Faculdade de Agronomia
Eliseu Maciel
Fundada em 1883

Current Agricultural Science and Technology
ISSN 2317-2436
http://periodicos.ufpel.edu.br/ojs2/index.php/CAST

Effects of nitrogen-modified atmosphere storage on physical, chemical and technological properties of Carioca bean (*Phaseolus vulgaris* L.)

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Received: 11 November 2013 / Accepted: 28 April 2014 / Available online: 03 September 2014

ABSTRACT

When stored under inappropriate conditions, the carioca bean (*Phaseolus vulgaris* L.) is affected by a hardening defect, which is accompanied by the coat color change that occurred as a function of phenolic compounds oxidation. The aim of this study was to evaluate the effects of nitrogen-modified atmosphere storage on coat color preservation and development of hard-to-cook defect in Carioca beans stored for 360 days. Beans (5 kg) were stored, in triplicate, in three different conditions: nitrogen-modified atmosphere (storage in 0.20-mm-thick polyethylene bags with nitrogen addition), normal atmosphere (storage in cotton bags) and control atmosphere at 5°C (control treatment; storage in 0.20-mm-thick polyethylene bags). Both beans stored under nitrogen-modified atmosphere and normal atmosphere were kept at 25°C and 75±5% relative humidity for 360 days. Storage in the nitrogen-modified atmosphere preserved 5.80 gkg^{-1} protein, 2.70 g kg^{-1} fat, a 9.54 coat color difference index (ΔE*), 4.89 g kg^{-1}gallic acid (phenolic content), a 540-second cooking time and a hardness of 19.09 N $grain^{-1}$ when compared to grain stored in the normal atmosphere (oxygen) for 360 days. Moreover, the storage in a nitrogen-modified atmosphere preserved the hydration coefficient and the electrical conductivity of carioca bean and slows the development of the HTC defect, at least for 360 days storage. This storage system also reduces the cellular stresses caused by storage in the normal atmosphere at 25°C.

Keywords: Carioca bean, Modified-atmosphere, Nitrogen, *Phaseolus vulgaris* L., Storage.

INTRODUCTION

Bean (*P. vulgaris* L.), one of the world's most popular members of the Fabaceae family (Castillo et al., 2010), is cultivated in all continents, mainly in developing countries where it is an important and cheap source of protein, dietary fiber and starch to a large population (Perla et al., 2003). There are many genotypes of beans that differ in size, shape and color of the tegument. The grain of the Carioca class is the most produced and consumed in Brazil, representing over 60% of bean production in the country (Conab, 2009). Carioca beans have a cream background with tan stripes. Their production occurs both in small farms, where farmers usually store the beans for their own consumption, and in large farms, where their production is sold entirely to processing industries. In both cases, the beans are often stored at improper temperature, humidity and light conditions. The changes that occur in the coat color and in the cotyledon of the grain are the most important and imply

serious damage to industries and consumers, due to changes in physical, chemical and technological properties and reduced consumer acceptability. This reduced acceptability is due to the preference of consumers for beans with short cooking times, thick broth after cooking, soft grains and, for beans of the carioca class, that present clear coat color.

The phenomenon of cotyledon hardening is known as the "hard-to-cook" (HTC) defect (Yousif and Deeth, 2003) and is responsible for an increase in cooking time and reduces the leaching of nutrients inside the grains (Barampama and Simard, 1993). The HTC defect is related to multiple mechanisms, such as starch gelatinisation, protein denaturation and cell wall changes (Liu, 1995; Shiga et al., 2004).The HTC defect in legumes has been shown to be associated mainly with alterations that occur in the cotyledons, whereas physical alterations in the cell structure of the seed coat cause the "hard-to-shell" (HTS) condition, which is related to the capacity of the grains to absorb water (Coelho et al., 2007; Hincks et al., 1987). In addition, phenolic compound oxidation is responsible for the darkening of the seed coat of the faba bean (*Vicia faba* L.) and is among the main factors responsible for changing the coat color of grains during storage (Nassar-Abbas et al., 2008a). Storage under adverse conditions of high temperature and high humidity renders the beans susceptible to the HTC defect (Kaur and Singh, 2007; Paredes-Lopez et al., 1989). Several changes that influence cooking and nutritional quality occur during storage.

An alternative for improving the preservation of beans during post-harvest is the storage under nitrogen-modified atmosphere, which consists of oxygen content reduction and an increase in nitrogen content in the storage environment. There are many studies that have evaluated the effects of temperature and relative humidity during storage on the physico-chemical and technological properties of common beans. However, there are few studies on the use of nitrogen-modified atmosphere storage. Nasar-Abbas et al. (2008a) assessed the changes in coat color and phenolic compounds of faba beans (*V. faba*) stored in different atmospheres. Brackmann et al. (2002) studied the conservation of three different genotypes of Carioca bean (*P. vulgaris* L.) stored under normal atmosphere, refrigeration and a nitrogen-modified atmosphere but did not cover the relationship between their physical, chemical and technological properties during 360 days of storage.

The aim of this study was to evaluate the effect of a nitrogen-modified atmosphere and storage time on the physical, chemical and technological properties of Carioca beans.

MATERIALS AND METHODS

Plant material

Carioca beans (*Phaseolus vulgaris* L.), cv. Pérola, were cultivated in an irrigation system and harvested when the moisture content was about 12.5% and, afterwards, submitted to a cleaning process. The grains were put in raffia bags and immediately transported to the Postharvest, Industrialisation and Quality of Grains Laboratory of DCTA-FAEM-UFPel where the experiment was carried out.

Storage conditions

Beans were placed in two different storage conditions, a nitrogen-modified atmosphere and a normal atmosphere (with oxygen), both in triplicate. In the nitrogen-modified atmosphere, 5 kg of grain was placed in 0.20-mm-thick polyethylene bags. The atmosphere modification was performed by a Webomatic® machine, which removed the oxygen inside the package, added nitrogen and immediately sealed the package. To evaluate the normal atmosphere, 5 kg of grain was stored in cotton bags and purged every 60 days of storage to avoid any interference in grain quality due to the presence of insects. Both treatments were kept protected from light with aluminum foil at 25°C and 75±5% relative humidity for 360 days. Another aliquot of 5 kg was maintained at 5°C for 360 days, which constituted the control sample. The determination of moisture, ash,

fiber, fat, hydration coefficient, electrical conductivity, hardness and morphology by optical microscopy were performed in triplicate at 360 days of storage for grains stored in both the two conditions and the control. The evaluation of grain coat color and cooking time was performed in triplicate on day 1 (initial), 120, 240 and 360 of storage.

Proximate composition

The water content of the beans during the storage period was determined using an oven method at 105±3°C, with natural air circulation for 24 hours, in accordance with the recommendations of ASAE (2000). These tests were carried out in triplicate and moisture content is expressed as percentage (%). The fat content was determined in accordance with the AACC method 30-20 (AACC, 1995). 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 ash content was determined by the method 08-01 of AACC (AACC, 1995). The fibre content was determined as described by Angelucci et al. (1987). The carbohydrate content was determined by difference testing.

Coat color

Color of grains was determined using a Minolta CR-310 chromameter (Minolta, Japan) using the granular-materials Attachment CR-A50. Data were collected for L*, a* and b* values. L* values represents lightness, a* values varies from green to red and b* values varies from blue to yellow. A white porcelain plate (L*= 99.41 a*= -4.91 b*= +7.33) was used for calibration.

To ascertain the practical significance of the changes in the objective measurements of common bean coat color during storage, the Color Difference Index (ΔE*) was calculated from the L*, a* and b* color coordinates using Eq. 1 (Nasar-Abbas et al., 2008a):

$$\Delta Eab^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (1)$$

where $\Delta L^* = L^*_1 - L^*_2$; $\Delta a^* = a^*_1 - a^*_2$; e $\Delta b^* = b^*_1 - b^*_2$.

Initial L*, a* and b* values (subscript by 1) and values at each storage interval (subscript by 2) were used to produce ΔEab* values; these values were used to compare post-harvest color changes in the samples.

Phenolic compounds

The total phenolic compounds were determined by the method of Zielinski & Kozlowska (2000). A defatted flour sample was extracted with 80% methanol (10 ml/ 0.5 g) for two hours at 4°C. After centrifugation at 7000 rpm for 20 minutes, 0.25 ml of the extract was mixed with 0.25 ml of Folin-Ciocalteau and 2 ml of distilled water. After 3 minutes at room temperature (25°C), 0.25 ml of a saturated solution (20%) of sodium carbonate (NA_2CO_3) was added. The sample was stirred for 30 minutes in a water bath at 37°C. The absorbance was measured at 750 nm using a UV-visible spectrophotometer. The result was expressed as grams of gallic acid per kg.

Hydration coefficient

The hydration coefficient was determined by soaking 20 g of bean grains at room temperature (25°C) in 100 ml deionised water (ratio 1:5). After 12 hours the beans were removed from the water and centrifuged at 1000 rpm for 1 minute to remove the free water and were then weighed again. The grain in weight was taken as the amount of water absorbed and expressed as the hydration coefficient (El-Refai et al., 1988; Nasar-Abbas et al., 2008b).

Electric conductivity

The electrical conductivity was determined from four replicates of 25 grains, weighed and immersed in 75 ml of water (in 250-ml beakers), placed in an incubator at 20°C

constant temperature and then incubated for 24 hours (ISTA, 2008). The solutions were shaken gently, and the electrical conductivity was determined from an unfiltered solution. The results were expressed in μS cm^{-1} g^{-1}.

Hardness

Grains (25 g) were soaked in beakers containing 200 ml distilled water, which were kept covered at room temperature for 12 hours. The grain samples were cooked for 16 minutes (initial cooking time) and analysed for their texture. Hardness of cooked grains was performed using a single grain placed on the base plate of a Stable Micro Systems Texture Analyser (model TA.XTplus, England). The cooked seeds were subjected to 80% compression with a cylindrical probe (38-mm diameter) at a crosshead speed of 1 mms^{-1} twice in two cycles using a 5-kg load cell. The textural parameters of hardness (maximum height of the force peak on the first compression cycle) were determined as described by Bourne (1978). Fifteen measurements were performed on each sample and the results were expressed in N $grain^{-1}$.

Cooking time

The cooking time of the grains stored under each condition was determined at days 1 (initial), 120, 240 and 360 using the method of Mattson (1946) and that was modified by Burr, Kon and Morris (1968), with some further modifications. Prior to cooking, grain samples (25 beans) were soaked in 80 ml deionised water for 12 hours. A Mattson-modified cooker was used to determine the cooking time of the individual beans. This cooker utilised 25 stain steel cylindrical holes, with 82-g piercing tip rods in contact with the surface of the bean. The cooker was then placed into a 2-l beaker containing 400 ml of boiling water. Bean grains were judged as 'cooked' when the 2-mm diameter piercing tip of the brass rods passed through the beans. Cooking time was reported as the time required for 50% of the grains to be cooked, as indicated by plungers dropping and penetrating individual beans.

Optical microscopy

Analysis by optical microscopy was performed at the Microscopy Laboratory of Embrapa Temperate Climate, Pelotas, according to the methodology described by Dawes (1971).

Statistical analysis

Analytical determinations of 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).

RESULTS AND DISCUSSION

Chemical composition

The proximate grain composition of carioca beans stored at 5°C (control), in the nitrogen-modified atmosphere and in the normal atmosphere for 360 days is presented in Table 1.There was a significant reduction in the lipid levels of the beans stored in the normal atmosphere for 360 days compared with the beans stored at 5°C (control) and the beans stored in the nitrogen-modified atmosphere. The crude protein content of grains stored in the normal atmosphere differed from the crude protein content of grains stored at 5°C (control), but no difference was observed between the grains stored under nitrogen-modified atmosphere and normal atmosphere. There was no significant difference in fibre, ash and carbohydrates content between the grains stored in the nitrogen-modified atmosphere and the normal atmosphere or the control treatment.

Table 1. Composition of Carioca bean grains stored at 5°C (control) in a nitrogen-modified and in a normal atmosphere for 360 days

Storage condition	Moisture (%)	Crude fibre	Crude protein	Total ash	Lipid content	Carbohydrate
		g kg^{-1}				
Control, 5°C	12.11±0.13[b]	68.70±6[a]	222.30±3[a]	43.40±0.4[a]	17.1[a]	527.40
Nitrogen-modified	12.61±0.07[b]	68.90±5[a]	220.40±4[ab]	44.50±0.3[a]	16.2[a]	523.90
Normal	13.69±0.21[a]	70.40±2[a]	214.60±1[b]	44.30±0.4[a]	13.5[b]	520.30

Simple arithmetic average of three replicates ± standard deviation accompanied by different uppercase letters in the same column differs by 5% according to Tukey's test.

The moisture content of the grains stored in the normal atmosphere increased at the end of the storage period due to hygroscopic equilibrium. The water absorbed by the grains was only possible due to the exchange of heat and water with the environment, which was not possible to observe in the nitrogen-modified atmosphere samples because of the isolation induced by the presence of an inert gas and polyethylene sealing. There was no significant difference in the fibre content of beans stored in either storage condition after 360 days of storage (Table 1). The protein content of the grains decreased during storage, from a baseline of 222.30 g kg^{-1} to 214.60 g kg^{-1} after 360 days, which was in agreement with the results reported by Coelho et al. (2009), who observed changes in protein content in Peruano and Paraíso bean grains after 135 days at a storage temperature of 29°C and 75% relative humidity. However, in this same study, the reduction did not influence the HTC defect development as the authors did not observe a significant increase in cooking time.

The ash values remained unchanged after 360 storage days, which is in agreement with the literature. Barampama and Simard (1995) observed ash content between 38 and 45 g kg^{-1} in four varieties of beans (*P. vulgaris* L.) cultivated in different regions of Burundi. A reduction in the lipid content was observed in grains stored under both conditions. The smallest decrease was found in grains stored in the nitrogen-modified atmosphere (Table 1) compared to grains stored at 5°C (control). Lipid content is an important parameter for evaluating the quality of stored grains because lipids are the most reactive components under adverse storage conditions and their degradation is responsible for changes in the percentage of free fatty acids, causing increased sensitivity of fatty acid oxidation and the alteration of functional properties (Anwar et al., 2007).

Coat color

Fig. 1 shows the L*, a*, b* and ΔE* values of beans stored in the nitrogen-modified atmosphere and in the normal atmosphere for 360 days. The coat color varied with the storage system. According to the results of the L* variable, the beans stored in the nitrogen-modified atmosphere showed higher brightness after 120, 240 and 360 days of storage compared to the beans stored in the normal atmosphere. Also, they presented smaller a* values for the three periods analysed, which means that these grains reddened more slowly than those in the normal grain storage atmosphere. The b* value is important to calculate the index of color difference; it can be seen that the beans stored in the normal atmosphere showed b* values well above the beans stored in the nitrogen-modified atmosphere (Fig. 1). These results are consistent with those of Nasar-Abbas et al.(2008a), who studied the effect of modified atmosphere storage on faba bean (*Vicia faba* L.) color with light protection and 12% moisture content at 30°C.

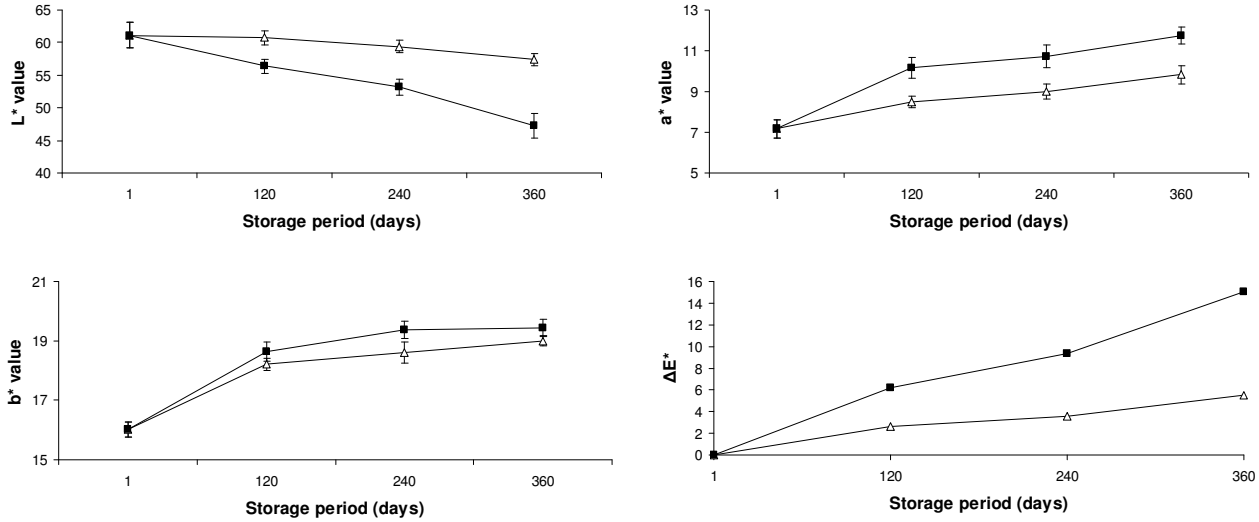

Figure 1.L*, a*, b* and ΔE* values for bean grains stored in a nitrogen-modified atmosphere (Δ) and in a normal atmosphere (■) for 360 days.

They found that storage in a nitrogen-modified atmosphere was the best system to preserve color, compared with a storage atmosphere comprised of carbon dioxide, oxygen and ethylene. Brackmann et al. (2002) found that storage in a nitrogen-modified atmosphere and in a refrigerated atmosphere preserves the color of the grain tegument of common bean (*P. vulgaris* L.).

Total phenolic compounds

Table 2 shows the total phenolic compounds content of Carioca bean stored at 5°C (control), in a nitrogen-modified atmosphere and in a normal atmosphere for 360 days. The darkening of the coat seems to be closely associated with the phenolic compounds oxidation. There was a reduction of total phenolic content of beans stored in both systems. However, the nitrogen-modified atmosphere better preserved the phenolic compounds content compared to the normal atmosphere. Actually, the phenolic content is probably the same, but the phenolics complexation with proteins and other macromolecular during cell stress makes them less extractable and, thus, it is verified a lower amount of phenolics when the Folin-Ciocalteau reaction is performed. The beans stored in nitrogen atmosphere showed a 29.52% reduction in phenolic content, whereas those stored in a normal atmosphere experienced a 49.77% reduction in phenolic content (Table 2).

These results are in agreement with those of Nasar-Abbas et al. (2008a), who reported that storage of faba bean (*Vicia faba* L.) for 12 months in a nitrogen-modified atmosphere better preserved the color and the total phenolic content compared to what occurred in grains stored under oxygen presence. The reduction in the total phenolic compounds concentration has been identified as being responsible not only for changing the coat color but also as being one of the factors that causes hardening of grains (Stanley, 1992). This is because monomeric phenols are polar compounds and, therefore, tend to be soluble in water. Thus, during the maceration process, these compounds migrate into the cotyledon interiors and interact with proteins and other macromolecular compounds in the cell (Aw and Swanson, 1985; Hincks and Stanley, 1987). Beninger et al. (2005) correlated the post-harvest browning of pinto beans with the increase in the dimer concentration

Table 2. Phenolic compounds in Carioca beans stored at 5°C (control), in a nitrogen-modified atmosphere and in a normal atmosphere for 360 days

Storage condition	Total phenolic compounds (g gallic acid kg^{-1} grain)
Control, 5°C	24.15±1.20[a]
Nitrogen-modified	17.02±0.76[b]
Normal	12.13±0.29[c]

Simple arithmetic average of three replicates ± standard deviation accompanied by different uppercase letters in the same column differs by 5% according to Tukey's test.

Physical properties

Table 3 shows some physical properties of beans stored at 5°C (control), in a nitrogen-modified atmosphere and in a normal atmosphere for 360 days. The hydration coefficient did not differ in grains stored in the nitrogen-modified atmosphere compared to the grains stored at 5°C (control). However, the grains stored in the normal atmosphere showed a lower hydration coefficient (Table 3). This fact is directly related to the development of the HTC defect because the HTC beans are characterised by limited cell separation and restricted gelatinisation, which are attributed partly to water competition between protein coagulation and starch swelling (Coelho et al., 2009; Liu, 1995). According to Moscoso et al. (1984), red kidney beans (*P. vulgaris* L.) stored at 32°C for 9 months showed a 10% reduction in hydration capacity compared with grains stored at 21°C. Yousif et al. (2003) also reported a significant reduction in the water absorption capacity of adzuki bean (*Vigna angularis*) stored for 6 months at 30°C compared to grains stored at 20 and 10°C. Thus, the more adverse the storage conditions are, the lower the hydration capacity is and, hence, the lower the hydration coefficient is. Nasar-Abbas et al. (2008b) found that the higher the storage temperature was, the lower the hydration coefficient in faba beans (*V. faba* L.) after 12 months of storage.

The electric conductivity of the beans stored in both conditions increased compared with the beans stored at 5°C (control). It was observed that the electric conductivity was higher in grains stored in the normal atmosphere than in the grains stored in the nitrogen-modified atmosphere, which is probably due to damage to the cell membrane, which facilitated the release of salts from the interior of the cells when the beans are hydrated. The conductivity increased 21.6% for grains stored in the modified and 41.5% in grains stored in the normal atmosphere compared to the storage at 5°C (Table 3).

The bean hardness, determined by the texture profile analysis, increased for beans stored in both conditions compared to the beans of the control treatment; however, the beans stored in the modified atmosphere had lower hardness than the beans stored in normal atmosphere for 360 days (Table 3).

This result is in agreement with Coelho et al. (2009), who found that the natural and accelerated aging of carioca bean caused an increase in the hardness of the grains. The hardness of the grains is related to the failure of the product to absorb water during the cooking process, which is a result of the HTC defect. Storage under adverse conditions has a definite effect on the change of the physical properties of the grains. The presence of oxygen seems to be one of those conditions that favor the appearance of the HTC defect.

Cooking time

Fig. 2 shows the cooking time of Carioca beans stored in the nitrogen-modified atmosphere and in the normal atmosphere for 360 days. The cooking time of the grains stored in both conditions increased up to 120 days, remained unchanged up to 240 days and increased significantly at 360 days of storage. At all storage times, the condition with the nitrogen atmosphere changed with a more delayed appearance of the HTC defect

compared to the normal atmosphere (Fig. 2), as was verified in the evaluation of physical parameters in Table 3. The results are similar to those found by Coelho et al. (2009), who found an increase in cooking time of black and carioca bean during aging both naturally and artificially. Brackmann et al. (2002) evaluated the cooking time of Carioca bean, cv. Pérola, stored for 9 months under different storage conditions, and determined a 27-minute cooking time for beans stored in a nitrogen-modified atmosphere and a 35-minutes cooking time for grains stored in a normal atmosphere (room temperature).

Optical microscopy

The optical microscopy photographs of the Carioca bean cotyledons are shown in Fig. 3. Image A shows part of the cotyledon of a bean grain stored in the nitrogen-modified atmosphere for 360 days. Image B illustrates a part of the cotyledon of a bean stored in the normal atmosphere for the same period. In both images, there are plenty of reserve cells in the cotyledons of the beans. On the periphery of the cotyledon there are sclerenchymas, which are dead cells at maturity. It is possible to perceive thickener and lignified cell walls in the inner layer of cotyledons from beans stored under normal atmosphere compared to beans stored under nitrogen-modified atmosphere, as indicated by arrows (Fig. 3B). The cell walls protect the cells, forming a kind of wrap. This is probably due to a response mechanism caused by the adverse storage environment. The adverse conditions promote the lignification process and cell wall thickening, which agrees with the results reported by Yousif et al. (2003).

The thickening and lignification of the cell wall observed in Fig. 3B during storage is attributed as the cause for the increased hardness and reduction of the hydration coefficient of grains, as shown in Table 3. The cell wall thickening provides the reduction of intercellular space that leads to a lower water hydration capacity. The presence of oxygen in the storage environment storage speeds up the metabolic processes and oxidative stresses in the beans. Thus, the lignification of the cell walls of the cotyledon cells can be understood as a way of protecting the cells from environmental stress.

Table 3. Changes in physical properties of Carioca beans stored under different conditions during 360 days

Storage condition	Physical properties		
	Hydration coefficient	Electric conductivity (μS cm^{-1})	Hardness (N $grain^{-1}$)
Control, 5°C	199±2[a]	106±3[c]	60.29±0.59[c]
Nitrogen-modified	196±3[a]	129±4[b]	98.04±1.98[b]
Normal	191±2[b]	150±6[a]	117.13±5.23[a]

Simple arithmetic average of three replicates ± standard deviation accompanied by different uppercase letters in the same column differs by 5% according to Tukey's test.

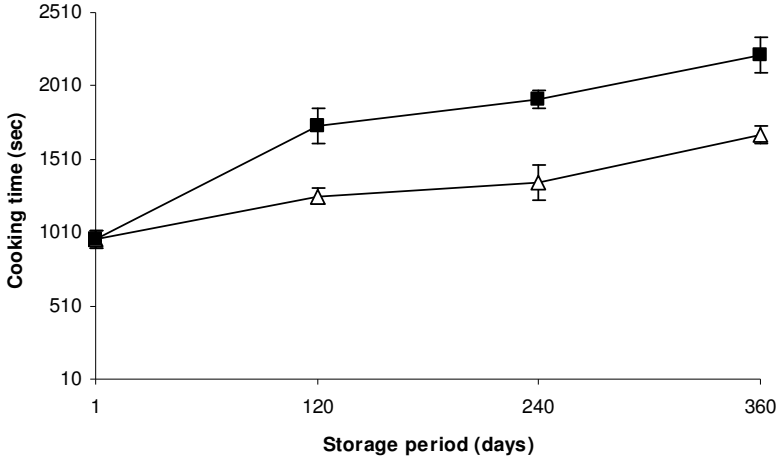

Figure 2. Cooking time (seconds) of Carioca beans stored in a nitrogen-modified atmosphere (Δ) and a normal atmosphere (■) for 360 days.

CONCLUSIONS

The storage of Carioca bean in a nitrogen-modified atmosphere preserves the protein and lipid contents, the hydration coefficient, the electrical conductivity, the hardness, the cooking time and the color of the grains, and slows the development of the HTC defect, at least for 360 days of storage. This storage system reduces the cellular stresses caused by adverse storage in the normal atmosphere (with presence of oxygen). Therefore, the storage of Carioca beans under nitrogen can maintain the quality of the grains for a higher period than the storage under conventional system (oxygen) and, thus, allow the supply of healthier and sensory acceptable grains for consumers for over the year. Further studies must be conducted in order to evaluate the effects of storage periods higher than 360 days under different atmospheres (nitrogen, carbon dioxide, oxygen) on the physical, chemical and technological properties of Carioca beans.

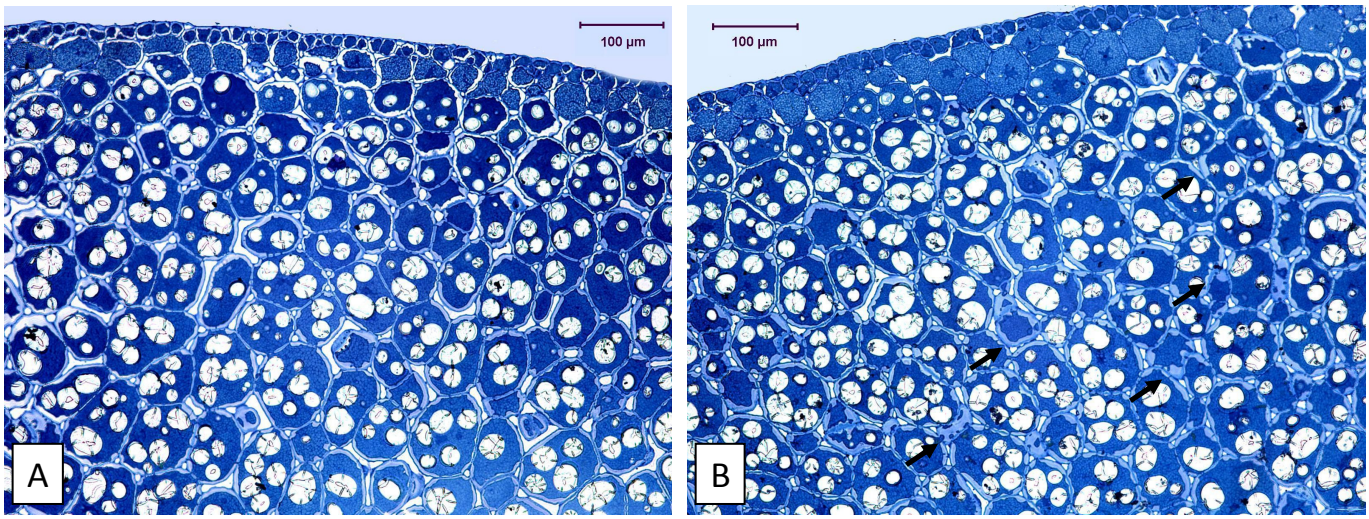

Figure 3. Optical microscopy photographs of bean stored in a nitrogen-modified atmosphere (A) and a normal atmosphere (B) for 360 days.

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