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

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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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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