LWT - Food Science and Technology 69 (2016) 593–600

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Do enzymatic or non-enzymatic pathways drive the postharvest darkening phenomenon in carioca bean tegument?

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ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form
13 January 2016
Accepted 31 January 2016
Available online 2 February 2016

Keywords:
Colour
Oxidoreductase
Principal response curves
Total phenolics

ABSTRACT

Four genotypes of carioca bean were accelerate aged (40 °C/75% relative humidity) and evaluated to determine if differences in postharvest darkening trait was an enzymatic or non-enzymatic process. Chromaticity a* was the colour parameter with major alterations, and increase in chromaticity b* often masked the reddish of tegument. Peroxidase activity was not detected. Polyphenoloxidase remained active along the storage time, but its activity was higher in the lighter genotypes. Flavonoid, proanthocyanidin and total phenol content were much higher in darker genotypes, although just this last component presented significant alterations. Genotypes showed different susceptibilities to the darkening independent of phenolic content. Results of principal response curves analyse suggested different pathways for the darkening process: darkening in lighter genotypes seem to be mostly due to polyphenoloxidase activity while in dark ones there are the combination of enzymatic and non-enzymatic oxidation.

1. Introduction

Carioca bean (*Phaseolus vulgaris* L.) is a Brazilian market class characterized by a light brown tegument with brown stripes. This grain is widely consumed in Brazil, although storage represents a significant problem due to its susceptibility to the postharvest darkening (PD), which reduces its value and market opportunity. Consumers and processors associate dark colour with old grain and poor cooking quality (Nasar-Abbas et al., 2009). Thus, studies of PD became an additional tool to select new genotypes, focusing on improvement of bean with the lightest background cream colour (Lopes et al., 2011).

During storage, complex reactions are activated inside the grains, initiating the darkening phenomena (Marles, Vandenberg, & Bett, 2008). Various groups of phenolic compounds have been found to contribute to non-enzymatic and enzymatic browning in different foods, mainly by their involvement in oxidative steps and subsequent changes in the flavonoid skeleton (Pourcel, Routaboul, Cheynier, Lepiniec, & Debeaujon, 2006). Considering that bean tegument is also rich in those compounds, researchers suggest that they may contribute to PD by way of quinone formation or similar enzymatic-mediated reactions.

Preliminary genetic analysis of a common bean line (pinto class) that darkens considerably more slowly than other genotypes suggest that the slow-darkening (SD) trait is simply inherited. The presence of the recessive allele of the bean tegument colour gene *J* (syn. *L*) has been associated with reduced levels of PD, and chemical analyses have associated this gene with proanthocyanidin production in yellow beans (Beninger & Hosfield, 1999). Marles et al. (2008) studying those same bean lines observed that the regular-darkening (RD) trait is strongly associated with a higher polyphenoloxidase (PPO) activity as compared to the activity in the recombinant inbreeding lines (RILs) expressing the SD trait.

In spite of the above reports that established the enzymatic oxidation and polymerization of polyphenols as responsible for the PD in common bean, Lopes et al. (2011) found no correlation

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http://dx.doi.org/10.1016/j.lwt.2016.01.079
0023-6438/

between the darkening process and PPO activity in carioca bean. Additionally, conflicting results have been reported regarding the changes in the phenolic compounds (Beninger et al., 2005) considering that most works did not use cultivars with genetic variability for this trait. Thus, the study of PD of carioca bean might be useful to identify mechanisms related to the maintenance of the lighter background colour along the storage.

The objective of the present work was to determine the colour changes of four carioca bean genotypes along the storage, as well as to establish whether the differences in PD trait is an oxidoreductase-mediated phenomenon or a non-enzymatic process.

2. Material and methods

2.1. Plant materials

Carioca beans were obtained from the Bean National Breeding Program Gene Bank of Embrapa Rice and Bean, Santo Antônio de Goiás, GO, Brazil. The genotypes used, characterized as contrasting for the PD phenomenon (Siqueira, Pereira, Batista, Oomah, & Fernandes, 2014), were BRSMG-Madrepérola, BRS-Pontal, Pérola and CNFC10467 (hereafter, without the prefix). After harvest, grains were subjected to natural drying and processing.

Beans were aged in the dark on a hot air oven at 40 ± 5 °C and 75% relative humidity to accelerate the darkening process. The analyses were performed at 0, 1, 2, 3 and 4 months of storage. The colour measurement was carried out on the whole grains, while the biochemical analyses were conducted on bean tegument flour. For that, beans were manually dehulled and the teguments separated from the cotyledons. Teguments were ground in an analytical mill (IKA® A11 basic, IKA® – WERKE GmbH & Co., Germany) to obtain milled flour, which was stored at −18 °C until the analyses.

2.2. Chemicals

Catechol (1,2-dihydroxybenzene), rutin, cyanidin chloride and bovine serum albumin V (BSA) were from Sigma–Aldrich (St. Louis, MO, USA). Tannic acid, hydrogen peroxide and Folin-Ciocalteu reagent were from Vetec Química Fina Ltda (Duque de Caxias, RJ, Brazil). All other reagents were of analytical grade and solutions were prepared with distilled water.

2.3. Colour determination

Colour measurement of bean tegument was evaluated in a ColorQuest XE colorimeter (Hunter Lab, Reston, USA) equipped with diffuse light (illuminant D65, 10° view angle), in the reflectance mode and in the CIE L* a* b* colour scale. The colorimeter was calibrated with a standard reference having values of L*, a* and b* corresponding to 97.55, 1.32 and 1.41, respectively. In addition, chroma C* (Equation (1)) and total colour change ΔE* (Equation (2)) were calculated from the CIE L* a* b* scale.

$$C^* = \sqrt{\left(a_t^{*2} + b_t^{*2}\right)} \quad (1)$$

$$\Delta E^* = \sqrt{\left(L_0^* - L_t^*\right)^2 + \left(a_0^* - a_t^*\right)^2 + \left(b_0^* - b_t^*\right)^2} \quad (2)$$

Where L^*_0, a^*_0, b^*_0 are the initial colour measurements of bean teguments and L^*_t, a^*_t, b^*_t are the colour measurements at a pre-specified time.

2.4. Oxidoreductase activities

Extracts were prepared from 1 g of tegument flour and 5 mL of sodium phosphate buffer 0.1 mol L^{-1} pH 6.0 (for PPO extract, the buffer was added of 1% – w/v – polyvinylpyrrolidone and 0.1% – w/v – sodium dodecyl sulfate), mixed and left under stirring for 30 min at 4 °C. The mixtures were centrifuged (Quimis Q222T, São Paulo, Brazil) at 10,000 × g and the supernatant was used as source of enzymes.

The assay of POD was done following the method described by Halpin and Lee (1987). In test tubes, it was added 50 μL of the crude extract and 1.2 mL of 0.1 mol L^{-1} catechol solution prepared in sodium phosphate buffer (0.1 mol L^{-1} pH 6.0). The reaction was started by the addition of 250 μL of hydrogen peroxide 0.05 mol L^{-1} and processed for 1 min at 25 °C. The absorbance was recorded at a spectrophotometer (BELphotonics 2000 UV) at 380 nm, and one enzyme unit (U) defined as an increase of 0.1 absorbance unit per min.

The enzymatic activity of PPO was determined according to the methodology described by Gomes, Oliveira, Carneiro, Barros, and Moreira (2001). 930 μL of catechol solution (80 mM) prepared in sodium phosphate buffer (0.1 M, pH 6.0) were added to 70 μL of crude extract. The reaction was performed at 25 °C for 1 min and after read at a spectrophotometer (BELphotonics 2000 UV) at 420 nm. One U was defined as an increase of 0.1 in absorbance per min of reaction.

Protein content of the enzyme extracts was determined by the biuret method (Gornall, Bardawill, & David, 1949) using BSA as standard. Specific activity of each enzyme was calculated by the relation of enzymatic activity and the amount of protein in the extract sample.

2.5. Flavonoid and proanthocyanidin determination

For determination of total flavonoid and proanthocyanidin content, bean tegument (0.5 g) was extracted at room temperature twice with 10 mL of a 80% (v/v) aqueous methanol (15 min) and then with 5 mL of the same solution (10 min) in an ultrasonic bath (Branson 2210, Connecticut, USA). After each extraction, the mixture was centrifuged at 2000 × g for 15 min and supernatant extracts combined and transferred to a 25 mL volumetric flask.

Flavonoid content was determined by the $AlCl_3$ method modified from the Pharmacopoeia Helvetica (Petry, Souza, Basani, Petrovick, & González-Ortega, 1998). 1 mL of 12% (v/v) $AlCl_3$ solution was added to 5 mL of crude extract, and the volume completed with 5% (v/v) acetic acid to 25 mL in a volumetric flask. After 30 min at 25 °C, the absorbance was read at 422 nm (Beckman DU-70 spectrophotometer). Total flavonoid content was expressed as rutin equivalent.

Proanthocyanidins present in crude extracts were determined according to the method described by Porter, Hrstich, and Chan (1986). 6 mL of the *n*-butanol/HCl reagent (950 mL of *n*-butanol and 50 mL concentrated HCl), 1 mL aliquot of the extract, and 0.2 mL of the iron reagent (2% – w/v-ferric ammonium sulphate in 2 mol L^{-1} HCl) were added o a 10 mL screw cap tube and contents vortexed. The tube was capped loosely, and placed in a boiling water bath for 50 min. Then, the tube was cooled and the absorbance at 550 nm was recorded using a Beckman DU-70 spectrophotometer. Cyanidin chloride (Sigma Aldrich Co.) was used to construct the standard curve.

2.6. Total phenolics determination

For total phenol content (TPC), crude extracts were prepared with 0.25 g of tegument flour and 5 mL of distilled water under

stirring in an orbital shaker (1 h/25 °C). The mixture was centrifuged at 5000 × g for 15 min and the supernatant used as source of phenols.

Folin-Ciocalteu assay was used to determine TPC in bean tegument extracts (Waterman & Mole, 1994). 0.5 mL extract samples were added to 0.5 mL of a 10% (v/v) Folin-Ciocalteu reagent. After 3 min, 0.5 mL of a 8% (v/v) sodium carbonate solution were added, and the contents vortexed. The absorbance was recorded at 760 nm at a spectrophotometer (BELphotonics 2000 UV) after 2 h of incubation at 25 °C. The results were expressed in mg of tannic acid equivalent per g of tegument.

2.7. Tegument pH determination

pH was measured on a slurry prepared with 0.3 g of bean tegument flour in 10 mL of distilled water.

2.8. Statistical analyses

All tests were conducted according to a completely randomized design considering separately each genotype. The experiments were performed at least in three replicate and results were expressed as mean ± standard deviation. Analysis of variance (ANOVA), Tukey's test and Pearson correlation were performed using the program Statistica 6.0 (StatSoft Inc., Tulsa. USA), with a significance level of 95%.

In order to analyse differences in TPC, colour parameters and PPO activity among the data sets from the bean genotypes, a multivariate Principal Response Curve (PRC) method was performed using the Canoco software package (Ter Braak & Šmilauer, 2012).

For the PRC analysis, two tests were performed to show the significance of treatment effects (Ter Braak & Šmilauer, 2012). Firstly, a Monte Carlo permutation test on whole time series (999 permutations under reduced model), following the PRC analysis ($p < 0.05$). This test assesses whether PRC explains a significant part of the treatment variance. Secondly, to verify which treatments resulted in a significantly altered chemical composition, Monte Carlo permutation tests (499 permutations), restricted the data to each sampling time.

PRCs were calculated on log(x+1)-transformed, centred and standardized data prior to analysis to obtain similar weight.

3. Results and discussion

3.1. Colour determination

Genotypes studied presented different tegument colours at harvest and all of them changed its colour after the storage time (Fig. 1). Considering that the four genotypes were grown in the same area and under the same climatic, soil conditions and crop management, the contrasts in tegument colour can be related to genetic characteristics (Araújo, Ramalho, & Abreu, 2012) since more than 18 genes are involved in the background colour, patterns of spots and stripes of bean tegument (Gepts & Debouck, 1991).

At harvest, L* varied according to genotype: 58.1 ± 1.0 for CNFC10467; 55.3 ± 1.9 for Madrepérola; 49.4 ± 1.6 for Pontal and 50.6 ± 1.6 for Pérola (Fig. 2-A). Cultivars with L* values exceeding 55 have a higher market value, although some authors use scores higher than 53 as standard value of L* (Ribeiro, Storck, & Poersch, 2008).

All studied genotypes gradually reduced their luminosity, becoming darker at the end of the storage period (Fig. 2-A). Along the storage time, genotypes CNFC10467 and Madrepérola were the lighter ones (higher L* values), while Pontal and Pérola showed lower L* values. Despite this division, the reductions in this colour parameter was equivalent for CNFC10467, Pontal and Pérola in about 12% L* reduction, and lower to Madrepérola (7.4%).

Chromaticity a*, which determines the reddish component, was the colour parameter with major alterations (43–77%) during storage (Fig. 2-B). The higher variation was observed for CNFC10467, which presented 77% increase in chromaticity a*. In general, the darker genotypes, Pontal and Pérola, presented the highest a* values, which suggest that this parameter should be considered in the colour evaluation of beans during PD studies as much as L*. In this respect, Nasar-Abbas et al. (2009) reported that over time faba bean decrease L* but increase a*, demonstrating that beans become darker reddish-brown. Díaz, Caldas, and Blair (2010) associated commercial classes of beans with red tegument to high anthocyanin content (0.15–0.21 g 100 g^{-1}) while Beninger and Hosfield (2003) associated the red colour due to presence of procyanidins.

Chromacity b*, ranging from blue to yellow, was also higher in all genotypes after storage (Fig. 2-C), although the variation in this parameter was lower than L* and a*. Along the storage, Pérola was the genotype with the higher b* values. Of great interest was the behaviour of CNFC10467, which presented the more pronounced changes, with about 36% increase in b* value compared to 11% increase in the other genotypes. Beans with yellow tegument or yellow in combination with other colour have been associated with low content of anthocyanins (Díaz et al., 2010) and tannins (Caldas & Blair, 2009).

C* values (Fig. 2-D), which indicate colour saturation, were higher for Pérola (20.9–24.9), followed by Pontal (20.0–23.7). This result was expected, since these genotypes are the darker ones. Nevertheless, the highest changes in C* were observed in CNFC10467, changing from 15.8 to 22.3.

Total colour change is provided by a joint evaluation of L* and chromaticities a* and b*. Substantial colour changes were found during storage, especially for CNFC10467 (9.5 ± 1.2), Pérola (8.2 ± 1.1) and Pontal (7.4 ± 2.3).

Although CNFC10467 was one of the genotypes with minor visual colour changes and remained light during the storage, it is important to note that when analysing the colour parameters, this genotype was the most susceptible to PD. However, the reduction of L* and increase of a* values have certainly been masked by a substantial and concomitant increase in b*, resulting in a visual masking of its PD and, consequently, featuring this genotype as a lighter background colour tegument, even after accelerated aging. On the other hand, Pérola had the highest a*, b* and C* values but was visually lighter than Pontal, probably due to its high yellowness.

Among the genotypes analysed, Madrepérola was the one with minor total colour changes, ΔE* 5.3 ± 2.1. This genotype has already been pointed as a grain with a very light cream background colour, persisting for a long time (Araújo et al., 2012). However, the maintenance of the lighter colour in Madrepérola is a consequence of minor colour alterations and not due to a combination of changes in the colour parameters resulting in the masking of PD, as occurred in CNFC10467. Colour similarities of these two lighter genotypes are high clarity and low redness of the grains at harvest. Therefore, in the present study the genotypes with lighter tegument as well as genotypes with darker tegument remained so throughout the storage time.

3.2. Oxidoreductases activities

Causes of colour changes in bean teguments are still poorly understood. However, researchers (Marles et al., 2008; Rios, Abreu, & Corrêa, 2002) attribute enzymatic oxidation as the possible

Fig. 1. Bean grains of genotypes freshly harvested (top row) and after four months of storage (bottom row) under accelerated aging conditions of 40 °C/75% relative humidity (from left to right: CNFC10467, Madrepérola, Pontal and Pérola).

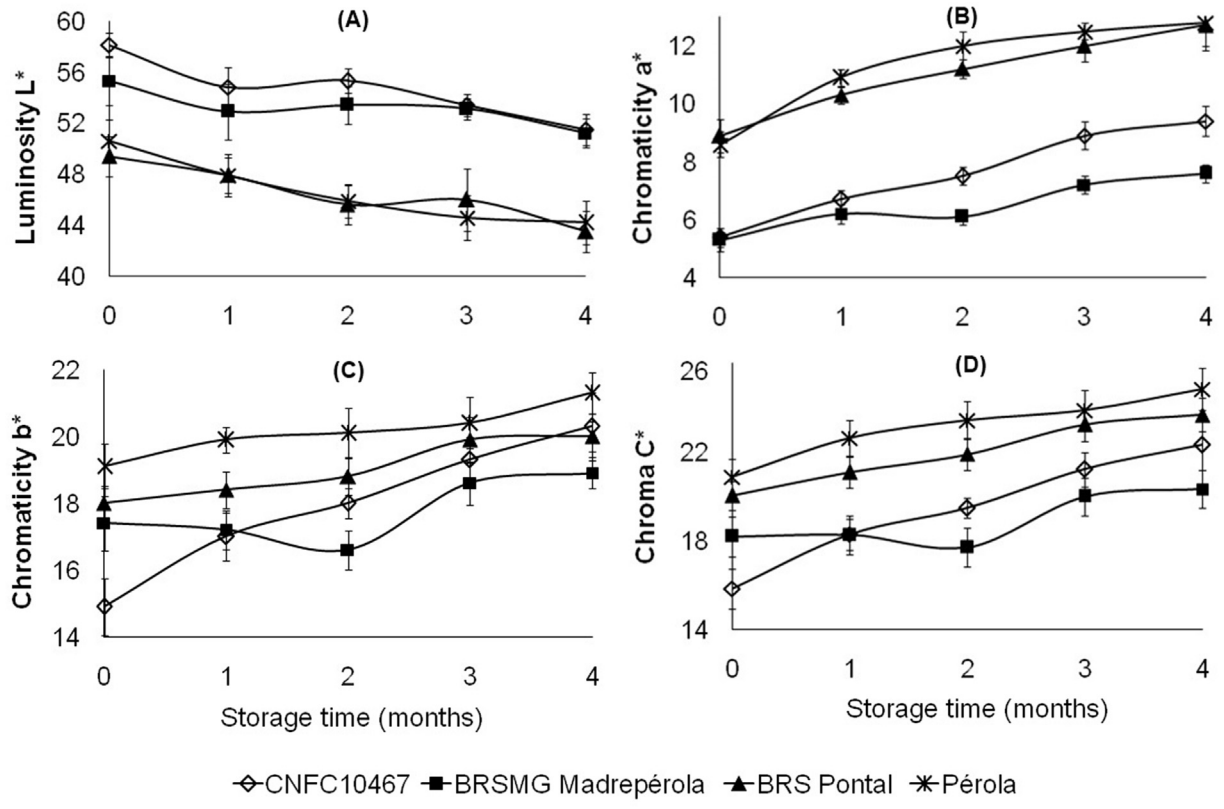

Fig. 2. Mean (n = 30; ± error bars) luminosity L* (A), chromaticity a* (B), chromaticity b* (C) and choma C* (D) of tegument of different carioca bean genotypes along four months of storage at accelerated conditions (40 °C/75% relative humidity). -◊- CNFC10467; -■- Madrepérola; -x- Pérola; -▲- Pontal.

darkening mechanism, due to formation of quinones. As grains age, the physical barrier that separates enzymes and substrates are lost, as a result of cell damage, enabling the oxidation of phenolic compounds by oxidoreductases (Pourcel et al., 2006).

POD activity was not detected in the tegument of the genotypes studied. Apparently, this result is inconsistent with those of Moura, Abreu, Santos, and Corrêa (1999) who studied three carioca genotypes and observed that those with darker tegument presented about 3-fold POD activity in comparison to the lighter ones and also observed a slight increase in POD activity after the storage period. Rios et al. (2002) also detected POD activity in three cultivars of common bean and this enzyme remained active over time. However, the above experiments were carried on the whole grain, which have the cotyledon as contaminant. It is probable that function of POD isoforms present in cotyledon are different from those in the tegument, and hence, their activation and inactivation profile are subjected to different routes of regulation.

Analysing the whole grain, Moura et al. (1999) observed that the cultivar with darker tegument presented higher PPO activity before and after storage, while the lineage with lighter tegument presented lower results of enzymatic activity. Marles et al. (2008) also observed that RD trait is strongly associated with a higher PPO activity on whole grain as compared to the activity in the RILs expressing the SD trait. Conversely, in this study PPO activity analysed separately in tegument was higher in CNFC10467 and Madrepérola (lighter tegument) and lower in Pontal and Pérola (darker tegument) during the complete aging process (Fig. 3-A). It is important to analyse the tegument separately, since this part of grain is the main modulator of interactions among the grain internal structures and the external environment, and thus, presents different regulation pathways from those of cotyledon.

Independent of the genotype, there was a tendency to increase PPO activity over time, despite no association was found between this enzyme activity and the L* values of the teguments. Though, these results suggest that PPO is not responsible for reducing the lightness of the grains. Lopes et al. (2011) studying 18 genotypes of carioca bean along the storage also observed that PPO activity in tegument is not directly associated with the PD phenomenon, due to the low magnitude of the scores in the correlation analysis between both factors ($r < -0.5$).

Parameters a*, b* and C* presented different significant ($p < 0.05$) correlations responses among the genotypes. However, considering correlation coefficients higher than $r = 0.60$, only CNFC10467 presented association of PPO activity and a* ($r = 0.81$), b* ($r = 0.78$) and C* ($r = 0.80$). It is important to highlight that PPO may be the principal responsible for the PD process of CNFC10467, causing its yellowish colour.

3.3. Phenolic compounds

Phenols are major contributors to tegument colour and pigment distribution or intensity (Caldas & Blair, 2009). Tegument colour in common bean is determined by activity of the flavonoid biosynthetic pathway resulting in the presence or absence of specific anthocyanins, condensed tannins and glycosidic flavonols (Díaz et al., 2010).

Flavonols may contribute to colour changes after quinone formation or similar PPO-mediated reactions, after a period of storage time (Marles et al., 2008).

The content of flavonoids in Pontal and Pérola was 2 or 3-fold as compared to CNFC10467 and Madrepérola (Fig. 3-B) independent of the storage time. Additionally, minor alterations were observed in the tegument flavonoid content along the storage. Similar results were observed by Beninger et al. (2005) in CDC Pintium, a RD genotype, which both aged and nonaged had 2-fold higher total flavonols compared with the 1533-15 SD genotype.

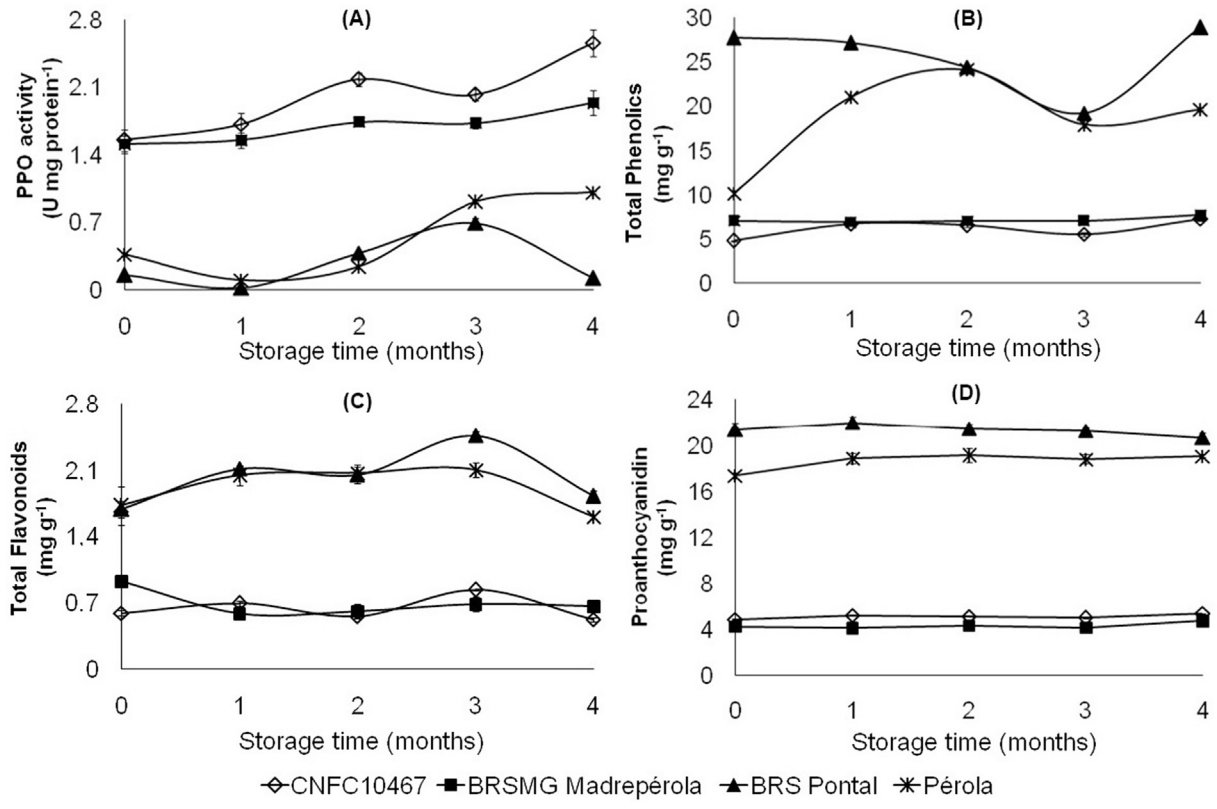

Fig. 3. Mean ($n \geq 3$; ± error bars) specific activity of polyphenoloxidase (A), total flavonoid (B), proanthocyanidin (C), and total phenol content (D) of tegument of different carioca bean genotypes along four months of storage at accelerated conditions (40 °C/75% relative humidity). -◊- CNFC10467; -■- Madrepérola; -x- Pérola; -▲- Pontal.

In general, carioca beans contains high levels of kaempferol glycosides (Ranilla, Genovese, & Lajolo, 2007), besides other flavonol glycosides, such as quercetin 3-O-glucoside and myricetin 3-O-glucoside (Mojica, Meyer, Berhow, & Mejía, 2015). However, some differences related to flavonols were reported, for instance, myricetin 3-O-arabinoside occurred in Pérola beans, whereas daidzin was detected only in Pontal cultivar. Additionally, the anthocyanins, petunidin glucoside and malvidin glucoside, were quantified in Pérola cultivar in very low amounts, 0.007 mg/g and 0.0014 mg/g tegument, respectively, but they were not identified in Pontal (Mojica et al., 2015).

Beninger and Hosfield (2003) isolated and identified flavonoids that contribute to colour in dark red kidney beans. Three yellow flavonol glycosides were characterized, but the major contribution to their red colour is due to the presence of procyanidins, also known as condensed tannins, and comprised of oligomers and polymers of flavan-3-ol subunits linked mainly through B-type linkages (Chen et al., 2014). They play an important role in the defence system of grains exposed to oxidative damage caused by environmental factors such as light, oxygen, free radicals and metal ions (Nasar-Abbas et al., 2009). Additionally, the oxidation of proanthocyanidins has been suggested as one of the causes of tegument PD (Beninger et al., 2005).

Proanthocyanidin content in Pérola and Pontal tegument was similar to the content reported by Díaz et al. (2010) in common beans (20 mg g^{-1}) and it was 4–5-fold higher than the content of proanthocyanidin present in CNFC10467 and Madrepérola tegument (Fig. 3-C). Ranilla et al. (2007) studied common beans with light brown tegument and observed that cultivars with the highest TPC had the highest tannin levels too. Independent of the genotype studied, the proanthocyanidin content remained constant along all the storage time. Beninger et al. (2005) also observed that aged and nonaged CDC Pintium teguments (RD) had approximately 3 times the concentration of proanthocyanidin as compared to 1533-15 (SD) and that, after aging, neither line showed a significant reduction in the amount of total proanthocyanidins.

Some differences in the amounts of monomeric proanthocyanidins, catechin and epicatechin, for Pontal and Pérola cultivars were detected by Mojica et al. (2015). The authors quantified those compounds by LC/MS and their results showed higher amounts of both flavan-3-ol in Pontal teguments (3.11 and 12.48%, catechin and epicatechin respectively) compared to Pérola cultivar (1.75 and 9.73%). It is worth to note that the amount of epicatechin was about four times higher than catechin in both cultivars (Mojica et al., 2015).

TPC (Fig. 3-D) was higher in Pontal and Pérola (darker tegument) and lower in CNFC10467 and Madrepérola (lighter tegument), inversely to the results of PPO activity (Fig. 3-A). Initially, Pontal presented the higher TPC, and kept with a high value along almost all the storage period. Pérola significantly increased ($p < 0.05$) the TPC along the storage, while CNFC10467 and Madrepérola presented minor alterations along the storage time.

Variations in TPC for Pontal and Pérola could be mainly due to the phenolic acids present in the tegument of both cultivars. Phenolic acids as chlorogenic acid, vanillic acid and *o*-coumaric acid expressed as % of total area under the curve have been quantified in Pontal, 6.06%, 3.18%, 0.23%, and Pérola 4.45%, 3.58%, 0.28%, respectively. Syringic acid was detected only in Pontal tegument, 11.9% (Mojica et al., 2015).

The above results demonstrate that phenols are an adequate biochemical parameter to distinguish lighter from darker bean genotypes. However, the content of those compounds is not a sufficient parameter to identify genotypes most prone to dark, since CNFC10467 and Madrepérola present similar TPC, flavonoids and proanthocyanidin, but are contrasting in the susceptibility to the PD. Additionally, Pontal present higher TPC, total flavonoid and proanthocyanidin than Pérola, but this last genotype had higher chromaticities a*, b* and C*. Therefore, the phenolic profile of bean tegument should be a more effective indicator of PD tendency, as phenolic compounds are related to different colour pattern, which may change according to the biochemical reactions they undergo.

The colour of phenolic compounds may also be influenced by pH, as oxidation rate by PPO is pH dependent (Jiménez & García-Carmona, 1999). Tegument pH of the genotypes significantly decreased ($p < 0.05$) over accelerated aging from about 6.2 to 5.7 (Fig. 4). The reduction in tegument pH was partially due to variation on phenolic acids concentration, as shown by the significant negative correlation ($p < 0.05$) between TPC with pH, mainly for Pontal and Pérola cultivars ($r = -0.82$ and $r = -0.87$, respectively).

3.4. Principal response curves

PRC, a time-dependent multivariate analysis, was conducted to find patterns in the changes of the phenolic constituents, colour parameters and PPO activity of bean genotypes during the accelerated aging process. PRC diagrams explained 97.7% of the variance and were highly significant (PRC1: 95.5%, $F = 967$, $p = 0.002$; PRC2: 2.2%, $F = 40.7$, $p = 0.002$).

Some variable weights (b_k) presented next to the PRC-1 diagram (Fig. 5-A) were positive and others were negative. The greater the variable weight the more the response for that variable resembles the deviation pattern (from the average, Cdt = 0 line) indicated on the PRC; negative weights show an opposite pattern. Variables weighing from −0.5 to +0.5 either show no response or a response that is unrelated to the pattern shown by the PRC (Van den Brink & Ter Braak, 1999). Therefore, proanthocyanidins, TPC and PPO activity, which had the highest weight values, were mainly responsible for the distinction of two groups of genotypes: Pontal and Pérola from CNFC10467 and Madrepérola. However, even Pontal and Pérola showed divergent pattern in most months. Flavonoids and chromaticity a* also contribute, but are less representative of PRC's deviation pattern.

The second axis explained 2.2% of the variance (Fig. 5-B). However, significant variations on PPO activity, proanthocyanidin and TPC make distinctions between Pérola and Pontal, and also between CNFC10467 and Madrepérola, suggesting that oxidative processes of phenolic compounds linked to PPO activity may contribute to different pattern of colour changes of these genotypes.

The above results reinforce the idea that PD phenomenon is

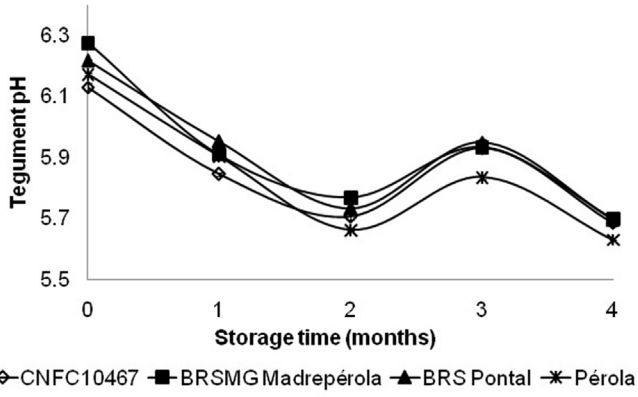

Fig. 4. Mean tegument pH (n = 6; ± error bars) of carioca bean genotypes along four months of storage at accelerated conditions (40 °C/75% relative humidity). -◊- CNFC10467; -■- Madrepérola; -x- Pérola; -▲- Pontal.

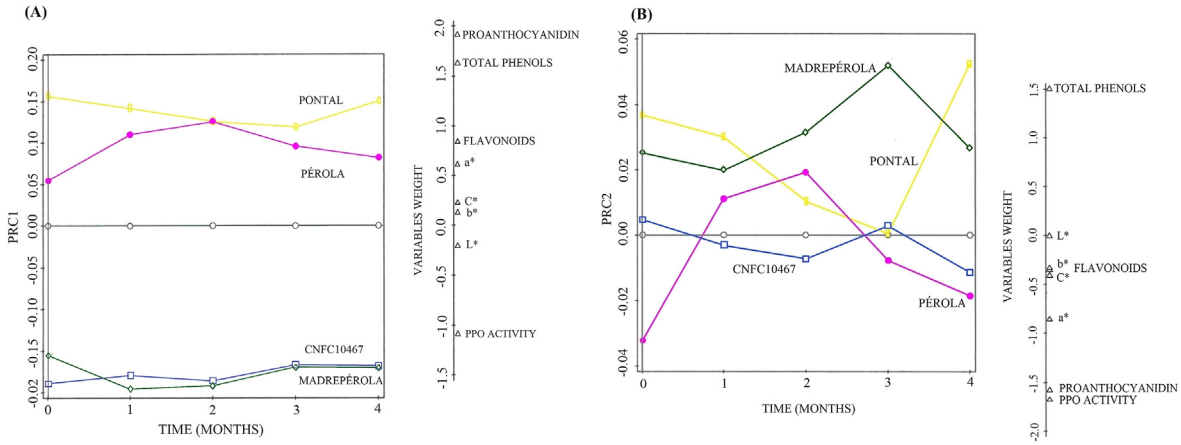

Fig. 5. First (A) and second (B) components of the principal response curves of genotypes data set.

mainly related to the phenolic constituents profile of the genotypes tegument and PPO activity. Quantitative differences in the amounts of phenolic compounds available for oxidation reactions are an important factor; however, the type of oxidation reaction, enzymatic or non-enzymatic, and the products formed will affect the final colour of the grain. Reddish-brown pigments are usually formed by proanthocyanidin oxidation, whereas yellowish pigments are a result of dimerization of two flavonoids or formation of flavan-3-ol-flavonol adducts, such as catechin-kaempferol dimer (Beninger et al., 2005). High amounts of yellow pigments increase chromaticity b* and mask the red colour, as occurred for Pérola and CNFC10467 genotypes.

These facts prove that there are different pathways to PD. In the case of CNFC10467 and Madrepérola, the darkening seem to be mostly due to PPO activity, but in the other genotypes there is a combination of enzymatic and non-enzymatic oxidation, mainly in the case of Pérola. Specific phenolic compounds present in the tegument play an important role as the substrate for the oxidation reactions and the type of pigments, which will determine the final colour of the beans. Further studies are needed to fully understand how differences in phenolic profile influence the colour changes during the PD.

4. Conclusions

From the results obtained, it can be concluded that there are different pathways for the postharvest darkening phenomenon among the studied genotypes. The postharvest darkening in some genotypes seems to be predominantly due to the polyphenoloxidase activity, whereas in others there are a combination of enzymatic and non-enzymatic oxidation. An important finding of this study was that the susceptibility of the genotypes to the postharvest darkening phenomena are not related to the lightness of its tegument, but it is mainly related to the phenolic constituent profile and PPO activity. Quantitative differences in the amounts of phenolic compounds available for oxidation reactions are an important factor; however, the type of oxidation reaction, enzymatic or non-enzymatic, and the products formed will affect the final colour of the grain.

Acknowledgments

Siqueira B. S. would like to acknowledge Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the fellowship support. The authors thanks Embrapa Rice and Beans for providing the bean grains.

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[1] Key references (marked with *) and their justification: The work of Pourcel et al. (2006) is useful to explain the mechanism of colour changes mediated by oxido-reductases. The works of Marles et al. (2008) and Nasar-Abbas, et al. (2009) are useful to demonstrate that there are different routes of darkening according to the bean type analyzed. The works of Mojica et al. (2015) and Ranilla, et al. (2007) are useful to explain the differences in the phenolic profile of some carioca bean genotypes.

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