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# Analyses of technological and biochemical parameters related to the HTC phenomenon in carioca bean genotypes by the use of PCA

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ABSTRACT

Carioca beans are subjected to the hard-to-cook phenomena (HTC), which results in loss of technological and sensorial qualities. To better elucidate this postharvest hardening process, changes in technological and biochemical properties of grains during storage at 40 °C and 75% relative humidity were investigated, and the results were analyzed using Principal Component Analysis (PCA). Results showed that the hardening process occurs at different intensity for each genotype, and those more susceptible to HTC presented higher water absorption index (WAI) and peroxidase activity. During the storage period, oxidoreductases remained active and total phenol content increased for all genotypes. PCA explained 92.2% of total variance and grouped hardness, cooking time and WAI on PC1 (67.8%) and peroxidase activity and total phenol content on PC2 (24.4%). PCA showed that the technological parameter more related to the HTC is WAI and that hardness observed in genotypes during storage cannot be attributed to changes in the total phenol content nor to oxidorreductase activities.



## 1. Introduction

The deterioration of beans during storage is considered as any degenerative change after the grain has reached its maximum quality and many biochemical mechanisms are involved in these changes. The deterioration rate depends on the environmental, especially high temperature and relative humidity, on their own chemical components and physical conditions of the grains at the beginning of storage (Delfino & Canniatti-Brazaca, 2010). Furthermore, the sensitivity of the grains to the deterioration process has been attributed to genetic traits (Santos, Menezes, & Villela, 2004; Siqueira et al., 2014) especially because since 1997, the breeding programs in Brazil has focused on improvement of bean technological quality, which highly impacted the genetic diversity of these grains to this feature (Chiorato et al., 2010).

The principal deterioration that occurs during storage is the textural defect known as hard-to-cook (HTC) phenomenon, which affects bean and other legume seeds. The development of the HTC is characterized by changes that make beans more resistant to softening during cooking, with consequent modifications in texture and palatability (Martín-Cabrejas, Esteban, Perez, Maina, & Waldron, 1997).

Although the hardening of beans has been attributed to structural alterations in the cotyledons, the mechanisms involved in the HTC defect were not elucidated satisfactorily. A possible interpretation of the mechanisms participating in the HTC defect may be that both nonenzymatic and enzymactic reactions are concurrently and sequentially participating in events leading to toughness in cotyledon of stored beans (Liu, 1995).

The nonenzymatic hypothesis relate the hardening to changes in the reserve materials such as starch and protein. The main argument contained in this hypothesis is that the insolubilization of cell wall components limits the water uptake by the cells, reducing or delaying gelatinization of starch and denaturation of protein, thus reducing the turgor pressure which forces cells away from each other. The insolubilization of the middle lamella, in this case, also contribute to the phenomenon by increasing the cohesion between the cells (Shiga, Lajolo, & Filisetti, 2004).

On the other hand, the stress of the harvesting process results in rupture of the membranes that separates enzymes and substrates/

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co-substrates, enabling the start of reactions suggested to contribute to hardening, which include oxidation of polyphenols assisted by oxidoreductases (Rivera, Hohlberg, Aguilera, Plhak, & Stanley, 1989), removal of methyl groups from pectins by pectin-esterases (Liu, Phillips, & Hung, 1992), production of insoluble calcium pectate through the activation of phytase (Galiotou-Panayotou, Kyriakidis, & Margaris, 2008), hydrolysis of storage proteins by proteases (Hohlberg & Stanley, 1987) and, less likely, oxidation of lipids by lipoxygenases (Stanley & Aguilera, 1985).

Although the above hypotheses have been established on the hardening phenomenon, the research that supports them presents several missing points. The first point to note is that most of the studies on this topic (Galiotou-Panayotou et al., 2008; Hohlberg & Stanley, 1987; Shiga et al., 2004) have been conducted on a single genotype, which was subjected to an aging process under adverse/tropical environmental conditions, and the comparisons were made between fresh and aged material. Furthermore, most studies carried the biochemical analyses on flour prepared with whole bean, instead of using the cotyledons separately. Conflicting results have been also reported mainly due to variations in the genetic material and inherent errors or analysis method. In this light, there is some disagreement regarding the role of those biochemical components on beans' hardening.

In view of the above and that there are few studies about the contribution of oxidureductases to the hardening phenomenon, the objective of this research was to assess the behavior of four carioca bean with contrasting susceptibilities to HTC, along the storage under adverse conditions and to use Principal Component Analysis (PCA) to identify the relationship of technological and biochemical parameters to the HTC phenomenon.

## 2. Materials and methods

### 2.1. Plant materials

Carioca beans grains were obtained from the Bean National Breeding Program Gene Bank of Embrapa Rice and Beans, Santo Antônio de Goiás, GO, Brazil. The cultivars used were BRSMG-Madrepérola, BRS-Pontal, Pérola and the lineage CNFC10467 (the name of the cultivars are used hereafter without the prefix). These materials were cultivated under the same conditions and adequate irrigation. After harvest, grains of each genotype were subjected to natural drying and processing.

The aging of grains was conducted at 40 ± 5 °C and 75% relative humidity (RH) according to methodology of Ribeiro, Prudencio, Miyagui, and Ribeiro (2009). The analyses were performed at 0, 1, 2, 3 and 4 months of storage and carried out on bean cotyledon flour, except the determination of cooking time (CT) and hardness, which were conducted on whole grains. To prepare cotyledon flour, randomly selected raw grains (150 g) of each bean cultivar were manually dehulled and the cotyledons were ground (IKA® All basic, Germany) separately.

### 2.2. Chemicals

Catechol (1,2-dihydroxybenzene) was from Sigma–Aldrich (St. Louis, MO, USA). Tannic acid, hydrogen peroxide and Folin-Cicalteou reagent were from Vetec Quimica Fina Ltda (Duque de Caxias, RJ, BRA). All other reagents were of analytical grade and solutions were prepared with distilled water.

### 2.3. Physicochemical properties

#### 2.3.1. Cooking time (CT)

In order to determine the CT, whole grains were previously soaked (Plhak, Caldwell, & Stanley, 1989) and then submitted to the cooking at the Mattson Bean Cooker (MBC). CT was defined as the 50% cooked point, indicated by plungers dropping and penetrating 13 of the individual grains.

#### 2.3.2. Texture analysis

A TA-XTplus texture analyzer (Stable Micro System Ltd., Surrey, UK) was used for the textural analyses of whole cooked grains. The analysis employed was the return-to-start method, measuring force under compression with a 2 mm cylindrical probe, recording the peak of maximum force. Whole grains were axially compressed to 90% of its original height. Force-time curves were recorded at a speed of 5 mm $s^{-1}$ and the results were the average of 30 measurements expressed in Newton (N). Grains used in the test were soaked and cooked according to Siqueira, Vianello, Fernandes, and Bassinello (2013).

#### 2.3.3. Water absorption index (WAI)

The WAI was determined according to methodology described by Okezie and Bello (1988) 0.2 g of cotyledon bean flour was mixed with 10 mL of distilled water for 1 min and centrifuged at 3000 × g for 10 min. The WAI was calculated according to Equation (1):

$$\text{WAI}\ \left(\text{g g}^{-1}\right) = \frac{\text{weight of wet sediment}\ \ (\text{g})}{\text{weight of initial sample}\ \ (\text{g})} \qquad (1)$$

#### 2.3.4. pH and moisture

Moisture content was determined by drying 1.0 g of bean cotyledon flour at 105 °C until constant weight (AOAC, 2000).

pH was measured on a slurry prepared with 3.0 g of bean cotyledon flour in 100 mL of distilled water (IAL, 1985).

### 2.4. Biochemical parameters

#### 2.4.1. Enzyme assays

Crude extracts were prepared with 1 g of cotyledon flour and 5 mL of sodium phosphate buffer 0.1 mol $L^{-1}$ pH 6.0. The mixtures were left under stirring on an orbital shaker for 30 min at 4 °C, centrifuged at 10,000 × g and the supernatant used as source of enzymes.

The assays of peroxidase (POD) activity were done following the method of Halpin and Lee (1987) and the enzymatic activity of polyphenoloxidase (PPO) was determined according the methodology of Gomes, Oliveira, Carneiro, Barros and Moreira (2001). Catechol was used as substrate in both assays and one enzyme unit (U) defined as an increase of 0.1 absorbance unit per min.

#### 2.4.2. Extraction and analysis of total phenols

Crude extracts were prepared with 0.25 g of cotyledon flour and 5 mL of distilled water under stirring for 1 h at 25 °C. The mixture was centrifuged at 5000 × g for 15 min and supernatant used as a source of phenols.

The Folin-Ciocalteu assay was used to determine total phenol content in the cotyledon flours based on a method by Waterman and Mole (1994). The results were expressed in terms of tannic acid equivalent (mg $g^{-1}$ of bean flour).

### 2.5. Statistical analyses

All data were expressed as the mean ± standard deviation. Analysis of Variance (ANOVA) and Tukey's test were performed, and significant differences were reported when $p < 0.05$.

PCA was performed on the eight constituents analyzed in this

study. Only principal components with eigenvalues higher than 1.0 (Kaiser's rule) were retained. All analyzes were performed using Statistica 7.0 Software (StatSoft Inc. Tulsa, OK, USA).

## 3. Results and discussion

### 3.1. Physicochemical properties

Cooking is intended to render beans palatable, digestible, and also to inactivate antinutritional factors. It is a hydrothermal process involving starch gelatinization, protein denaturation, and texture changes that results in softening of the grains (Kinyanjui et al., 2015). Considering the CT of grains at harvest, it is possible to divide genotypes into three groups: CNFC10467 showed medium susceptibility to cooking (20.1 ± 0.5 min); Madrepérola and Pérola showed normal resistance to cooking (27.4 ± 0.6 min and 27.9 ± 2.0 min, respectively); and Pontal showed medium resistance to cooking (33.9 ± 2.0 min) according to the classification of Ramos Júnior, Lemos, and Silva (2005).

The extend of the effect of storage under high temperature and high humidity (HTHH) on CT varied with the genotype, but all stored beans required prolonged CT ($p < 0.05$) compared to freshly harvest beans (Fig. 1-A). After one month of storage Pontal distinguished itself as the most resistant to the cooking process. The other genotypes presented similar behavior of gradual increase, reaching about 55 min of CT at the end of storage period, which were 2 or 3 times higher than those of the fresh beans. Consistent with our data, previous researches (Carbonell, Carvalho, & Pereira, 2003; Siqueira et al., 2014) reported increases from 3 to 6 times in the CT for beans that were stored under conditions of HTHH. Those studies indicated CT increases during storage depending on genotypes and their analysis of variance revealed CT was dependent on genotype, storage time and their interaction.

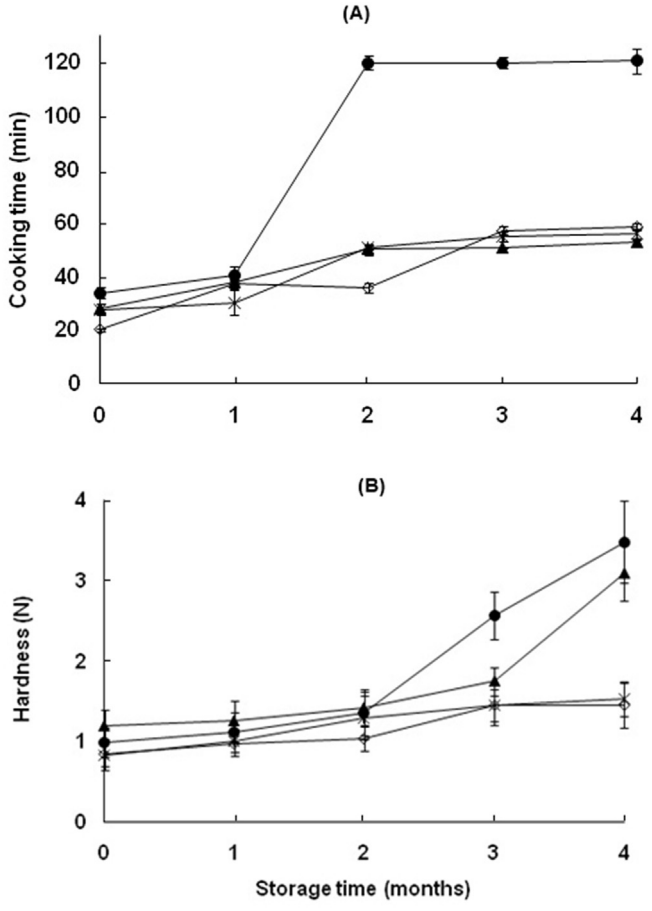


**Fig. 1.** (A) Mean cooking time (min; n = 3; ± error bars) and (B) hardness (N; n = 30; ± error bars) of cooked grains of different carioca bean genotypes along four months of storage at accelerated conditions (40 °C/70% RH). -◊- CNFC10467; -x- Madrepérola; -▲- Pérola; -●- Pontal.

Hardness of cooked grains accessed instrumentally at harvest ranged from 0.86 ± 0.2 N to 1.2 ± 0.2 N. Along storage all genotypes became significantly ($p < 0.05$) harder (Fig. 1-B). After two months of storage at HTHH conditions, hardness of Pérola and Pontal increased drastically, becoming 157% and 251% higher at the end of storage period, respectively. The pronounced increase in hardness of these genotypes is probably due to genetic characteristics of grains or due to their susceptibility in interaction of genetic and environmental factors (Carbonell et al., 2003).

Comparing the results of CT with those of hardness, it can be seen that instrumental texture analysis (ITA) could better distinguish genotypes susceptible to the HTC phenomenon (Pérola and Pontal) from those less susceptible (CNFC10467 and Madrepérola). Although CT determined in MBC is a trait evaluated by many breeding programs to access HTC tendency (Romero Del Castillo, Valero, Casañas, & Costell, 2008), it has been recently reported that it is not an adequate method to estimate cooking quality of beans (Siqueira et al., 2013). Therefore, this protocol is being progressively substituted by the ITA (Nasar-Abbas et al., 2008) due to its characteristics of fast, practical execution and high experimental accuracy. Aguilera, Hau, and Villablanca (1986) has already pointed that the force required to puncture individual grains in a ITA is a well accepted indicator of texture quality.

Moisture content is an important parameter affecting the quality of stored grains and the adequate value for long term storage is less than 15% (Rani, Chelladurai, Jayas, White, & Kavitha-Abirami, 2013). Moisture of cotyledon flour of fresh grains (Fig. 2-A) were similar for all genotypes (9.1 ± 0.2 to 9.8 ± 0.3 g 100 $g^{-1}$), except for Madrepérola (11.6 ± 0.1 g 100 $g^{-1}$). After the first month of storage moisture of cotyledon flour significantly ($p < 0.05$) decreased, and then increased gradually again. Rani et al. (2013) observed that grains with moisture contents of 16–20% showed a decrease in moisture content during the initial weeks of storage, irrespective of the storage temperature. This behavior must be due to balance between moisture content of grains and the environment in which they were stored.

Fresh grains diverged in pH values (Fig. 2-B). CNFC10467 remained with the higher pH values during storage period, in contrast to Pérola which remained with the lower pH values. Along the storage period the genotypes presented variations in the pH values, which should be due to formation and degradations of compound inside the grains. On the other hand, pH changes has been pointed to highly affect some functional properties, which should influence in the cooking quality (Aguilera, Estrella, Benitez, Esteban, & Martín-Cabrejas, 2011).

The use of legumes depends on their capacity to absorb water and soften sufficiently during soaking and cooking because failure to do so reduces quality in processed products (Ruiz-Ruiz, Dávila-Ortíz, Chel-Guerrero, & Betancur-Ancona, 2012). In relation to this technological property, Pérola and Pontal presented higher ($p < 0.05$) WAI compared to Madrepérola and CNFC10467 independently of storage period (Table 1). The differences in WAI among the genotypes should be due to their chemical composition mainly in the carbohydrate fraction. Pectin is present in seed legumes, mainly in the water-soluble form, which allows the absorption of water by the grains. The presence of insoluble pectin has been cited as a limiting factor to water inlet in bean grains (Yousif & Deeth, 2003; Ndungu, Emmambux, & Minnaar, 2011). Additionally,

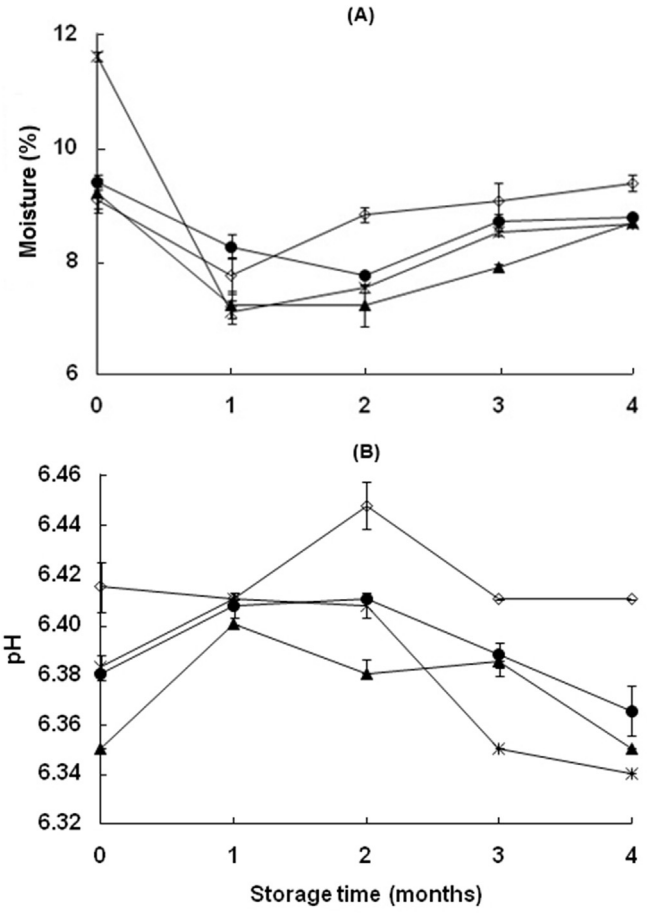


**Fig. 2.** (A) Moisture content (%; n = 3; ± error bars) and (B) pH (n = 6; ± error bars) of cotyledon bean flour of carioca bean genotypes along four months of storage at accelerated conditions (40 °C/70% RH). -◊- CNFC10467; -x- Madrepérola; -▲- Pérola; -●- Pontal.

the starch composition (amylose: amylopectin ratio), molecular structure of amylopectin (chain length, extent of branching and molecular weight), and granular architecture (crystalline and amorphous regions) of bean starch direct influence the water intake (Cereda, 2001).

Pontal reduced its WAI after the third month of storage and Madrepérola increased its WAI after the first month of storage. WAI of the other genotypes remained constant along all storage period, in agreement with the results of Delfini, De, and Canniatti-Brazaca (2008) who did not observe changes in WAI of carioca beans stored at ambient conditions for 6 months. Although Plhak et al. (1989) observed HTC black beans, obtained by storage for 7 months at HTHH (30 °C/80% RH) conditions, had significantly greater initial rates and final values of water absorption than control beans, stored at low temperature and low humidity (15 °C/35% RH), Carbonell et al. (2003) observed on their research that the correlation between hydration capacity with CT was not significant. They indicated that there is no direct relationship between the grain hydration capacity with the CT, suggesting that a particular cultivar may have low hydration capacity and this does not indicate the CT trend. However, it is worth mentioning that the above reports analyzed the WAI in whole grains and the WAI of this study was obtained from cotyledon flour.

**Table 1**
Water absorption index of cotyledon bean flour of different carioca bean genotypes along four months of storage at accelerated conditions (40 °C/70% RH).[a]

| Storage time (months) | Genotype | | | |
|---|---|---|---|---|
| | CNFC10467 | Madrepérola | Pérola | Pontal |
| 0 | $1.68^{aC}$ | $1.67^{bC}$ | $2.38^{aB}$ | $3.29^{aA}$ |
| 1 | $1.70^{aD}$ | $1.94^{aC}$ | $2.45^{aB}$ | $3.24^{aA}$ |
| 2 | $1.65^{aD}$ | $1.92^{aC}$ | $2.49^{aB}$ | $3.16^{aA}$ |
| 3 | $1.65^{aD}$ | $1.91^{aC}$ | $2.36^{aB}$ | $2.75^{bA}$ |
| 4 | $1.69^{aD}$ | $1.95^{aC}$ | $2.38^{aB}$ | $2.81^{bA}$ |
| SD[b] | 0.06 | 0.12 | 0.25 | 0.09 |

[a] Results are mean of three determinations. Data followed by the same capital letter in the row and the same lowercase letter in the column are not significantly different (Tukey's test, $p > 0.05$).
[b] Pooled standard deviation.

### 3.2. Biochemical parameters

In relation to biochemical parameters, in the beginning of storage period genotypes could be divided in a group with high total phenol content (Madrepérola and Pontal) and other with low total phenol content (Pérola and CNFC10467) (Fig. 3). Until the first month of storage, all genotypes significantly increased ($p < 0.05$) total phenol. After one month of storage, genotypes maintained their phenol content almost constant, except Pérola which increased ($p < 0.05$) this biochemical parameter until the third month of storage. The results corroborate those reported by Sievwright and Shipe (1986). who demonstrated that the measurable total condensed tannin and its fractions both increased until reaching a plateau and then declined in black bean stored at 30 °C and 40 °C and 80% RH when compared to control. Early research (Stanley, 1992) on black and white bean stored at 30 °C and 85% UR for one year proposed the increase in cotyledon polyphenols may be due to migration of tannins from the tegument to this part of the grain resulting in the formation of cell wall and middle lamella macromolecule-tannins complexes.

Many studies indicate the hardening of common beans may occur due to changes in polyphenols (Nasar-Abbas et al., 2008; Stanley, 1992). According to Martín-Cabrejas et al. (1997) there is alteration in the polyphenol content in the whole grain and that such changes may indicate postharvest physiological activity and formation of tannins and lignin by oxidation and polymerization of polyphenolic compounds in the grain. The formation of those compounds can be the result of a response initiated by stress-

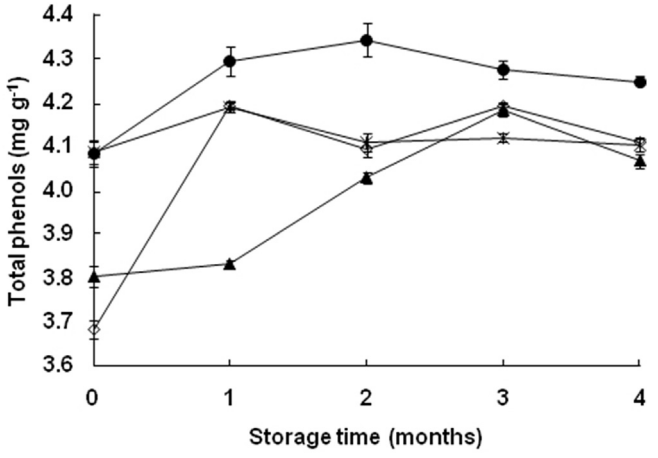


**Fig. 3.** Total phenol content (mg $g^{-1}$; n = 3; ± error bars) determined in bean cotyledon flour of different carioca bean genotypes along four months of storage at accelerated conditions (40 °C/70% RH). -◊- CNFC10467; -x- Madrepérola; -▲- Pérola; -●- Pontal.

adverse conditions, and could be responsible, at least in part, by HTC indices that develop in common beans during extended storage.

Besides the changes in the content of polyphenols, it has also been reported the involvement of certain enzymes in the HTC phenomenon of common beans. During the aging process, heat and high humidity could cause the activation of enzymes present in the cotyledon, triggering numerous reactions (Liu, 1995). Additionally, the deterioration of grains, determined primarily by the interaction between genetic inheritance, grain hydration degree and environmental temperature, favor the disintegration of the membrane system, altering the structure of the cell wall and middle lamella components (Santos et al., 2004).

Considering the possibility that enzymatic oxidation of polyphenols is one mechanism involved in the HTC phenomenon, PPO and POD were investigated. CNFC10467 presented the lower PPO activity ($p < 0.05$) along all storage period, followed by Pérola and Madrepérola (Fig. 4-A). These three genotypes presented PPO activity almost constant during storage, but Pontal presented a different behavior, exhibiting a peak of activity after one month of storage (4.7 ± 0.3 U).

POD activity was more intense than PPO in the four bean genotypes (Fig. 4-B) over the storage period. POD activity significantly increased ($p < 0.05$) until the first month of storage, excepted for CNFC10467, which kept this enzyme activity approximately constant along the storage period. After the first month of storage, the genotypes decreased POD activity, keeping a constant value until the end of the storage. Again, Pontal and Pérola presented the higher enzyme activities along storage period, about 2 and 3-fold higher than other genotypes, respectively. The results corroborate those of Hohlberg and Stanley (1987) who stored black bean for 10 months under different environmental conditions and observed POD activity in all bean cotyledon extracts, although no significant differences was noted among samples.

POD is thought to catalyze the polymerization of phenylpropanoid precursors of lignin (Abeles & Biles, 1991). Thus, the importance of this enzyme in bean hardening has been emphasized in the literature due to its involvement in the last step in lignin formation. Lignification hypothesis associate the development of hardening in common beans to polymerization of phenolic compounds coming mainly from teguments, mediated by oxidoreductases and by cross linking between phenolic compounds and proteins of the cell wall cotyledons (Nasar-Abbas et al., 2008).

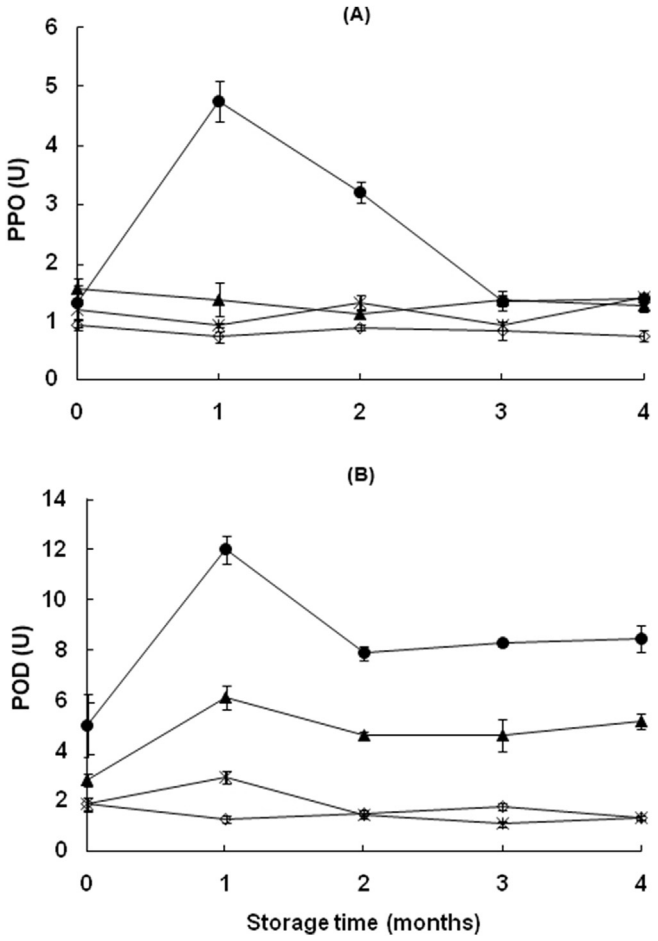


**Fig. 4.** (A) Polyphenoloxidase (U; n = 5; ± error bars) and (B) peroxidase (U; n = 5; ± error bars) activities of cotyledon bean flour of different carioca bean genotypes along four months of storage at accelerated conditions (40 °C/70% RH). -◊- CNFC10467; -x- Madrepérola; -▲- Pérola; -●- Pontal.

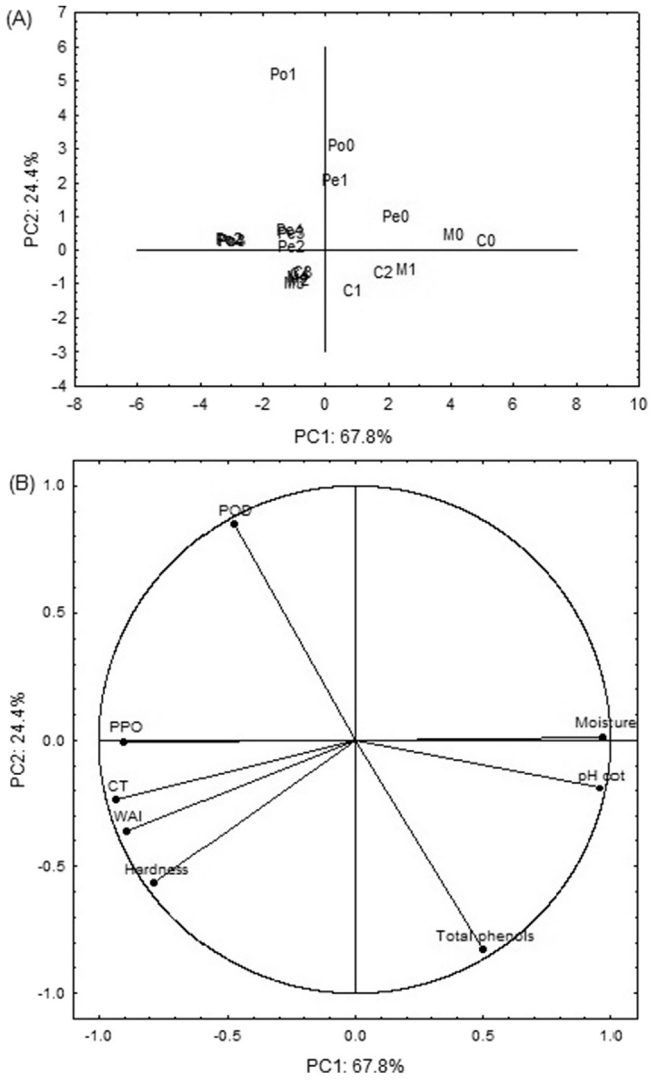


**Fig. 5.** (A) Score plot of PC1 versus PC2 from carioca bean genotypes stored at accelerated aging conditions. (B) Loading plot of PC1 versus PC2 from technological and biochemical parameters of carioca bean genotypes stored at accelerated aging conditions. (POD = Peroxidase; PPO = Polyphenoloxidase; WAI = Water Absorption Index; pH cot = cotyledon pH; Po = Pontal; Pe = Pérola; C = CNFC10467; M = Madrepérola; 0, 1, 2, 3 and 4 = months of storage).

**Table 2**
Correlation coefficients between technological and biochemical characteristics of carioca bean genotypes.[a]

| | Moisture | Total phenol | PPO | POD | pH cot | Hard | CT | WAI |
|---|---|---|---|---|---|---|---|---|
| Moisture | 1.00 | 0.43 | **−0.85** | −0.50 | **0.88** | **−0.74** | **−0.95** | **−0.87** |
| Total phenol | | 1.00 | −0.44 | **−0.90** | 0.66 | 0.03 | −0.24 | −0.16 |
| PPO | | | 1.00 | 0.38 | **−0.88** | 0.69 | **0.77** | **0.74** |
| POD | | | | 1.00 | −0.59 | −0.13 | 0.28 | 0.13 |
| pH cot | | | | | 1.00 | −0.67 | **−0.81** | **−0.77** |
| Hard | | | | | | 1.00 | **0.83** | **0.88** |
| CT | | | | | | | 1.00 | **0.92** |

[a] Significant correlations ($p < 0.05$) with absolute value > 0.70 are in bold. PPO = Polyphenoloxidase; POD = Peroxidase; pH cot = cotyledon pH; Hard = Hardness; CT = Cooking time; WAI = Water absorption Index.

Lignin serves as a matrix around the polysaccharides components of some plant cell walls, providing extra rigidity and compression strength, besides making the walls hidrophobic and water impermeable (Whetten & Sederoff, 1995).

Additionally, increase in POD activity due to adverse conditions indicate a protective role of the enzyme against stress (Lima, Brasil, & Oliveira, 1999). The plants, when subjected to stress, can modulate the defense response, such as increasing oxidoreductases activities in order to overcome stresses and return to normal metabolism (Soares & Machado, 2007). POD act as a barrier against the detrimental effects of stress by breaking down toxic substances generated like peroxides and phenolics (Lima et al., 1999).

### 3.3. Principal component analysis

PCA was performed on the standardized data to explore their underlying complex interrelationships, due to it often reveal previously unexpected associations among variables and thereby allows interpretation that would not be possible otherwise (Purcena, Di Medeiros, Leandro, & Fernandes, 2014).

PCA analysis generated two factors with eigenvalues exceeding 1.0 (Kaiser's rule) that accounted for 92.2% of the total variance. The first component (PC1) accounting for 67.8% of total variance was defined by moisture, WAI, hardness and CT. The second component (PC2, 24.4%) was influenced by total phenol content and POD activity.

The PCA showed three groups (Fig. 5-A). The first and second group corresponded to genotypes fresh harvest (T0, T1), and the difference between them is that the first is composed by Pontal and Pérola, while the second is composed by Madrepérola and CNFC10467. The third group formed a larger group than the others and its samples were quite similar to each other. It included all the genotypes stored for more than two months (T2, T3 and T4).

The loading plot of PC1 versus PC2 (Fig. 5-B) shows the main relationship between variables and principal components, and also highlights relationships between the variables themselves. The variables located on left-hand side (hardness, CT and WAI) correlated positively with the genotypes exhibiting the HTC defect (Fig. 5). This group also correlated negatively with moisture and cotyledon pH which were the variables responsible for the grouping of the fresh harvest Madrepérola and CNFC10467 at the right-hand side of PC1.

Correlation analysis showed similar observations (Table 2). Hardness was highly positively correlated with CT and WAI and negatively correlated with moisture and cotyledon pH. The relation of hardness to CT was expected, since CT is extensively used to estimate grain cooking quality in breeding programs (Romero Del Castillo et al., 2008), despite not being a sensitive and accurate method (Siqueira et al., 2013). On the other hand, the relation between WAI and hardness has been justified by Aguilera et al. (2009) to protein denaturation and also the carbohydrate content that could influence on the water holding. Jackson and Varriano-Marston (1981) found that intact beans stored at HTHH absorbed more water than the fresh beans. Hincks, McCannel, and Stanley (1987) hypothesized that membrane damage or deterioration is responsible for the increase in this parameter in stored beans.

According to correlation analysis (Table 2) and PCA (Fig. 5-B), total phenol content and POD are negatively correlated. In fact, polymerization of phenols within the cell walls has been suggested to be the major occurrence responsible for bean hardening (Martín-Cabrejas et al., 1997). Polymerization of phenols in the cell walls of plant tissue is believed to occur through the oxidative coupling of free phenoxy radicals, mediated by PPO or POD. Lignin or lignin-like polymers produced within the cotyledons would give greater rigidity and hydrophobicity to the cell walls, thus disallowing bean softening (Plhak, Staley, Hohlberg, & Aguilera, 1987). However, in this study total phenols and POD activity were shown not to be biochemical components directly related to the HTC phenomenon. By all indications, these parameters are most related to the defense against the oxidative stress of the natural aging process then to the hardening of grains. Plhak et al. (1987) studying the relationship between total extractable phenols and POD activity in whole black bean to the HTC defect also observed that high levels of this enzyme were not associated with storage conditions promoting HTC beans.

## 4. Conclusion

In conclusion, carioca bean genotypes behave differently along the storage at adverse conditions, proving that genetic variability should be considered during studies of the aging process. WAI is the property most related to the textural changes of beans, and, despite the POD activity and total phenol content of bean cotyledon have been negatively correlated, these parameters are not directly related to the grain hardening.

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