/60 N, 21 min/84 N and 37 min/117 N for hermetic storage at 5 °C, nitrogen-modified atmosphere at 15 °C and normal atmosphere at 25 °C, respectively.

2.3. Starch isolation

The samples (300 g) of each treatment, containing compounds of a mixture of 100 g of grains collected from each storage bag, were ground using a laboratory mill (Perten, 3100). Subsequently, the bean flour was embedded in distilled water containing 0.16% sodium hydrogen sulphite for 24h at 4°C. Addition of sodium hydrogen sulphite to the steep water assists in breaking down the protein-starch matrix and also checks the microbial growth (Singh, Sandhu, & Kaur, 2004). Afterwards, the steep water was drained off, and 400 mL of distilled water and grains were ground in a laboratory blender. The ground slurry was screened through a 200 mesh sieve. The material remaining on the sieve was washed thoroughly with distilled water. The filtrate slurry was allowed to stand for 3 h. Then, the supernatant was removed and the settled starch layer was resuspended in distilled water and centrifuged in wide-mouthed cups at $1200 \times g$ for 20 min. The upper non-white layer was scraped off. The white layer was resuspended in distilled water and recentrifuged at $1200 \times g$ for 15 min. Then, the upper non-white layer was scraped off again and the starch was collected and dried in an oven at 40 °C for 12 h.

2.4. 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 centrifugal tubes. The suspensions were heated at $90\,^{\circ}\text{C}$ for $30\,\text{min}$. The gelatinised samples were then cooled to room temperature and centrifuged at $1000\times g$ for $20\,\text{min}$. The supernatant was dried at $110\,^{\circ}\text{C}$ until a constant weight was achieved so that the soluble fraction can be quantified. The solubility was expressed as the percentage of dried solid weight based on the weight of the dry sample. The swelling power was represented as the ratio of the weight of the wet sediment to the weight of the initial dry sample (deducting the amount of soluble starch).

2.5. X-ray diffraction

X-ray diffractograms of the starches were obtained 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 $30 \, \text{kV}$, a current of $30 \, \text{mA}$ and a scan speed of $1^{\circ}/\text{min}$. The relative crystallinity (RC) of the starch granules was calculated as described by Rabek (1980) by the following equation: RC (%) = $(A_{\text{c}}/(A_{\text{c}} + A_{\text{a}})) \times 100$, where A_{c} is the crystalline area and A_{a} is the amorphous area on the X-ray diffractograms.

2.6. Differential scanning calorimeter (DSC)

Gelatinisation characteristics of bean starches were determined using Differential Scanning Calorimetry (TA-60WS, Shimadzu, Kyoto, Japan). Starch samples (approximately 3.5 mg, dry basis) were weighed directly in an aluminium pan (Mettler, ME-27331), and distilled water was added to obtain an aqueous suspension containing 70% water. The pan was hermetically sealed and allowed to equilibrate for one hour before the analysis. An empty pan was

used as a reference. The sample pans were then heated from 10 to 95 °C at the rate of 5 °C/min. The onset temperature of gelatinisation $(T_{\rm o})$, the peak temperature $(T_{\rm p})$, the conclusion temperature $(T_{\rm c})$ and gelatinisation enthalpy (ΔH) were determined. The range of gelatinisation was calculated as $T_{\rm c}-T_{\rm o}$.

2.7. Pasting properties

The pasting properties of the starch samples (3.0 g, 14% moisture content (wet basis)) were determined by using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Australia) and profile Standard Analysis 1. Parameters including pasting temperature, peak viscosity, holding viscosity, breakdown, final viscosity and setback were recorded.

2.8. Scanning electron microscopy (SEM)

The morphology of the starch granules was examined by the scanning electron microscope (Shimadzu, SSX-550). Starch samples were initially suspended in acetone to obtain a 1% (w/v) suspension and kept in 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 one hour. Subsequently, all of the samples were coated with gold and examined in the scanning electron microscope under an acceleration voltage of 15 kV and a magnification of $1000\times$ and $2000\times$.

2.9. Statistical analysis

Analytical determinations for the samples were performed in triplicate and standard deviations were reported. 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. X-ray diffraction, swelling power and solubility

Table 1 shows the intensity of the main peaks verified on X-ray diffractograms, the relative crystallinity, the swelling power and the solubility of the starches isolated from bean grains stored for 360 days under the three different atmosphere conditions. The X-ray diffraction patterns of the starches isolated from the carioca bean grains that were stored for 360 days under hermetic conditions at 5 °C, nitrogen-modified atmosphere at 15 °C and normal atmosphere at 25 °C, are presented in Fig. 1. The starches show the conventional "C" pattern characteristic of legume starches (Fig. 1). This "C" pattern is a crystalline polymorph that is considered to be a mixture of "A" and "B" polymorphs, which are characteristic of cereals and tuber starches, respectively (Gernat, Radosta, Damaschun, & Schierbaum, 1990; Lawal & Adebowale, 2005).

The X-ray diffraction properties provide ample evidence of an ordered structure of the starch granule. The X-ray diffraction pattern of bean starches showed peaks at diffraction angles 2θ of 15° , 17° , 20° , 23° and 26° , which is similar to those observed by Ancona,

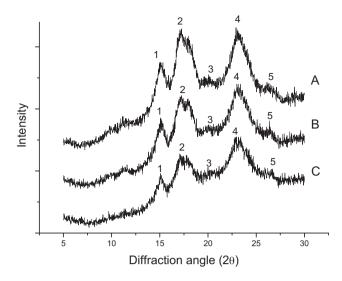


Fig. 1. X-ray diffraction patterns of starch isolated from bean grains stored under different atmosphere conditions for 360 days: (A) starch isolated from grains stored under hermetic conditions at $5\,^{\circ}$ C; (B) starch isolated from grains stored in nitrogen-modified atmosphere at $15\,^{\circ}$ C; and (C) starch isolated from grains stored in normal atmosphere at $25\,^{\circ}$ C.

Campos, Guerrero and Ortíz (2011) studying Lima bean (*Mucuna pruriens*) and Velvet bean (*Phaseolus lunatus*) starches. The peak intensity detected in X-ray diffractograms is determined according to the height of each peak. The numbers 1–5 (15°, 17°, 20°, 23° and 26°, respectively) represent the peaks detected in the X-ray diffractograms (Fig. 1). The starch peak values were observed in the following order: hermetic storage at 5 °C > nitrogen-modified atmosphere at 15 °C > normal atmosphere at 25 °C. The highest values were found for peak 3 (Table 1). The starch isolated from bean grains stored under hermetic conditions at 5 °C showed the highest peak values for the three peaks evaluated.

Table 1 shows the relative crystallinity of bean starches calculated as the ratio between the area of five peaks (crystalline area) verified in the X-ray diffractograms and the total area (crystalline and amorphous area). The relative crystallinity was observed in the following order: normal atmosphere at 25 °C > nitrogen-modified atmosphere at 15 °C > hermetic storage at 5 °C. The increase in the relative crystallinity of the starch was verified in the grains stored under nitrogen at 15 °C and under normal atmosphere at 25 °C and is probably due to the reduction in amylose content and change in amorphous and crystalline regions. According to Zobel (1988a,b), the crystallinity is associated with the amylopectin component, while the amorphous regions mainly represent amylose. Hoover and Ratnayake (2002) studied the relative crystallinity of starch isolated from different Canadian bean cultivars and verified that the pinto bean, which is physically very similar to the carioca bean, had a relative crystallinity of approximately 25%. These authors justified the differences in relative crystallinity between starches as a factor that is affected by: (1) crystal size, (2) amount of crystalline regions (influenced by amylopectin content and amylopectin chain length),

Intensity of the main peaks of X-ray diffractograms, relative crystallinity, swelling power and solubility of bean starches.

Storage condition	Intensity (CPS)					Relative crystallinity (%)	Swelling Power (g/g) ^a	Solubility (%) ^a
	1	2	3	4	 5			
Hermetic at 5 °C	912	1180	810	1190	750	36.04	9.34 ± 0.12 b	2.50 ± 0.25 a
Nitrogen at 15 °C	766	946	716	1038	742	42.19	$9.42 \pm 0.13 b$	2.63 ± 0.14 a
Normal at 25 °C	586	744	636	882	632	47.3	10.05 ± 0.10 a	2.67 ± 0.25 a

CPS, counts per second.

a Different letters in the same column differ statistically (p < 0.05). Results are the means of three determinations \pm the standard deviation.

(3) orientation of the double helices within the crystalline domains, and (4) extent of interaction between the double helices. Garcia and Lajolo (1994) studied the changes in the starch of HTC beans and found a much stronger birefringence in HTC starch granules, suggesting that the isolated starch of these grains have a higher degree of crystallinity. Therefore, the results showed that the starch from grains stored under normal atmospheric conditions at 25 °C were more influenced than the starch isolates from beans stored under the nitrogen-modified atmosphere at 15 °C (Table 1), possibly due to the faster development of the HTC defect in grains stored under the conventional system.

There was no difference between the swelling power of the starches isolated from grains stored under hermetic conditions at 5°C and under nitrogen-modified atmosphere at 15°C. However, the starch isolated from grains stored under normal atmospheric conditions at 25 °C presented higher swelling power (Table 1). This increase in the swelling power of starch isolated from grains stored under normal atmospheric conditions at 25 °C can be attributed to the increased crystallinity and changes in the arrangements of the crystalline regions of the starch. According to Singh et al. (2003) and Tester and Morrison (1990), the swelling behaviour of starch is due to the property of its amylopectin content, and amylose acts as both a diluent and inhibitor of the swelling. Thus, as already verified in relative crystallinity, the results of swelling power suggest that the grains stored under normal atmosphere at 25°C have lower amylose content than the samples stored under the other two conditions. Starch solubility results from the leaching of amylose, which dissociates from and diffuses out of granules during swelling. This leaching represents a transition from order to disorder within the starch granules that occurs when starch is heated with water (Tester & Morrison, 1990). The solubility of starches isolated from the grains stored under the three different conditions did not differ statistically (p < 0.05). The differences of the swelling and solubility behaviours of the starches between botanical sources and among the cultivars of any one botanical source are caused by the differences in the amylose and lipid contents, as well as the granule organisation (Singh et al., 2003).

When stored under normal atmosphere at 5 °C for 12 months, faba bean (*Vicia faba* L.) grains showed a good preservation of the physical–chemical parameters (Nasar-Abbas et al., 2008b, 2009), however, storage at 5 °C is uneconomical in high scale, being subjected to storage at room temperature (25 °C). The results showed that the storage of carioca beans under nitrogen at 15 °C, which is economically viable, was more favourable in preserving the properties of starch than in normal atmospheric conditions at 25 °C, because the swelling power and solubility were more similar to the storage in hermetic atmosphere at 5 °C.

3.2. Differential scanning calorimetry

Differential scanning calorimetry provides quantitative measurements of heat flow associated with gelatinisation, where the endothermic peaks are indicative of melting. The gelatinisation temperature is characteristic of the starch type and depends on the glass transition of the amorphous fraction of the starch. Starch gelatinisation and melting is an important phenomenon occurring in various food processing operations because it provides unique textural and structural characteristics for the products (Sozer, Dalgiç & Kaya, 2007). Table 2 presents the gelatinisation parameters of the onset temperature (T_0), peak temperature (T_p), conclusion temperature (T_c), and gelatinisation enthalpy (ΔH) of starches that were isolated from carioca bean grains and stored for 360 days under hermetic conditions at 5 °C, nitrogen-modified atmosphere at 15 °C and normal atmosphere at 25 °C. DSC thermograms of the starches isolated from carioca bean grains are presented in

Table 2Thermal analysis of the gelatinisation of starches isolated from beans stored under different atmosphere conditions for 360 days.

Storage condition	<i>T</i> _o (°C)	<i>T</i> _p (°C)	<i>T</i> _c (°C)	$\Delta T (T_{\rm c} - T_{\rm o})$	Δ <i>H</i> (J/g)
Hermetic at 5°C	72.17	75.12	77.90	5.73	1.73
Nitrogen at 15°C	72.13	74.87	77.29	5.16	2.87
Normal at 25°C	71.73	74.73	77.34	5.61	3.49

Fig. 2. The thermograms are typical for legume starches where the endotherm is attributed to the gelatinisation of amylopectin crystalline lamellae.

The starch from beans stored under nitrogen at 15 °C presented a gelatinisation onset temperature that is similar to starches from beans stored under hermetic conditions at 5 °C. However, storage of the starches in normal atmospheric conditions at 25 °C presented a lower gelatinisation onset temperature (Table 2). Hoover and Ratnayake (2002) evaluated the gelatinisation parameters of starches of different cultivars and found an onset temperature of 64.1 °C for black beans and 72.5 °C for pinto beans. The results are different from that observed by Yousif, Batey, et al. (2003), who studied the effects of three different temperatures (10, 20 and 30 °C) and two different relative humidities (40 and 65%) for the storage on the starch properties of adzuki beans (Vigna angularis) that were stored for 6 months, and reported that the higher storage temperature, the greater the onset temperature and peak temperature. However, Paredes-López, Maza-Calviño, and González-Castañeda (1989) did not detect an alteration of gelatinisation temperature with the development of the hardening defect. The highest conclusion temperature was observed in the starch from the beans stored under hermetic conditions at 5 °C (Table 2). The conclusion temperature of the starch isolated from beans stored under the nitrogen-modified atmosphere at 15 °C and in the normal atmosphere at 25 °C was very similar. Su, Lu, and Chang (1997) reported conclusion temperatures of 78.0 °C and 78.4°C for the red kidney and pinto beans, respectively. A difference in $T_c - T_o$ (Table 2) suggests that the degree of heterogeneity of the crystallites within the granules of the starches is not different, because there was no difference in ΔT between the starches. The reduction in ΔH on starch from the carioca beans stored under hermetic conditions at 5 °C (Table 2) may be due to the disruption of the crystallites that are less stable or smaller in size, showing a lower crystallinity and thus requiring less energy to disrupt.

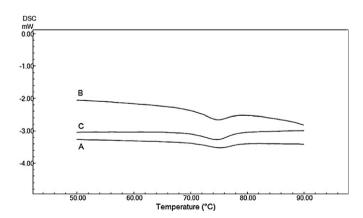


Fig. 2. DSC curves of starch isolated from bean grains stored under different atmospheric conditions for 360 days: (A) starch isolated from grains stored under hermetic conditions at 5° C; (B) starch isolated from grains stored in nitrogen-modified atmosphere at 15° C; and (C) starch isolated from grains stored in normal atmosphere at 25° C.

Table 3Pasting characteristics of starches isolated from beans stored under different atmospheric conditions for 360 days.

Storage condition	Parameter ^a									
	Pasting temperature (°C)	Peak viscosity (cP)	Holding viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)				
Hermetic at 5 °C	$80.90 \pm 0.00 a$	$2819 \pm 35 \text{ a}$	$1932\pm28a$	$887 \pm 7 \text{ b}$	$4358 \pm 53 \text{ a}$	$2425\pm24a$				
Nitrogen at 15 °C	81.37 ± 0.37 a	2753 ± 27 a	$1801\pm32b$	$952\pm4a$	$4008\pm31~b$	$2207\pm1b$				
Normal at 25 °C	$81.00\pm0.05~a$	$2808\pm21a$	$1846\pm3b$	$962\pm18~a$	$4040\pm0.5\;b$	$2194\pm2b$				

^a Different letters in the same column differ statistically (p < 0.05). Results are the means of three determinations \pm the standard deviation.

3.3. Pasting properties

The pasting temperature, peak viscosity, holding viscosity, breakdown, final viscosity and setback of starches isolated from carioca bean grains stored for 360 days under different atmosphere conditions and evaluated in RVA are presented in Table 3. There was no statistical difference in the pasting temperature and peak

viscosity between starches isolated from grains stored under the three different atmospheres. Higher values of holding viscosity, final viscosity and setback parameters were found for the starches isolated from grains stored under hermetic conditions at $5\,^{\circ}$ C. There were no differences in the aforementioned parameters between the grains stored under the nitrogen-modified atmosphere at $15\,^{\circ}$ C and under normal atmosphere at $25\,^{\circ}$ C. According

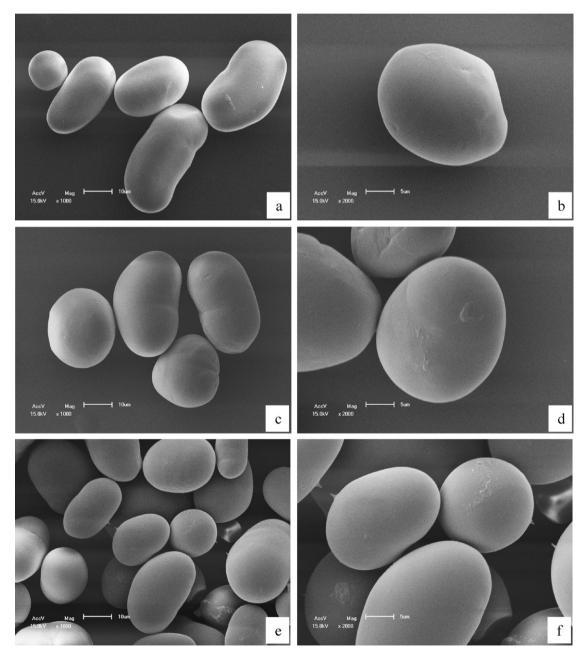


Fig. 3. Scanning electron micrographs of starches isolated from beans stored for 360 days under different conditions: hermetic, 5° C, $1000 \times$ (a); hermetic, 5° C, $2000 \times$ (b); nitrogen, 15° C, $1000 \times$ (c); nitrogen, 15° C, $1000 \times$ (d); normal, 25° C, $1000 \times$ (e); normal, $1000 \times$ (f).

to Kuakpetoon and Wang (2001), the amorphous lamella mainly consists of amylose, and the regions around it consist of branches of amylopectin. Amylose molecules reassociate more easily due to their linear structure and are primarily responsible for the occurrence of setback (retrogradation). Therefore, the starch isolated from grains stored under hermetic conditions at 5°C showed higher setback due to lower crystallinity and possibly by higher amylose content. The starch isolated from grains stored under hermetic conditions at 5 °C showed lower breakdown than the starches from grains stored under the nitrogen-modified atmosphere at 15 °C and under normal atmosphere at 25 °C, which showed similar parameters. The increased breakdown of the starch isolated from beans that were stored under the nitrogen-modified atmosphere at 15 °C and under normal atmospheric conditions at 25 °C shows that starches are less stable during continued heating and shearing. The changes in the pasting properties are most likely due to changes in crystallinity of the starches isolated from grains stored under the three different atmospheric conditions.

3.4. Scanning electron micrographs

The scanning electron micrographs (SEM) of the starches that were isolated from carioca bean grains and stored for 360 days in hermetic conditions at 5 °C, nitrogen-modified atmosphere at 15 °C and in normal atmosphere at 25 °C are shown in Fig. 3. The letters (a), (c) and (e) show the starch granules of the grains stored under hermetic conditions at 5 °C, nitrogen-modified atmosphere at 15 °C and normal atmosphere at 25 °C, respectively, in an approximation of $1000\times$. The letters (b), (d) and (f) show the starch granules of the grains stored under hermetic conditions at 5 °C, nitrogen-modified atmosphere at 15 °C and normal atmosphere at 25 °C, respectively, in an approximation of $2000\times$.

The starch granules obtained from the stored beans were very similar, even under different conditions. The granules were oval to spherical shaped with a smooth surface and absence of fissures, as verified by Kaur and Singh (2007) in normal and chemically hardened kidney beans. The starch granules of the beans stored under normal atmosphere at $25\,^{\circ}\mathrm{C}$ seems to be more uniform than the starch granules from the two other storage conditions, which showed a visible tip where it was adjoined with another granule. However, this characteristic was not verified in literature since there are few works about the morphological properties of starch isolated from stored bean grains.

4. Conclusion

The properties of bean starch are influenced by the storage conditions. The starch isolated from grains stored in a nitrogen-modified atmosphere at 15 °C did not differ in solubility and pasting properties compared to grains stored in the normal atmosphere at 25 °C. However, the pasting properties of these two conditions differ from that of the hermetic conditions at 5 °C. The starch isolated from grains stored under the hermetic conditions at 5 °C showed the lowest crystallinity, swelling power and heat that is necessary for gelatinisation, followed by the starch isolated from grains stored under nitrogen-modified atmosphere at 15 °C. The starch from the grains stored under normal atmosphere at 25 °C showed the highest crystallinity, swelling power and heat necessary for gelatinisation.

Studies related to the effects of storage conditions on the properties of bean starch are important in understanding the changes of this bean fraction that occurred with the hardening defect. Furthermore, elucidating the effects of storage conditions makes it possible for the standardisation of bean starch used in industry.

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