Physicochemical, antioxidant and cooking quality properties of long-term stored black beans: effects of moisture content and storage temperature

[1*]Ferreira, C.D., [1]Ziegler, V., [2]Paraginski, R.T., [1]Vanier, N.L., [1]Elias, M.C. and [1]Oliveira, M.

[1]*Department of Agroindustrial Science and Technology, Federal University of Pelotas, 96010-900, Pelotas, RS, Brazil*

[2]*Federal Institute of Education Science and Technology Farroupilha, Campus Alegrete, 97555-000, Alegrete, RS, Brazil*

Abstract

The objective of this study was to evaluate the main physicochemical and cooking quality changes in black beans stored at 14% and 17% moisture contents and at different temperatures (11, 18, 25 and 32ºC) during 12 months. The proximate composition, the fat acidity, the electric conductivity, the leaching of solutes, the total phenolics and proanthocyanidins, the cooking time and the hardness of freshly harvested and stored black beans were determined. The protein, fat and ash contents remained unchanged ($p \leq 0.05$) during storage, regardless of the moisture content and storage temperature. The fat acidity was greatly impacted by the moisture content. Electric conductivity, the leaching of solutes, the cooking time and hardness presented higher increases at 17% moisture content and temperatures of 25 and 32ºC. The extractability of the total phenolics and proanthocyanidins decreased during storage impacted by both high moisture content and high storage temperatures. A similar reduction trend was determined in the antioxidant capacity of black bean extracts.

Introduction

Beans possess excellent nutritional value, since they are a good source of sulfur amino-acids-rich proteins, dietary fibers, and slow digestible starch (Shiga and Lajolo, 2006; Boye *et al*., 2010; Du *et al.*, 2014). More recently, studies have shown the importance of bioactive compounds of beans as antioxidant, anti-inflammatory, and anticarcinogenic agents (Campos-Vega *et al*., 2010; Díaz *et al*., 2010; Hayat *et al.*, 2014). The main bioactive compounds found in beans are flavonoids (anthocyanins and proanthocyanidins) and phenolic acids (mainly ferulic, caffeic, sinnapic and gallic acids) (Hayat *et al.*, 2014). They are predominantly found in the seed coat (Nasar-Abbas *et al*., 2009).

Important changes in the technological, sensory and nutritional quality properties of beans occur as a function of storage. The reduction in bean quality during storage at inadequate conditions is mainly associated with two distinct but complementary phenomena: the hard-to-shell defect (HTS), that arises from seed coat hardening; and the hard-to-cook defect (HTC), that is associated with the reduced water absorption capacity of cotyledons (Nyakuni *et al.*, 2008; Shiga *et al*., 2009). Performing a deeper prospect of the factors responsible for the hardening defect of

beans during storage, the following phenomena may be listed: (1) complexation reactions between pectin-cation-phytate (Njoroge *et al*., 2014); (2) cell wall lignification (Shiga, 2004); (3) interactions between proteins and starch (Liu *et al.*, 1992; Rupollo *et al*., 2011); and (4) interactions between tannins and other phenolic compounds with proteins. The appearance and development of HTS and HTC defects in stored beans result in the increased cooking time, reduced palatability, decrease in nutritional value, and the consumers' acceptability (Nasar-Abbas *et al*., 2008a; Njoroge *et al.*, 2014).

Grain moisture content and storage temperature are key factors to be controlled during the storage period. According to results presented by Rani *et al.* (2013) and Chidananda *et al*. (2014), storage at 12-14% maintains the quality of the grains for a longer period due to the decreased metabolism of the grains and associated fungi, preventing the formation of an undesirable flavor and aroma. As related to the effects of storage temperature on bean quality, previous studies were performed by Yousif *et al.* (2003); Maurer *et al.* (2004) and Granito *et al*. (2008). They revealed that temperatures around 30ºC might significantly impact the nutritional and cooking quality properties of beans stored for periods between 3.5 and 6 months.

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*Corresponding author.
Email: *cristiano.d.f@hotmail.com*
Tel/Fax: +00555332757284

The insolubilization of phenolic compounds have been determined by Granito *et al.* (2008) and Nasar-Abbas *et al.* (2009) in black beans (*Phaseolus vulgaris* L.) and faba beans (*Vicia faba* L.), respectively. Remarkably, these are associated with high storage temperatures. As related to the cooking quality of stored beans, Maurer *et al.* (2004) determined an increase of 41 minutes in the cooking time of black beans stored at 65% relative humidity and 29ºC for 3.5 months. According to Chidananda *et al.* (2014), the changes that occurred in the quality of stored beans were favored by the increase in the grains' respiration rate and enzymatic activity These could be increased 2-3 times when the moisture content was increased from 12-14% to 18-20% levels and the temperature was increased from 10-20°C to 30-40°C.

Brazil is one of the three largest producers of beans in the world, with approximately 3.2 million tones produced per year (FAO, 2014). In order to maintain the supply of beans throughout the year, Brazilian stockists and food industries store the grains in bags or silos. These are common storage methods used worldwide. During the long-term storage beans are subjected to the most variable temperature conditions (ranging from 10-45ºC), depending on the region. It is presumable that if the temperature conditions are not proper for grain preservation during long-term storage, the moisture content of the grains should be set at low levels aiming to increase beans conservability at high temperature conditions. But this is not what really happens; as an example, the Brazilian bean's legislation (MAPA, 2008) has not established a moisture content limit for bean marketing; therefore, grains can often be found packed with moisture contents ranging from 14% to 17% or even higher. Some food industries that market grains with moisture contents around 17% justify that the cooking quality properties of black beans stored at high moisture content levels are better than the cooking quality properties of black beans stored at low moisture levels.

The aim of this study was to evaluate the physicochemical, antioxidant and cooking quality properties of black beans stored at 14% and 17% moisture contents at different temperatures (11, 18, 25 and 32°C) during 12 months of storage.

Materials and Methods

Materials and storage conditions

Black beans from the Diamante Negro cultivar produced in 2013 growing season at Erechim (27º 38' 03" S, 52º 16' 26" W, 783 m altitude) in the State of Rio Grande do Sul, Brazil, were used. The grains were mechanically harvested when the moisture content was 17%, placed into raffia bags and immediately transported to the Laboratório de Pós-Colheita, Industrialização e Qualidade de Grãos from DCTA-FAEM-UFPel, where the experiment was carried out. After being subjected to the cleaning step, an aliquot of the grains was kept at 17% moisture content while another aliquot was dried at 35°C until 14% moisture content was achieved. Afterwards, both aliquots were purged using aluminium phosphide to prevent the interference of insects in the experiment. Black beans with 14% and 17% moisture content were then stored in polyethylene bags of 0.2 mm-thick plastic film with a capacity for 0.9 kg of grains at 11, 18, 25 and 32°C for 12 months, in triplicate. The grains were stored in chambers with controlled temperature and air humidity according to each grain moisture and temperature used. This procedure was used for maintain the hygroscopic equilibrium of grains during the storage time. The stored black beans were maintained and protected from light by covering the polyethylene bags with aluminium foil. Evaluations were performed on freshly harvested black beans (initial treatment, non-stored grains) and on grains stored during 4, 8 and 12 months. For analysis, the grains were ground in a laboratory mill (Perten 3100, Perten Instruments, Huddinge, Sweden), and equipped with a 70 mesh sieve to obtain flour with uniform particle size.

Proximate composition

The moisture content of the black beans during the storage period was determined using a drying oven set at 105ºC, with natural air circulation for 24 h, following the recommendations of the American Society of Agricultural Engineers (ASAE, 2000). The moisture content was expressed as a percentage (%). The fat content was determined following the method 30-20 of the American Association of Cereal Chemists (AACC, 1995). The nitrogen content was determined according to 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 according to the AACC method 08-01 (AACC, 1995).

Fat acidity

The fat acidity was determined following the titrimetric procedure described in the AACC method 02-01A (AACC, 2000). The titratable acidity was expressed as the mg of sodium hydroxide required to neutralize the acids in 100 g of sample, using a phenolphthalein solution as an indicator.

Electric conductivity

The electric conductivity was determined following the method described in the (Vieira *et al.*, 1999). Four replicates of 25 grains were weighed and immersed in 75 mL of deionized water (within beakers of 250 mL-capacity), and incubated for 24 hours at 20°C. The solutions were gently shaken and the electric conductivity was determined using a conductivitmeter without filtering the solution. The results were expressed in $\mu S.cm^{-1}$.

Solutes leaching

The determination of the leaching of the solutes was performed according to Nasar-Abbas *et al.* (2008a). Beans (10 g) were immersed in 50 ml of deionized water for 18 hours. After the immersion period, the water for hydration was collected and taken to an oven with air circulation at 105°C for 24 hours. The results were expressed in mg per g of black bean (dry weight basis).

Total phenolics, proanthocyanidins and antioxidant activity determinations

Extraction of phenolics

Phenolics were extracted according to the method described by Nasar-Abbas *et al.* (2008b), with some modifications. Black bean flour (2.0 g) was mixed with 10 mL of an acetone: water (70:30 v/v) solution. The material was shaken for 20 min and centrifuged at 7500 x g for 15 min. The supernatant was collected and the residue resuspended again in 10 mL of an acetone: water (70:30 v/v) solution. This extraction procedure was performed for three times; and the supernatants obtained from each extraction were combined and concentrated until dry by using a rotary evaporator at 35°C (Heidolph, Larorata Model 4000, Germany). The dry phenolics were resuspended in 30 mL of an acetone: water (70:30 v/v) solution.

Total phenolics content

The total phenolic compounds were quantified in accordance to the Folin-Ciocalteau method described by Zieliński and Kozłowska (2000), with some modifications. Extracts (100 μL) were added to 400 μL of distilled water. Afterwards, 250 μL of the 1 N Folin-Ciocalteau reagents were added. After 8 min of stabilization, 1.25 mL of a 7% sodium carbonate solution (w/v) was added. The material was agitated and left under darkness for 2 h. Absorbance was then measured at 725 nm. Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of black bean (dry weight basis).

Total proanthocyanidin content

Proanthocyanidins (PAs) were quantified according to the method described by Porter *et al.* (1985). Absorbance was measured at 550 nm (UV 17000 spectrophotometer, Shimadzu, Japan). The quantification was performed based on a calibration curve of leucocyanidin. Results of the triplicate analysis were given as mg of leucocyanidin equivalents per 100 g of black bean (dry weight basis).

DPPH• and ABTS•+ scavenging activities

The ability of black bean phenolic extracts to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH) and 2.2 azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was measured. The DPPH radical scavenging activity was determined based on the method of Brand-Williams *et al*. (1995). The ABTS radical scavenging activity was measured by using the method described by Re *et al*. (1999). Both DPPH and ABTS free radical scavenging activities in an acetone: water (70:30 v/v) solution were expressed as mg of trolox equivalents (TE) per 100 g of black bean (dry weight basis).

Cooking time

The cooking time of black beans was determined in non-stored grains (initial treatment) and in grains stored for 4, 8 and 12 months, in accordance to the method established by Mattson (1946), with some modifications described by Burr *et al.* (1968). Prior to cooking, the grains (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 utilized 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 2000 mL beaker containing 400 ml of water at 90°C, remaining at in boil until end of cooking. The bean grains were judged as 'cooked' when the 2 mm diameter piercing tip of the brass rods passed through by 50% of the beans, as indicated by the plungers dropping and penetrating individual beans, taking note of time.

Hardness

Grains 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 21.3 minutes (initial cooking time) and analyzed for their texture. The hardness of the 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 mm.s^{-1} twice in two cycles using a 5 kg load cell. The textural parameter of hardness (maximum height of the force peak on the first compression cycle) was determined as described by Bourne (1978). Results were expressed in N.grain^{-1}.

Statistical analysis

Analytical determinations for the samples were performed in triplicate, and standard deviations were reported. A comparison of the means was ascertained with Tukey's test to a 5% level of significance using an analysis of the variance (ANOVA). Pearson's correlation was used to determine the association between cooking time and hardness (p $\leq$ 0.01). The regression model with the highest R² value (coefficient of determination), the best significance of model fit and the best significance of the model parameters were selected.

Results and Discussion

Proximate composition

Black beans used in the present study presented average protein, ash, and fat contents of 25.6, 4.6 and 1.4 g/100 g, respectively. No changes (p > 0.05) in proximate composition were observed during storage of the black beans, even in those grains stored at the highest moisture content and temperature (17% moisture content and 32°C) (data not shown). Similar results were found by Berrios *et al.* (1999) in black beans stored with 9.25% moisture content under ambient conditions (23-25°C). The authors reported protein, ash and fat values of 25.9, 4.6 and 1.6 g per 100 g of black bean, respectively, with no changes (p > 0.05) in these constituents even after two years of storage. Nasar-Abbas *et al.* (2008a) evaluated the effects of storage temperature during long-term storage on the composition of faba beans, and reported no changes in the protein, ash and fat contents in faba bean stored at 27°C during 12 months.

Fat acidity

The fat acidity is one of the main parameters used to determine the quality of stored grain. Although beans exhibit a low fat content, the acidification of fat constituents is a major cause of the hardening of beans, because the acidification of tissues is related to pectin insolubilization and partial denaturation of proteins (Liu *et al.*, 1992). Free fatty acids are also responsible for unsuitable flavors and aromas (Toci *et al.*, 2013). The fat acidity results for black beans stored at 14% and 17% moisture contents at different temperatures
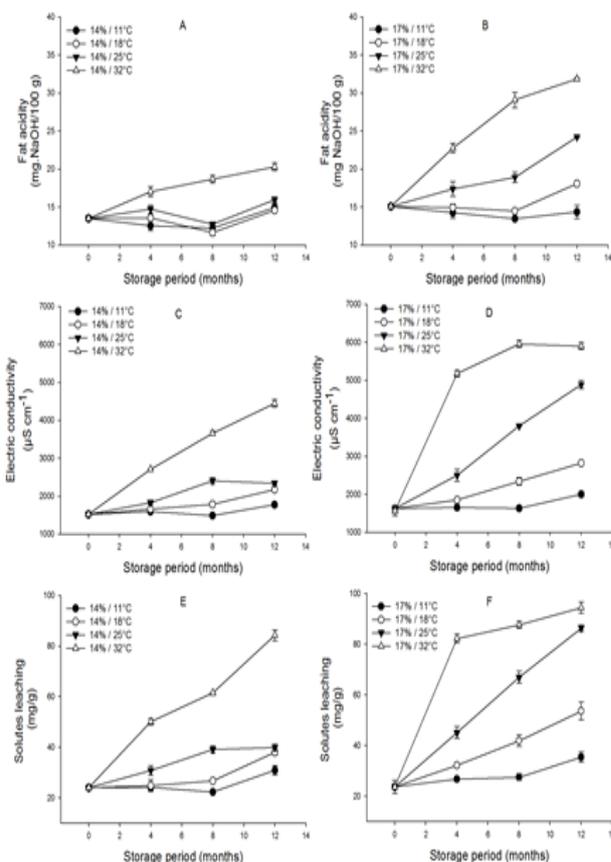

Figure 1. Fat acidity (mg NaOH/100 g) (A and B), electric conductivity (μS.cm^{-1}) (C and D) and the leaching of solutes (mg/g) (E and F) of beans stored at 14% (A) and 17% (B) moisture content at different temperatures (11, 18, 25 and 32°C) during 12 months.

during 12 months are presented in Figures. 1A and 1B, respectively. Grains stored at 14% moisture content and 32°C presented an increase (p ≤ 0.05) in fat acidity even after 4 months of storage, while no difference (p > 0.05) in fat acidity was determined in black beans stored at the same moisture content of 14% but at the lower temperatures of 11, 18 and 25°C during 12 months of storage (Figure 1A). For those grains stored at 17% moisture content, an increase (p ≤ 0.05) in fat acidity was determined for all the studied storage temperatures, except 11°C (Figure 1B). This increase was accelerated at the highest storage temperatures of 25 and 32°C, accounting for an 80.3%- and a 105.6%-increase in the 12th month of storage, respectively, as compared to black beans in the initial treatment.

Similar results were found by Rani *et al.* (2013), which evaluated the effects of different storage temperatures (10-40°C) and moisture contents (12-20%) on pinto bean fat acidity. The authors reported an initial fat acidity of 7.9 mg KOH per 100 g of pinto bean, with a two-fold increase in the moisture contents of 16% and 18% after just 16 weeks of storage. An

increase in fat acidity was related to the increase in grain intrinsic lipase activity and/or to the increase in lipase activity of associated microorganisms. High moisture contents and temperatures favored lipase activity and, thus, the hydrolysis of lipids, liberating the free fatty acids (Kapchie *et al.*, 2013; Toci *et al.*, 2013).

Electric conductivity and solutes leaching

The electric conductivity and the leaching of solutes of black beans stored at 14% and 17% moisture content at different temperatures are presented in Figure 2C, 2D, 2E and 2F. According to Hentges *et al.* (1991), the electric conductivity indicated the levels of ions released from the grains to the water of hydration. These ions (mainly divalent cations) are a result of phytates' hydrolysis. During storage, phytates are redistributed in the middle lamella forming insoluble complexes with pectin. There was an increase (p ≤ 0.05) in the electric conductivity of black beans after 4 months of storage for those grains stored at 14% moisture content and 32°C (Figure 2C). Black beans at 17% moisture content showed a remarkable increase (p ≤ 0.05) in electric conductivity when stored at 25°C and 32°C during 4 months, compared to the initial treatment (Figure 2D). At 17% moisture content; and after 12 months of storage, grains maintained at 18, 25 and 32°C showed a higher (p ≤ 0.05) electric conductivity than in the initial treatment. Beans stored for 12 months at 17% moisture content exhibited a 321.5%- and a 388.1%-increase in electric conductivity when stored at 25 and 32°C, respectively. Similar results were found by Nasar-Abbas *et al.* (2008a) in faba beans (*Vicia faba* L.) stored for 12 months. The authors reported an electric conductivity of 1115 μS.cm⁻¹ and 2523 μS.cm⁻¹ in faba beans stored at 25°C and 37°C, respectively, which corresponded to increases of 105.0% and 305.1%, respectively, when compared to freshly harvested faba beans.

Freshly harvested black beans exhibited 24.0 mg of the leaching of solutes per 1 g of bean, regardless of moisture content (Figure 2E and 2F). The higher increases in the leaching of solutes , as well as that determined for fat acidity and electric conductivity, were observed in black beans stored at 17% moisture content and 32°C and 25°C. This corresponded to a 370.0%- and 398.7%-increases in the leaching of solutes after 12 months of storage, respectively, as compared to freshly harvested black beans. These results were similar to those determined by Berrios *et al.* (1999), which reported lower values for the leaching of solutes in black beans stored under hypobaric refrigeration conditions (10.5 mg of solutes
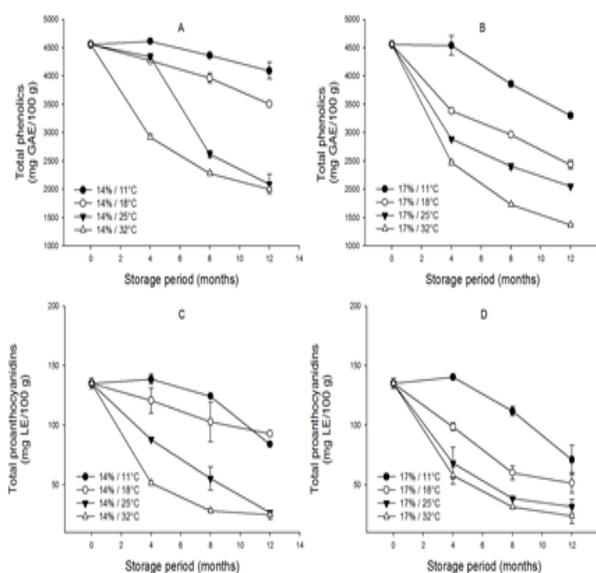

Figure 2. Total phenolic content (mg GAE/100 g) (A and B) and proanthocyanidins (mg LE/100 g) (C and D) of beans stored at 14% and 17% moisture content at different temperatures (11, 18, 25 and 32°C) during 12 months.

leaching per 1 g of sample) than in black beans stored at ambient air conditions (18.6 mg of solutes leaching per 1 g of sample). Nasar-Abbas *et al.* (2008a) found a significant (p ≤ 0.05) 215.1%- and 324.2%-increase in the leaching of solutes in faba beans (*Vicia faba* L.) stored for 9 months at 25 and 37°C, respectively. According to Berrios *et al.* (1999) and Nasar-Abbas *et al.* (2008a), increases in the electric conductivity and the values for the leaching of solutes occurred as a function of a cell disruption phenomena. The higher the cell disruption, the higher would be the electric conductivity and the leaching of solutes. The hydrolysis by phytase provided the release of cations such as Na⁺, K⁺, Ca²⁺ and Mg⁺² from the cell wall structure, increasing the electric conductivity and the leaching of solutes (Maria *et al.*, 2007). Some of these ions might react with pectic acids, forming insoluble pectates that hamper the water intake by grain and cell separation during cooking (Njoroge *et al.*, 2014).

Total phenolic compounds and proanthocyanidins

The total phenolic and proanthocyanidin contents in black beans stored at 14% and 17% moisture contents at different temperatures are presented in Figure 2. According to Luthria and Pastor-Corrales (2006) and Lin *et al.* (2008), the main phenolics found in black beans included the flavonoids anthocyanins 3-O-glucoside, delphinidin, malvidin and petunidin, and the phenolic acids named p-coumaric, sinnapic, caffeic and ferulic.

Freshly harvested black beans at 14% and 17% moisture content exhibited 4559 and 4561 mg of

GAE per 100 g of dry sample, respectively (Figure 2A and 2B). These results were higher than those found by Granito *et al*. (2008), which reported a total phenolics content of 1917 mg of TAE (tannic acid equivalents) per 100 g of sample in freshly harvested black beans (L-140 variety). For grains stored at 14% moisture content, a notable 34.5%-decrease (p ≤ 0.05) in total phenolics content was determined in the fourth storage month for grains maintained at 32°C, compared to freshly harvested black beans. In the eighth month of storage, grains stored at 14% moisture content and 25°C also exhibited a notable 41.2%-decrease (p ≤ 0.05) in the total phenolic content, compared to freshly harvested black beans; while the total phenolic content of black beans stored at 14% moisture content and at the lowest temperatures of 11 and 18°C exhibited a much lower trend to decrease during 12 months of storage (Figure 2A). The highest moisture content of 17% clearly intensified the reduction in the total phenolic content of black beans (Figure 2B). Even those grains stored at 17% moisture content and just 18°C presented a significant (p ≤ 0.05) 25.86%-decrease in the total phenolic content after 4 months of storage, compared to freshly harvested beans (Figure 2B). The greatest reductions in the total phenolic content at the 12th month of storage were determined in grains stored at the following conditions of moisture content and temperature: 14% and 25°C (54.1% decrease); 14% and 32°C (55.6% decrease); 17% and 25°C (55.0% decrease); and 17% and 32°C (70.0% decrease).

Granito *et al.* (2008) observed an 18.6%-decrease, a 46.7%-decrease, and a 72.3%-decrease in the total phenolic content in black beans after 150 days of storage at 30ºC/11% relative humidity, 50ºC / 11% relative humidity, and 50ºC / 80% relative humidity, respectively, as compared to freshly harvested beans. The decrease in the phenolic content during storage was attributed to the complexation of phenolics with cell wall constituents that hinder their extractability by a solvolytic solution. According to Acosta-Estrada *et al*. (2014), phenolic compounds possess a great reactivity with the cell wall structural components, such as cellulose, hemicellulose, pectin and proteins, that act as a physical and chemical barrier.

Freshly harvested black beans at 14% and 17% moisture content exhibited 135 and 132 mg of leucocyanidin equivalents per 100 g of dry sample, respectively (Figure 2C and 2D). Boateng et al. (2008) reported a total proanthocyanidin content of 51 mg of leucocyanidin equivalents per 100 g of black bean, respectively. Higher values observed in the present study for freshly harvested beans, compared to results presented by Luthria and Pastor-Corrales

(2006) and Boateng *et al*. (2008), might be due to genetic factors, such as environmental, maturation and post-harvest conditions. Similar trends described for the total phenolic content was determined for black bean proanthocyanidins, as shown in Figure 2C and 2D. After 12 months of storage, the greatest reductions in the proanthocyanidin content were determined in black beans stored with the following storage conditions: 14% moisture content and 25°C (59.7% decrease); 14% moisture content and 32°C (63.0% decrease); 17% moisture content and 25°C (57.2% decrease); and 17% moisture content and 32°C (71.3% decrease). Even at the lowest moisture content of 14% (Figure 2C) there was a remarkable decrease in the proanthocyanidin extractability of grains stored at 25ºC and 32ºC, showing that storage temperature exhibited an important role in the phenolic complexation with cell wall constituents during the long-term storage of black beans.

Similar results were found by Nasar-Abbas *et al*. (2008b), which studied the effects of storage atmosphere on the proanthocyanidin content of faba beans (*Vicia faba* L.). The authors reported a 65.8%-reduction in the proanthocyanidin content of faba beans stored for 12 months with an oxygen atmosphere at 12% moisture content and 30ºC. According to Stanley (1992), proanthocyanidins were present in a greater concentration in the seed coats of beans and could easily bind to cell wall components or other macromolecules, such as proteins, pectins and cellulose, when beans were stored at high moisture contents and/or high temperatures. This, in turn, reduced the proanthocyanidin extractability. Proanthocyanidins play an important role in preventing beans to oxidative stresses, such as the presence of light, oxygen and metal ions, which act mainly in the seed coat of the grain.

ABTS$^{•+}$ and DPPH$^{•}$ scavenging activities

The ABTS and DPPH radical scavenging activities from black beans stored at 14% and 17% moisture contents at different temperatures are presented in Figure 3. In general, there was a decrease in both the ABTS and DPPH radical scavenging activities of phenolic extracts obtained from stored black beans, compared to those extracts from freshly harvested black beans. This behavior could be expected, since the ABTS and DPPH radical scavenging activities are known as positively correlated to the total phenolic content of beans (Takahata *et al*., 2001; Granito *et al.*, 2008). In the present study, a positive correlation was determined between the total phenolic content and the ABTS$^{•+}$ scavenging activity (r^2 = 0.92, p ≤ 0.001), and between the total phenolic content and

the DPPH• scavenging activity ($r^2 = 0.99$, $p \leq 0.001$).

Freshly harvested black beans exhibited an ABTS•+ scavenging activity of 1963.4 mg of a Trolox equivalent per 100 g of dry sample and a DPPH• scavenging activity of 1412.9 mg of a Trolox equivalent per 100 g of a dry sample, regardless of the moisture content (Figure 3). The values obtained for the DPPH• scavenging activity were lower than the values observed for the ABTS•+ scavenging activity, which is a result of the chemical structure of these two free radicals. The ABTS•+ has, in its structure, a more unstable peroxyl radical, which reacts faster with antioxidants, while the DPPH• is a more stable nitrogen radical, which reacts slowly with the antioxidant, when compared to the peroxyl radical. This is mainly due to steric inaccessibility, where smaller molecules exhibit a better affinity for the site of radical (Prior *et al.*, 2005). Similar results of the ABTS•+ scavenging activity were reported by Açar *et al.* (2009), when studying the antioxidant activity of colored beans from different varieties. The authors reported an ABTS•+ scavenging activity of 1391.1 mg of Trolox equivalent per 100 g of dry sample for black bean sample. The black beans exhibited a high phenolic content; but during the storage, these molecules were degraded or complexed with structural components, such as proteins, pectins, cellulose and hemicellulose. These were partially extracted and less available for the ABTS•+ and DPPH• scavenging activities.

Cooking time and hardness

The cooking time and hardness of long-term stored black beans are presented in Tables 1 and 2, respectively. The cooking time of freshly harvested beans was 21.3 minutes. After 12 months of storage there was an increase ($p \leq 0.05$) in the cooking time for the black beans, compared to the initial treatment, regardless of moisture content and storage temperature. The moisture content of 17% promoted a notable influence in the cooking time, since the grains stored at 17% moisture content were classified as hard-to-cook (cooking time higher than 180 min) after 12 months of storage at 18, 25 and 32°C, while the 14%-moisture content-stored grains did not present grains classified as hard-to-cook during 12 months of storage even at the highest temperature studied (Table 1). Paredes-López *et al.* (1989) reported a 6 times-increase in the cooking time of beans stored at 40°C and 80% relative humidity for 135 days compared to cooking time of beans at the beginning of storage period. A greater increase in the beans' cooking time during storage was determined at higher storage temperatures. In the present study,
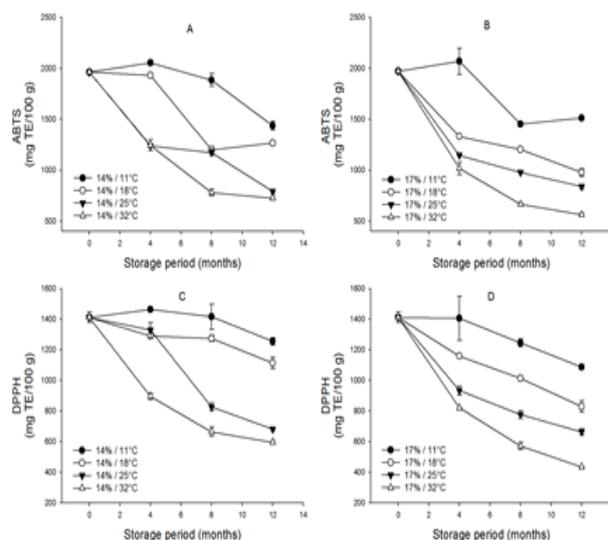

Figure 3. ABTS•+ (mg TE/100 g) (A and B) and DPPH• (mg TE/100 g) (C and D) scavenging activities of beans stored at 14% and 17% moisture contents at different temperatures (11, 18, 25 and 32°C) during 12 months.

it was clear that 17% moisture content favored the elevation in the beans' cooking time during storage, as compared to 14% moisture content.

The hardness of black beans represented the required force for deforming grain structure after the cooking process, when compressing 90% of grain height with a cylindrical probe. The hardness of freshly harvested black beans was 41.52 N, regardless of moisture content (Table 2). After 12 months of storage, an increase ($p \leq 0.05$) in bean hardness was observed for all studied storage conditions, since more pronounced in those grains stored at 25 and 32°C.

Among the major changes that occur in stored beans there are: (1) an increase in acidity; (2) a cell disruption and leakage of solutes; (3) a reduction in protein solubility; (4) a lignification of cell wall constituents, insolubilization of phenolic compounds and a seed coat hardening; and (5) changes in starch properties (Liu *et al.*, 1992; Maurer *et al.*, 2004; Shiga, 2004; Maria *et al.*, 2007; Njoroge *et al.*, 2014). Black beans have high amounts of proanthocyanidin and phenolic compounds. During the storage period this can be chemically or enzymatically oxidized, providing a greater rigidity to the cellular structure, thus reducing the water permeability (Stanley, 1992; Granito *et al.*, 2008; Nasar-Abbas *et al.*, 2008b;). According to Shimelis and Rakshit (2005) the reduced water permeability directly influences the hydration phase, and promotes the increase in cooking time and hardness of beans. Similar behavior was observed in the present study (Table 1).

Previous studies revealed a positive correlation

Table 1. Cooking time (minutes) of beans stored at 14% and 17% moisture contents
at different temperatures (11, 18, 25 and 32°C) during 12 months.

Treatment	Storage period			
	Initial	4 months	8 months	12 months
14% moisture content				
11°C	A 21.33±2.08 b[a]	D 30.00±2.65 a	C 33.67±0.58 a	D 31.67±4.16 a
18°C	A 21.33±2.08 d	D 31.67±2.52 c	C 41.33±3.21 b	C 73.67±3.21 a
25°C	A 21.33±2.08 d	B 45.67±4.16 c	B 72.33±4.93 b	B 90.33±2.52 a
32°C	A 21.33±2.08 c	A 76.33±2.08 b	B 67.67±6.81 b	A 133.67±5.51 a
17% moisture content				
11°C	A 21.33±2.08 c	CD 32.33±0.58 b	C 42.00±2.65 a	D 36.67±3.51 ab
18°C	A 21.33±2.08 c	BC 39.67±3.06 b	A 123.33±10.41 b	*
25°C	A 21.33±2.08 c	A 75.67±1.53 b	A 125.00±5.00 a	*
32°C	A 21.33±2.08 a	*	*	*

[a] Data with different capital letters in the same column and lowercase letters in the same line indicated significant differences (p < 0.05)
*Grains that presented cooking time over 180 minutes and classified as hard-to-cook.

Table 2. Hardness (N) of beans stored at 14% and 17% moisture contents at different
temperatures (11, 18, 25 and 32°C) during 12 months.

Treatment	Storage period			
	Initial	4 months	8 months	12 months
14% moisture content				
11°C	A 41.52±13.56 b[a]	E 44.44±13.30 b	E 44.40±11.70 b	E 57.77±12.09 a
18°C	A 41.52±13.56 b	CD 60.69±12.28 a	D 61.57±12.63 a	DE 67.13±13.78 a
25°C	A 41.52±13.56 d	C 68.58±12.32 c	C 78.37±7.69 b	C 90.91±16.52 a
32°C	A 41.52±13.56 c	B 93.70±20.59 b	B 98.33±16.09 b	B 118.24±21.39 a
17% moisture content				
11°C	A 41.52±13.56 c	E 48.24±10.67 bc	D 57.20±11.76 ba	E 63.31±18.53 a
18°C	A 41.52±13.56 c	DE 51.62±14.06 b	D 59.95±10.16 b	CD 80.47±11.61 a
25°C	A 41.52±13.56 c	B 82.65±14.18 b	B 89.36±9.63 b	B 105.64±14.81 a
32°C	A 41.52±13.56 c	A 122.39±11.82 b	A 129.43±10.69 b	A 142.75±14.06 a

[a] Data with different capital letters in the same column and lowercase letters in the same line indicated significant differences (p < 0.05)

between cooking time results obtained from the Mattson procedure and grain hardness determined in a texture analyzer (Wang *et al.*, 2003; Shimelis and Rakshit, 2005; Nasar-Abbas *et al.*, 2008). A positive correlation (r² = 0.83, p ≤ 0.001) was determined between the cooking time and hardness. According to Nasar-Abbas *et al.* (2008a), the reduced quality of the cooking and hardness of the grains was mainly attributed to a reduction in the hydration capacity, which had a negative correlation with solids and electrolytes leached, favored in drastic storage conditions. This behavior was also observed in the present study.

Conclusion

Even with the concern that bean legislation in many countries does not establish a maximum moisture content to be adopted for the marketing of beans, food industries may consider 14% moisture content and temperatures up to 18°C in order to properly maintain bean quality during long-term storage. This statement is supported by fat acidity, electric conductivity, the leaching of solutes, the total

phenolics, total proanthocyanidins, cooking time and the measurements of hardness. Not significant difference in proximate composition in function of moisture content and storage temperature. Both the 17% moisture content and the highest temperatures of 25 and 32ºC favored increase in fat acidity and changes in cell membrane, which was indirectly evaluated by electric conductivity and determinations of the leaching of solutes.

The extractability of the total phenolics and total proanthrocyanidins was reduced as a function of both grain moisture content and storage temperature, which is associated with their complexation with cell wall constituents. As a result of this decrease in the extractability of phenolics, the antioxidant capacity of phenolic extracts was also decreased, following a similar trend.

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