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Six months under uncontrolled relative humidity and room temperature changes technological characteristics and maintains the physicochemical and functional properties of carioca beans (*Phaseolus vulgaris* L.)

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ABSTRACT

Carioca beans contribute to health maintenance around the world, and the evaluation of commercial postharvest storage (CPS) ensures their quality. This study aimed to evaluate the effect of CPS on technological, physicochemical and functional properties of carioca beans. Two genotypes (Pontal–PO and Madreperola–MP beans) were stored under CPS or controlled conditions and were evaluated after harvest and after three- and six-months storage. PO and MP hardened with time, but the cooking time did not differ. PO is darker than MP and both darkened over time. Storage time affected pH and acidity of the beans and MP presented better physicochemical properties than PO, with lower activity of peroxidase ($p = 0.004$) and polyphenoloxidase ($p = 0.001$) enzymes. Glycosylated kaempferol was suggested as a possible chemical marker to differentiate the aging of PO and MP beans. In conclusion, besides the technological differences, the storage was able to prevent physicochemical and functional alterations of beans.

1. Introduction

Common beans (*Phaseolus vulgaris* L.) are widely appreciated in developing countries for their affordability, long shelf life and protein content. Carioca variety has a seed with light beige colored tegument with tan stripes when recently harvested, but presents a quick darkening during storage, which is generally associated to the grain hardening phenomena. However, the technological, physicochemical and functional quality of the grains are determined by the interaction between the genotype and the storage conditions; therefore, not all grains stored for a long period become dark or difficult to cook (Siqueira, Pereira, Batista, Oomah, Fernandes, & Bassinello, 2014).

In this sense, new carioca bean cultivars have been developed to postpone the darkening and hardening over time (Siqueira et al., 2014). For instance, BRSMG Madreperola bean (MP) and BRS Pontal bean (PO) are beans obtained by selectively bred crossing 2 lineages with contrasting ability to resist to darkening and hardening during storage, wherein MP is a cultivar with slow darkening and hardening during storage (Carneiro et al., 2012; Siqueira et al., 2014). In addition, MP beans present a short growth cycle, around 88 days, with high productive potential and a light beige tegument with light brown stripes. It can be preserved for a long period of time, which is considered its main advantage in relation to other carioca bean cultivars (Carneiro et al., 2012).

The loss of quality during storage is characterized by alterations on technological properties, such as increased degree of darkness, hardness and longer cooking time. These possible changes are mainly related to high temperature (> 25 °C) and high relative humidity (> 65%) in the presence of light and oxygen, which results in the hard-to-cook phenomenon (HTC) (Junk-Knievel, Vandenberg, & Bett, 2007) due to the hardshell, modification of content and metabolism of phenolic compounds, loss of phytate and soluble solids (Rios, Abreu, & Correa, 2003). The bean aging process is characterized by increased cross-links between polysaccharides and other cell wall components, such as protein and phenolic compounds. Increasing these bonds would cause the polysaccharides and proteins to become insolubilized, thus causing waterproofing of the cell wall, which would make it difficult for the water to entry into the cells and harden the seed by limiting starch

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gelatinization and protein denaturation (Pirhayati, Soltanizadeh, & Kadivar, 2011).

The time of storage needs to be controlled, since the longer the storage period, the greater the damage on water absorption and digestibility, and the greater the phenolic content increase, which causes darkening and hardening in beans over time (Rios et al., 2003). Pectin and lignin form the cell wall of plant cells, together with cellulose and glycoproteins and may also be affected by the storage time of beans, but the process of tissue lignification is not the only factor that increases the hardness over time (Siqueira et al., 2014).

Nutritionally, storage time is related to loss of phytate, tannins and lipids (Chiaradia & Gomes, 1997). In addition, large protein molecules can suffer natural enzymatic hydrolysis, forming small polypeptides during storage, which may alter the peptide functional properties. However, we have already demonstrated that storage up to six months does not affect the antioxidant and anti-inflammatory effect of carioca bean peptides (Alves, de Mejía, Vasconcelos, Bassinello, & Martino, 2016).

It has been demonstrated that, after six months of storage under environmental conditions, common beans may present the HTC phenomenon and increased cooking time. For most carioca beans, only three months of storage would be enough to observe hardness (Alvares, Pereira, Melo, Miklas, & Melo, 2020). However, there is little information about the effect of commercial postharvest storage (CPS) (uncontrolled relative humidity and room temperature) on hardening, darkening, physicochemical and functional properties of carioca beans. Therefore, this study aimed to evaluate the effect of CPS up to six months on technological, physicochemical and functional properties of two carioca beans with contrasting darkening and hardening resistance during storage. In this study, we hypothesized that CPS could contribute to the darkening and hardening of carioca beans, however, CPS would not affect their physicochemical and functional properties.

2. Materials and methods

2.1. Materials

Carioca bean cultivars (PO and MP), with contrasting darkening behavior during storage, were harvested at Capivara Farm of Embrapa Rice and Beans, in Santo Antonio de Goias, GO, Brazil, during the fall season of 2013. The genotypes were selectively bred by crossing 2 lineages (MP: AN 512666–0 and AN 730031, and for PO: BZ3836/FEB 166/NA 910523) (Del Peloso et al., 2004; Carneiro et al., 2012). The best lineages were selected after six generations, based on productivity potential and resistance to diseases (Carneiro et al., 2012).

The postharvest practices to process grains and performing technological, physicochemical and functional analyzes were done by using the following equipment: a drying oven MA-035/5, an automatic Mill MA 090 CFT and an water bath MA 093 (Marconi®, Piracicaba, SP, Brazil); a dielectric moisture meter (Grainer II, Kett®, US); a pH meter (Kasvi®, São José do Pinhais, PR, Brazil), a Mattson cooker apparatus with 25 plungers of 90 g and 1.0 mm of diameter of pin tip; a HunterLab Colorimeter (ColorQuest® XE, Reston, Virginia, USA); a block digester, nitrogen distiller and vacuum pump TE-0581 (Tecnal®, Piracicaba, SP, Brazil); muffle furnace Q318M and Soxhlet apparatus Q308B (Quimis®, Diadema, SP, Brazil); horizontal homogenizer (BHB_800, Benfer®, São Paulo, SP, Brazil); Plasma Emission Spectrophotometer (Perkin Elmer-Optima DV 3300, Norwalk, Connecticut, USA); UV/VIS Spectrophotometer (Multiskan GO, Thermo Scientific®, Waltham, Massachusetts, USA); sonicator (USC 1600A, Unique®, Indaiatuba, SP, Brazil); Ultra-Performance Liquid Chromatography (UPLC) analysis was performed using an ACQUITY UPLC™ system (Waters Corporation, Milford, MA, USA), coupled to a quadrupole-time-of-flight (QTOF) (Waters Corporation).

All solutions were prepared with deionized water (PURELAB®, ELGA Labwater, Canada). Methanol (99.8% PA), NaCl (> 99% PA), nitric acid (65% PA) and HCl (37% PA) were purchased from Neon® (Suzano, SP, Brazil); anhydrous sodium carbonate (99.5% PA) from Proquimios® (Rio de Janeiro, RJ, Brazil); sodium hydroxide in micropearls (99% PA) from Êxodo Científica® (Sumaré, SP, Brazil); anhydrous sodium acetate (> 99% PA), phytic acid (myo-inositol hexaphosphate) and vanillin (99%) from Sigma Aldrich® (San Louis, MO, USA); potassium hydroxide (KOH) in micropearls (85% PA) from Biotec® (Guarulhos, SP, Brazil). The Total Dietary Fiber Assay Kit was purchased from Sigma Aldrich®, Resistant Starch Assay Kit from Megazyme® (Wicklow, Ireland) and the AG® 1-X4 Resin from Bio-Rad Laboratories (São Paulo, SP, Brazil). All other chemicals and reagents used were of analytical grade.

2.2. Storage conditions of the carioca beans

After harvesting, drying and cleaning procedures, PO and MP beans were stored for three and six months, in sealed polypropylene packaging, at room temperature (22 ± 3 °C), without humidity control (< 65% of relative humidity) and protected from sunlight and heat, which simulated conventional conditions of storage. The storage procedure aimed to reproduce the commercial storage period usually applied or indicated by Brazilian market. After storage, the beans were cooked in a pressure cooker (1:2 bean: water; 120 °C, 50 min), dried for 8 h, at 60 °C and milled (sieve of 600 μm, size 30 mesh) to obtain the whole flour, which was packed under vacuum in polypropylene packaging and stored at − 20 °C, until the time for analysis.

For technological (hardness, cooking time and instrumental color parameters) and some physicochemical (pH, acidity and peroxidase and polyphenoloxidase enzymatic activity) analyses, the beans were stored under controlled temperature and humidity conditions (21 °C and 60% of relative humidity) and were evaluated after three and six months storage. The data were compared with the analyses performed after harvest (room temperature; 22 ± 3 °C, without humidity control). Instrumental color analysis was performed in raw and cooked beans.

2.3. Technological properties of carioca beans

2.3.1. Grain hardness measurement

For hardness measurement, beans were soaked for 18 h and cooked in distilled water in a hot air oven for 2 h at 105 °C. Texture analysis of cooked beans was performed with a TA-XTplus texture analyser (Stable Micro Systems Ltd., Surrey, UK). The return-to-start analysis method measured force under compression with a 2 mm cylindrical probe (*P*2), recording the peak maximum force. Whole beans were axially compressed to 90% of its original height. Force-time curves for 30 measurements of individual cooked beans were recorded at speed 5 mm/s and the results expressed in Newton (N). Three sampling repetitions of each genotype were analyzed (Siqueira et al., 2014).

2.3.2. Bean cooking time

Bean cooking time was obtained by cooking beans in Mattson cooker apparatus. The beans previously soaked in deionized water for 18 h at room temperature were placed on the support plate of the apparatus with each plunger over a bean. The cooking time was defined as the time between the start of boiling and the fall of the 13th plunger (Ribeiro, Cargnelutti Filho, Poersch, & Rosa, 2007).

2.3.3. Integument color

The instrumental color of raw and cooked beans was determined using a HunterLab Colorimeter, with the CIELAB system, according to Bento et al. (2020), to obtain the values of the coordinates L* (luminosity), a* (red/green) and b* (yellow/blue) by reflection with diffuse illumination (illuminant D65, viewing angle 10°). The difference between each coordinate (L*, a* and b*) during the storage time was calculated by the difference between the color after storage and the color of the freshly harvested beans. The equations used to calculate

each parameter are presented: Total color difference $(\Delta E*) = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2(\Delta b^*)^2}$; Chroma $(C*) = \sqrt{(a^{*2} + b^{*2})}$ and Hue angle $(H^{\circ}) = \text{arctg}\left(\frac{b^*}{a^*}\right)$.

2.4. Physicochemical properties of carioca beans

2.4.1. Acidity and pH

For sample preparation, 10 g of the bean flour sample was dissolved in 100 mL of water and the procedures followed the methodology proposed by AOAC Official Method 942.15B (1997). For acidity, three drops of 1% phenolphthalein alcoholic solution were added and the solution was titrated with 0.1 M (0.4% w/v) sodium hydroxide. The result was expressed in mL percent of the molar solution. For the determination of pH, the solution was homogenized and the pH was measured with the aid of a pH meter.

2.4.2. Determination of peroxidase and polyphenoloxidase activity

The peroxidase and polyphenoloxidase activity were evaluated in accordance with Moura, Abreu, Santos, and Correa (1999), with modifications. For enzyme extraction, 1000 mg of bean flour was homogenized in 10 mL of 1.2% (w/v) sodium phosphate buffer, pH 6.5 and stirred for 30 min at 4 °C. The extract was centrifuged at 14000 × g for 20 min at 4 °C. The obtained supernatant was filtered and used to determine the enzymatic activity.

The concentration of peroxidase was measured in UV/VIS spectrophotometer at 470 nm and the concentration of polyphenoloxidase was measured at 420 nm. The enzymatic activity of peroxidase and polyphenoloxidase was expressed in U/mg bean flour sample. An enzyme unit was considered as the amount of enzyme which caused an increase of 0.001 absorbance units per minute of reaction, under the conditions of the test. The results were obtained in duplicate.

2.4.3. Chemical composition

The chemical composition of whole bean flour was determined in triplicate, using the analytical procedures recommended by AOAC (2016) for moisture (AOAC Official Method 950.46), mineral residue (ash) (AOAC Official Method 920.153), lipid content (AOAC Official Method 923.05) and protein (AOAC Official Method 945.18), using the Micro Kjeldahl method for content determination through total nitrogen quantification (conversion factor for vegetable proteins: 5.75). Total dietary fiber, soluble and insoluble fractions were determined through the enzymatic gravimetric method (AOAC Official Method 900.02) with specific Total dietary fiber assay kit (Sigma Aldrich®), in duplicate. For enzymatic hydrolysis α-amylase, protease and thermoresistant amyloglucosidase were used. The total dietary fiber content was obtained by adding the soluble and insoluble fractions.

The carbohydrate content was calculated by the difference between the total sample and the sum of the content of protein, lipid, dietary fiber, water and ash. The conversion factors of 4, 4 and 9 kcal/g for carbohydrates, proteins and lipids, respectively, were considered for the energy value.

2.4.4. Resistant starch

The resistant starch (RS) content was determined according to AOAC (2016) (AOAC Official Method 2002.02), using the RS assay kit (Megazyme®). Briefly, the enzymatic hydrolysis of non-resistant starch (NRS) was performed through the simultaneous action of pancreatic α-amylase (10 mg/mL) and amyloglucosidase (AMG) (3 U/mL), by incubating the sample for 16 h, at 37 °C. Subsequently, the NRS was separated by centrifugation at 1500 × g, for 10 min, and the pellets containing the RS were purified with ethanol and re-suspended with 2 mL of 13.2% (w/v) KOH. This solution was neutralized with 9.9% (w/v) sodium acetate buffer and the starch was hydrolyzed to glucose with AMG. D-Glucose was measured with glucose oxidase/peroxidase reagent (GOPOD). The concentration of RS was measured in UV/VIS spectrophotometer at 510 nm, and the content was expressed as g/100 g carioca bean flour on a dry weight basis. The results were obtained in duplicate and presented as mean and standard deviation.

2.4.5. Mineral content

The contents of iron, calcium, zinc, copper, manganese, magnesium, sodium, potassium and phosphorus were determined according to Gomes and Oliveira (2011), in triplicate. All glassware used was previously demineralized at 10% (v/v) nitric acid solution for 12 h and dried in air circulation oven. The samples (1 g) were weighed in triplicate in digestion tubes and 10 mL of concentrated nitric acid were added. Subsequently, the samples were heated in digester block with exhaustion at initial temperature of 80 °C. The temperature was increased progressively up to 160 °C, and the samples were exposed to this temperature for 16 h, until a clear solution was formed. The tubes were cooled at room temperature and the content transferred to a 50 mL volumetric flask tube, washed with deionized water and agitated in vortex to avoid losses. This solution was used for reading the mineral content, using plasma emission spectrophotometry.

2.4.6. Phytate

Phytate content was determined in triplicate by ion exchange and UV/VIS spectrophotometry, according to Latta and Eskin (1980), with modifications. For the extraction of phytates, 0.1 g of sample was weighed and 15 mL of 2.4% (v/v) HCl were added and remained under horizontal agitation for 12 h. The extract was filtered through a vacuum pump (using a 3 μm porosity filter) and purified using ion exchange column, with stationary phase consisting of AG® 1-X4 Resin. The column was preconditioned with 11.7% (w/v) NaCl, and the extract obtained from the previous steps was applied to it. The inorganic phosphors were eluted with 0.29% (w/v) NaCl, followed by elution of the retained phytates with 11.7% NaCl. Phytate was determined by UV/VIS spectrophotometry, at 500 nm. An analytical curve of phytic acid at concentrations of 10 –100 μg/mL was created, using the Linear regression equation ($y = -0.0038x + 0.4963$; $R^2 = 0.994$) to express the phytate content in mg /100 g of flour.

2.4.7. Condensed tannins

The content of total tannins was evaluated by Vanillin reaction (Price, Van Scoyoc, & Butler, 1978). Tannins were extracted from samples (200 mg) in 1% (v/v) HCl in methanol, under automatic agitation, for 20 min, and the temperature was maintained at 30 °C. After extraction, the solutions were centrifuged at 1006 × g for 20 min. The supernatant (1 mL) was mixed with 2.5 mL of 1% (w/v) solution of vanillin in methanol and 2.5 mL of 8% (v/v) HCl in methanol. After 20 min under rest, the absorbance was measured in an UV/VIS spectrophotometer at 500 nm. A standard curve of catechin was used ($y = 0.5002x + 0.0052$; $R^2 = 0.999$) and the results were expressed as mg equivalent of catechin per 100 g of sample. The standard curve was prepared, using a stock solution of 0.1% (w/v) catechin in methanol, and aliquots of 2.5, 5, 10, 20, 25 and 50 mL were removed from the concentrated solution. The volume was adjusted to 100 mL with methanol in a volumetric flask. The reaction was performed under the same conditions described for the sample.

2.5. Functional properties of carioca beans

2.5.1. Bioactive potential

The UniProtKB Database (www.uniprot.org) was used to select proteins from common beans, and their amino acid sequences were used to investigate the profiles of protein potential biological activity, using BIOPEP database (http://www.uwm.edu.pl/biochemia/biopep/start_biopep.php). The bioactive potential of peptides sequenced found in common bean protein was calculated as follows: % occurrence of frequency = number of amino acids of each bioactive effects/ total number of amino acids with bioactive effects.

2.5.2. Total phenolics

The total phenolics were determined using Folin–Ciocalteu reagent, according to Singleton, Orthofer, and Lamuela-Raventós (1999). For the extraction of phenolic compounds, 1 g of the sample was diluted in 20 mL of 60% (v/v) aqueous methanol solution, for 20 min, with horizontal agitation, and filtered through a vacuum pump (using a 3 μm porosity filter). Aliquots of 100 μL of extract were mixed by vortex with 1 mL Folin–Ciocalteu (0.25 N), for 10 s. After 3 min, 1 mL of 7.5% (w/v) aqueous sodium carbonate was added. After 7 min, 5 mL of deionized water were added, and the mixture was kept under agitation for 30 min, at room temperature. The absorbance was read in an UV/VIS spectrophotometer, at 765 nm. The results were expressed in mg of gallic acid equivalents per gram of flour (mg GAE/g), using a standard curve obtained by different concentrations of gallic acid ($y = 30.763x + 0.0818$; $R^2 = 0.996$).

2.5.3. Phenolic compound concentration and identification

The concentration of phenolic compounds was determined using Folin–Ciocalteu reagent, as described by Singleton et al. (1999). For this analysis, samples (400 mg) were extracted with 10 mL of a methanolic solution with methanol:water:cloridric acid volumetric ratio of 50:48:2. The material was sonicated for 1 h, and the extract was centrifuged (2683 × g). An aliquot (1.5 mL) was filtered in PTFE 0.22 μm and placed in vials for UPLC-QTOF analysis. Chromatography was performed in a Waters Acquity UPLC BEH column (150 mm × 2.1 mm × 1.7 μm), fixed temperature of 40 °C. The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), gradient of 0–15 min (2 – 95%) of B; 15.1–17 min (100%) of B; 17.1–19.1 min (2%) of B, flow of 0.4 mL/min and injection volume of 5 μL. The Electrospray Ionization mode was acquired at 110–1180 Da, temperature stationary source at 120 °C, desolvation temperature at 350 °C, gas desolvation flow of 350 L/h, extraction cone of 0.5 V, capillary voltage of 2.6 kV. The cone tension of 35 V, collision energy of 5 eV was used as low scan. For high scan cone tension, it was 35 V with power ramp of collision of 20–40 eV. Leucine enkephalin was used as lock mass in the MS^E acquisition mode, under the following conditions: retention time of 1.2 to 7.0 min, 120 to 1200 Da, and level 5 of noise elimination. The instrument was controlled by Masslynx 4.1 software system (Waters Corporation) and the chromatograms were analyzed by the MarkerLynx software system for the discrimination of potential chemical markers.

2.5.4. Antioxidant capacity

The antioxidant activity was evaluated by oxygen radical absorbance capacity (ORAC), according to Prior et al. (2003), using 20 μL of 6-hydroxy-2,5,7,8-tetramethylchroman 2-carboxylic acid, 1–8 μM final concentration (Trolox standard; $y = 0.9905x - 8.172$; $R^2 = 0.989$) and whole bean flour (1 mg). ORAC results were expressed in mmol Trolox equivalent/g of dry flour.

2.6. Statistical analysis

For the analysis of the results, the variation sources bean genotype × time were evaluated, and the bean genotype*Time interaction was used as residue, since the experiment was carried out without repetition.

The Shapiro-Wilk normality test was used. For parametric data, the *one-way* Analysis of Variance (ANOVA) was performed and, for qualitative results (Genotype) significant at 5% probability, the analysis was conclusive, while for quantitative results (Time) linear regression equations were used for analyze the effects of independent variables on responses. The results were presented as the mean ± SD. Nonparametric data were evaluated by Kruskal-Wallis test and, for significant results at 5% probability, the analysis was conclusive. The results were presented as the median. The Statistical Analysis System (SAS®-Institute Inc., North Carolina, USA, 1989), online version was used. The characterization of phenolic compounds was performed using principal component analysis (PCA), and orthogonal projections to conduct latent structures-discriminant analysis (OPLS-DA) for S-plot graphics construction and distinction of potential markers.

Table 1
Technological and physicochemical characteristics of carioca beans.

	Bean cultivar		
	BRS Pontal	**BRSMG Madreperola**	***p*(F)**
Hardness (N)	3.99 ± 0.65	3.40 ± 0.75	0.013*
Cooking time (min)	57.37	57.30	0.337 ns
pH	6.31 ± 0.20	6.27 ± 0.15	0.399 ns
Acidity (%)	12.33 ± 1.45	11.40 ± 1.01	0.072 ns
Peroxidase (U/mg of sample)	19.74	0.76	0.004*
Polyphenoloxidase (U/mg of sample)	1.45 ± 0.40	0.44 ± 0.16	0.001*
	Regression model	**R^2**	
Hardness (N)	2.990 + 0.008* Time	0.609	0.014
pH	6.476 – 0.002* Time	0.665	0.003
Acidity (%)	10.498 + 0.015* Time	0.798	0.004

Values represent means ± SD or median for instrumental color parameters. Controlled conditions were: 21 °C and 60% of relative humidity. Regression models were performed as a function of time (days) and their respective coefficients of determination (R^2) and probability levels (p) are shown. * indicates significant difference ($p < 0.05$).

3. Results and discussion

3.1. Technological properties of carioca beans

3.1.1. Hardness and cooking time

All technological characteristics were evaluated according to genotype and time of storage. Hardness was higher ($p = 0.013$) for PO genotype compared to MP and it was affected by storage time (up to six months) independent of the genotype ($R^2 = 0.609$). The cooking time was preserved ($p > 0.05$) regardless of the storage time or genotype evaluated (Table 1). Although the seeds of PO were harder, it did not affect its cooking time. Similar to the present study, Siqueira et al. (2014) evaluated 5 types of carioca beans and the hardening (evaluated by cooking time) did not differ.

Hardening over time is related to hydration defects (Rios et al., 2003). In this sense, high temperature (> 25 °C) and relative humidity (> 65%) in the presence of light and oxygen are the main factors that impair water absorption, which results in texture defect during storage and contributes to the hard-to-cook phenomenon due to alterations in the phenolic content by lignification and loss of phytate (Rios et al., 2003; Junk-Knievel et al., 2007). The lignification process relates the hardening with the polymerization of the phenolic compounds from the shells, mediated by polyphenoloxidases, and the formation of cross-links between phenolic compounds and cell wall proteins of the cotyledons. Peroxidase is involved in the polymerization reaction of phenolic compounds, its increased activity could be associated with the lignification process of the cell wall (Varriano-Martson & Jackson, 1981). However, Siqueira et al. (2014) demonstrates that the hardness observed in genotypes during storage cannot be attributed solely the process of tissue lignification, but is probably related to other metabolic pathways, such as storage conditions and the genetic characteristics of beans.

3.1.2. Instrumental color

Luminosity (L*) values significantly differed for MP beans compared to PO, with higher brightness value ($p < 0.001$) for MP, for both raw and cooked beans. L* values decreased linearly ($R^2 = 0.934$) with storage time for the raw of carioca beans and the regression model was

Table 2
Instrumental color parameters for carioca beans, raw and cooked.

	Bean cultivar		
	BRS Pontal	BRSMG Madreperola	*p*(F)
	Luminosity (L*)		
Raw	45.92 ± 3.47	51.39 ± 1.76	< 0.001*
Cooked	39.77 ± 2.63	47.23 ± 1.85	< 0.001*
	Chroma values (C*)		
Raw	20.54 ± 1.09	19.35 ± 0.83	0.001*
Cooked	15.34 ± 1.77	15.99 ± 1.46	0.156 ns
	Hue angle values (H°)		
Raw	61.84	69.27	0.004*
Cooked	56.81 ± 1.33	65.60 ± 0.93	< 0.001*
	ΔE*		
Raw	5.32 ± 3.78	2.69 ± 1.95	0.019*
Cooked	3.50 ± 2.58	3.14 ± 2.09	0.504 ns
	Regression model	**R^2**	
L* - Raw	52.023 – 0.037*Time	0.934	0.032
C* - Raw	18.706 + 0.014*Time	0.963	< 0.001
C* - Cooked	13.966 + 0.019* Time	0.669	0.004

Values represent means ± SD or median for instrumental color parameters. Controlled conditions were: 21 °C and 60% of relative humidity. Regression models were performed as a function of time (days) and their respective coefficients of determination (R^2) and probability levels (*p*) are shown. * indicates significant difference ($p < 0.05$).

adjusted (Table 2). Chroma (C*) values, an index of color saturation or intensity, was different ($p = 0.001$) regarding the type of raw bean and the PO presented the highest C* value. The cooked beans did not differ ($p > 0.05$) and regarding the time of storage, raw and cooked beans interacted with time and differed ($R^2 = 0.963$ and $R^2 = 0.669$, respectively) for C* value, indicating an increase in this characteristic with increasing storage time of beans, regardless PO or MP. The interaction of Hue angle (H°) values with genotype was significant for both raw and cooked beans ($p = 0.004$ and $p < 0.001$, respectively) and for both, MP presented the highest values. All genotypes darkened after six months storage; however, the degree of darkening was different for each genotype, and the cooked bean follow the same pattern of raw bean (Table 2). It was possible to confirm the better stability of BRSMG Madreperola to the darkening process. Further, our results were similar to those encountered by Bento et al. (2020) and Siqueira et al. (2014).

The total color difference index (ΔE*) of the raw samples differed ($p = 0.019$) between genotypes and PO presented the highest value. ΔE* values did not differ ($p > 0.05$) between genotypes for cooked beans. Siqueira et al. (2014) evaluated 5 types of carioca beans and, although hardening (evaluated by cooking time) did not differ, MP also stood out by its higher luminosity values and less darkening, while PO presented the worse results.

3.2. Physicochemical properties of carioca beans

3.2.1. pH, acidity and enzymatic activity

Comparison of the pairs demonstrated that pH and acidity did not differ ($p > 0.05$) between PO and MP beans, but both were influenced by the storage time and the regression model was adjusted ($R^2 = 0.665$ and $R^2 = 0.798$, respectively) (Table 1). The pH of the grains tends to decrease over time and consequently the acidity tends to increase. This result is in agreement with data reported in the literature, the pH reduction of stored beans and the acidity increase in over storage time are a characteristic of aged beans. The physicochemical change occur mainly due to inadequate storage conditions and/or storage for long time (Silochi et al., 2016).

The PO cultivar is described for its rapid darkening and hardening in relation to MP and the results demonstrate the activity of peroxidase and polyphenoloxidase significantly enriched ($p = 0.004$ and $p = 0.001$, respectively) in the PO compared to MP beans (Table 1), although there is no interaction with the storage time ($p > 0.05$). Despite the remarkable darkening of PO bean, the time of storage was suitable to preserve hardening, regardless of the time of storage of the beans or its interactions. It is interesting, since consumers consider darkening as a characteristic of aging and hardening of beans (Siqueira et al., 2014).

3.2.2. Macronutrients and dietary fiber

The chemical composition of the common beans, PO and MP was evaluated and no statistical differences ($p > 0.05$) were observed for moisture, ash, protein, total dietary fiber, soluble and insoluble dietary fiber, carbohydrates or resistant starch, compared with time of storage (Fig. 1). Regardless of the type of bean, lipid concentration decreased over the storage time of 0, 3 and 6 months (PO: 1.35 ± 0.03, 1.29 ± 0.02 and 1.20 ± 0.4, respectively; MP: 1.31 ± 0.13, 1.30 ± 0.17 and 1.17 ± 0.17 g/100 g, respectively; $p = 0.020$) and the regression model was adjusted: Lipids = 1.34–0.02*Time ($R^2 = 0.910$). However, this reduction between 0, 3 and 6 months was an inexpressive nutritional loss, mainly considering that common bean presents low lipid content. The main effect of storage seems to be on the lipid content, in which a reduction may be observed, even under controlled conditions (Ribeiro, Prudencio-Ferreira, & Miyagui, 2005). Lipids are an important parameter of bean quality during storage, since they are the most reactive components under adverse conditions. Lipid degradation affects free fatty acid content and increases sensitivity to fatty acid oxidation (Anwar, Chatha, & Hussain, 2007). The lipid

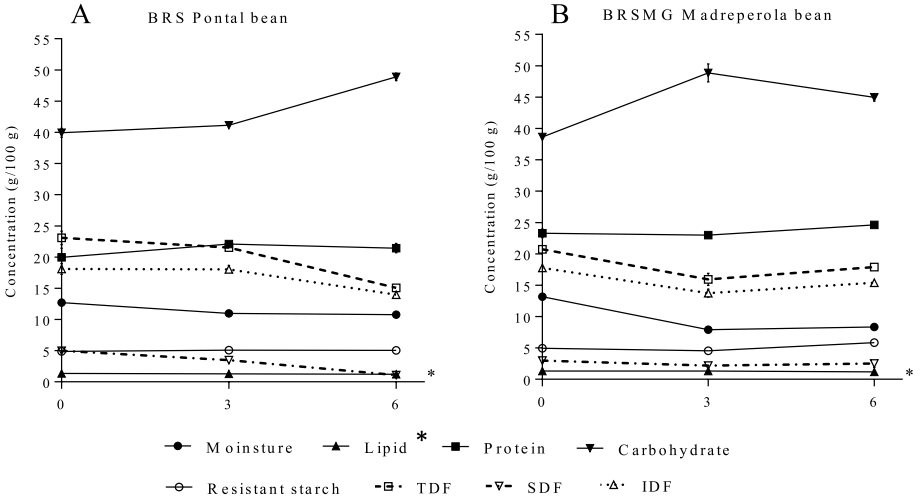

Fig. 1. Macronutrients composition of carioca beans stored up to six months. TDF: total dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber. Numbers 0, 3 and 6 indicate months of storage. Results are presented by g/100 g of dry sample. * Indicate difference ($p < 0.05$) along storage time with Bean*Time interaction of variation. There are no differences between the types of bean ($p > 0.05$).

content of beans is linked to general problems in food quality, as these constituents may undergo a series of deterioration processes resulting in undesirable changes in flavor, aroma and color, mainly oxidative rancidity. Even the low lipid content, it contributes to grain deterioration, along with carbohydrates. One of the oxidative rancidity problems in beans is caused by the enzyme lipoxygenase, which works catalytically on the polyunsaturated fatty acids. However, each bean genotype may have a different behavior of lipoxygenase activity over time (Silva et al., 2020).

Besides, the initial moisture content (Time 0: PO = 12.71 ± 0.07 and MP = 13.16 ± 0.06 g/100 g) was similar to that observed for carioca beans stored under normal and modified conditions (Vanier, Rupollo, Paraginski, de Oliveira, & Elias, 2014). For Pinto bean, similar to carioca, the lower moisture content (12 and 14%) was maintained throughout the storage period (360 days), while samples with higher initial moisture content (16–20%) decreased during the initial weeks of storage (Rani, Chelladurai, Jayas, White, & Kavitha-Abirami, 2013). Moisture content around 14% may increase the microbial stability of Common beans during storage (Uebersax & Siddiq, 2012). In addition, low moisture content (< 16%) is important to reduce hardness during storage.

As aforementioned, no effect on protein was observed for PO and MP beans submitted to a short storage period (six months) (Fig. 1) and the same was verified by Rani et al. (2013) for beans stored for four months. However, Vanier et al. (2014) observed decreased protein content after 12 months, under high relative humidity (75 ± 5%). As suggested by Vanier et al. (2014), reduced proteins may be associated with the decreased tissue pH, which is inversely proportional to the time of storage, and may partially contribute to the development of the HTC phenomenon.

The carbohydrate content of PO and MP remained unchanged, which helps to prevent hardness, since changes in this macronutrient due to starch retrogradation contributes to hardness during storage (Ferreira et al., 2017) and loss of capacity to form a thick broth, which is an important quality aspect for American Latin consumers (Sánchez-Arteaga, Urías-Silvas, Espinosa-Andrews, & García-Márquez, 2015).

No change on dietary fiber content was observed in the present study (Fig. 1), and PO and MP beans presented lower soluble/insoluble dietary fiber rate (r) (r < 0.75), which indicates low propensity to hardening, since rates higher than 0.75 can contribute to hardness during storage (Reyes-Moreno, Paredez-López, & Gonzalez, 1993). In addition, storage period and temperature seem to be crucial for alterations on dietary fiber content, as verified in beans stored for a prolonged time and at higher temperatures (36 °C; 24 months) (Reyes-Moreno et al., 1993).

Similarly to dietary fiber, no effect due to storage time was observed for starch content (p > 0.05). This result was expected, since chemical or structural alteration in starch depends on environmental conditions (Siqueira et al., 2014). The resistant starch content of PO and MP ranged from 4.89 ± 0.09 to 5.03 ± 0.06 and 4.95 ± 0.14 to 5.85 ± 0.22 g/100 g, respectively, from 0 to 6 months of storage, without differences among beans (p > 0.05). These results were higher than the values observed by Almeida Costa, Queiroz-Monici, Reis, and de Oliveira (2006), of 2.33 ± 1.23 mg/100 g for cooked carioca beans. These differences can be attributed to the methods of analysis and cooking process, since Almeida Costa et al. (2006) cooked the beans after soaking them for a period of 16 h (1:2 w/v). Other factors, such as genetic, crop management or environmental aspects, may be related to these differences.

3.2.3. Mineral content

The storage time did not affect mineral content, as shown in Supplementary (I). Some variations are expected for minerals when environmental conditions promote membrane damage (Parmar, Singh, Kaur, & Thakur, 2017). Thus, the storage conditions used in the present study were suitable to preserve the mineral content of carioca beans.

The time of storage did not affect the ash or mineral content, which indicates that storage up to six months, under environment conditions, is enough to preserve the evaluated minerals (Supplementary (I)). In fact, even long time of storage (up to 360 days), at room temperature (25 °C), seems to be suitable to preserve mineral content, as observed by Vanier et al. (2014). Regarding bean type, some differences were observed. At periods 0, 3 and 6 months, MP presented higher concentrations of phosphorus (14.63, 12.19 and 21.28% higher, respectively) (p = 0.047), while PO presented higher concentrations of calcium (23.81, 10.53 and 35.29% higher, respectively) (p = 0.033) and manganese (29.71, 23.03 and 31.04% higher, respectively) (p = 0.016) (Supplementary (I)).

3.2.4. Phytate and condensed tannins

No statistical difference (p > 0.05) was observed for phytate content between storage time and type of bean (Fig. 3A). The tannin content was higher in the PO than MP (p = 0.042), however, the beans were not affected by the commercial postharvest storage time (p > 0.05) (Fig. 3B). Polymeric phenolics, such as tannins, are relatively polar and soluble in water and exhibit mobility in environments with high water activity, unlike the findings of the present study, since relative humidity was 60%. As for the chemical aspects, the pectin and lignin that form the cell wall of plant cells may be affected by the storage time of beans. However, the hardness observed in genotypes during storage cannot be attributed solely to the process of tissue lignification and the ability to decompose pectic substances during the cooking process, but it is probably related to the set of characteristics that make up each variety of beans (Siqueira et al., 2014). It is suggested, for example, that the darkening of different lines of carioca beans is related to the differing amounts of proanthocyanidins (syn. condensed tannins) in the seed coats (Beninger et al., 2005). Further, the loss of bean quality, including hardening, is related to the activation of enzymes (Silva et al., 2020) and changes in lignin and phytates (Siqueira et al., 2014).

3.3. Functional properties of carioca beans

3.3.1. Bioactive potential

According to the UniProtKB database, there are > 250 identified amino acid sequences from *Phaseolus vulgaris* L. beans, which are generally classified for their functions, including storage protein, carbohydrate metabolism, defense, stress response and nitrogen metabolism (Luna-Vital, Mojica, De Mejía, Mendoza, & Loarca-Piña, 2015). Although most of the reported proteins are related with metabolic functions in the plant (Luna-Vital et al., 2015), we summarized that common beans generally present proteins with a huge number of amino acid sequences that can be used to generate bioactive peptides (Supplementary (II)). As demonstrated in Fig. 2, these proteins have been reported to present peptides with bioactive potential (BIOPEP database, http://www.uwm.edu.pl/biochemia/biopep/start_biopep.php), mainly related to glycemic control by the inhibition of dipeptidyl peptidase IV (DPP-IV) and dipeptidyl peptidase III (DPP-III), and blood pressure control by the inhibition of angiotensin converting enzyme I (ACE-I). In addition, other potential functional properties include antioxidative effects, carbohydrate metabolism and modulation of immune system (Fig. 2).

In fact, common beans have been described as a potential source of bioactive peptides (Luna-Vital et al., 2015; Gomes et al., 2020). Long time of storage can promote natural enzymatic hydrolysis, which may alter the functional properties of peptides. We have previously demonstrated that short time of storage (up to six months) generated different amino acid sequences but did not affect the potential of common bean to produce peptides with functional properties (Alves et al., 2016). In fact, we have previously demonstrated, in mice, that whole bean flour and bean protein hydrolysate (700 mg/kg/day) reduced glycemia, total cholesterol, lipid peroxidation and inflammation

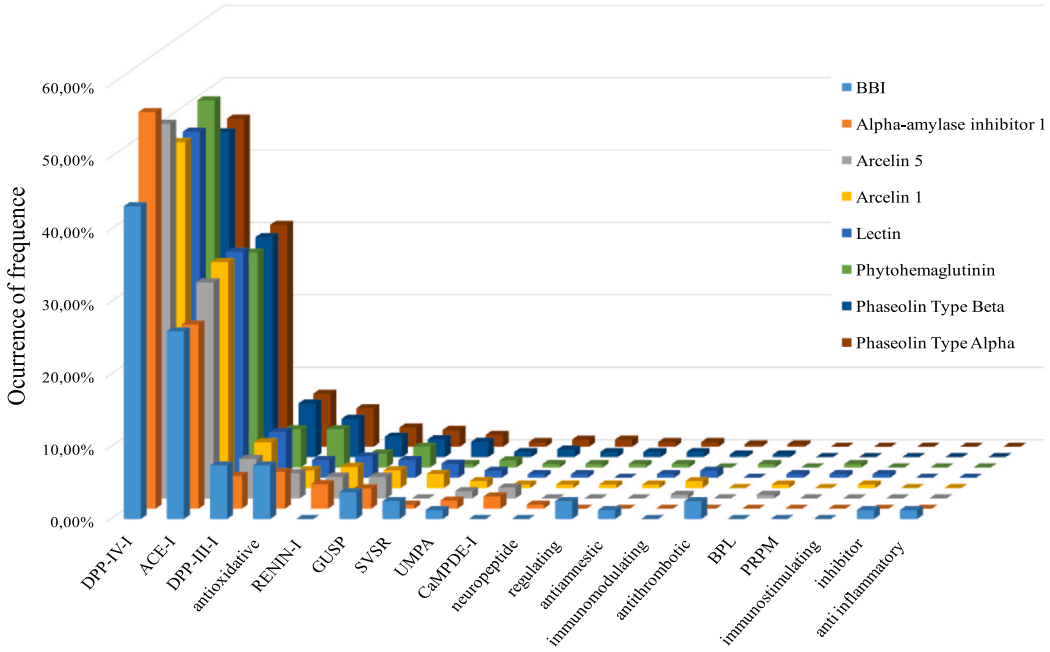

Fig. 2. Bioactive potential of peptides sequenced found in carioca beans. Sequences identified in UniProtKB® Program and analyzed in BIOPEP® database. DPP-IV: dipeptidyl peptidase IV inhibitor; ACE inhibitor: angiotensin-converting-enzyme inhibitor; DPP-III: dipeptidyl peptidase III inhibitor; Renin-I: renin inhibitor; GUSP: Glucose uptake stimulating peptide; SVSR: Stimulating vasoactive substance release; UMPA: Activating ubiquitin-mediated proteolysis; Regulating: peptide regulating the stomach mucosal membrane activity; BPL: bacterial permease ligand; PRPM: Peptide regulating phosphoinositol metabolism.

(de Lima et al., 2019). In addition, bean protein hydrolysate reduced weight gain and food intake (de Lima et al., 2019). It was also able to prevent endothelial dysfunction and, consequently, the atherogenic risk, by reducing inflammation, tumor necrosis factor-α and angiotensin II, and increasing endothelial nitric oxide synthase (Gomes et al., 2020), which demonstrates the ability of beans to improve human health and prevent chronic diseases.

3.3.2. Phytochemicals and antioxidant capacity

No statistical difference ($p > 0.05$) was observed for total phenolic content between storage time and type of bean (Fig. 3C). The antioxidant capacity by ORAC was higher in the PO than MP ($p = 0.012$) (Fig. 3D). However, the beans were not affected by the commercial postharvest storage time ($p > 0.05$).

PO and MP (at time zero) presented total phenolic content (1.32 ± 0.2 and 1.25 ± 0.04 mg EqGA/g, respectively) similar to that observed for PO bean (without storage) (1.33 ± 0.15 mg EqGA/g) (Dias et al., 2015). Reduced total phenolic content of stored beans is usually expected, due oxidation, which is associated with darkening and hardening during storage (Vanier et al., 2014). According to Vanier et al. (2014), the total phenolic content is probably the same, but these compounds may form complexes with proteins and other macromolecules during cell stress, which makes them less extractable. Therefore, a lower amount of phenolics is detected.

According to PCA, the separation between MP and PO indicates that these cultivars differ in the types of phytochemicals, which can be due to the intensity or absence of characteristic ions, including primary metabolites. In addition, the types of phytochemicals of PO bean seem to be affected by storage time, since there is a separation at PC2 (10.18%) related to 0, 3 and 6 months of storage. The slight difference between the time of storage for MP at PC2 and the almost absence of variation at PC1 (47.26%) reveals a constant phytochemical composition during the period of storage analyzed (Fig. 4C).

The OPLS-DA comparison demonstrates, through S-plot graphic, that citric acid (t_r 1.32, *m/z* 191.02), catechin (t_r 3.09, *m/z* 289.07) and kaempferol 3-hexoside (t_r 4.69, *m/z* 447.09) were the representative chemical markers for chemical differentiation between the varieties (Fig. 4A & B). The glycosylated kaempferol concentration of PO beans presented variation during storage (6 months), constituting a chemical marker for a differentiation of the effect of storage on the evaluated cultivars (Fig. 4A & B). As discussed below, glycosylated kaempferol is a natural phenolic compound of *Phaseolus vulgaris* L. However, the slight variation of MP phenolic compounds showed by PCA and OPLS-DA agrees with the fact that MP is more resistant to aging than PO. Therefore, we suggest glycosylated kaempferol as a possible marker that differentiates the behaviors of PO and MP beans during commercial postharvest storage.

In the present study, the phytochemical analysis was performed in the whole grain after storage and cooking process, based on the preparation habits of consumers. However, most of the available studies performed these determinations in the isolated coat or cotyledon. Ranilla, Genovese, & Lajolo (2007), for example, evaluated 25 Brazilian cultivars of raw *Phaseolus Vulgaris* L, including BRS Pontal, stored up to one year at 4 °C and relative humidity of 85%. Mojica, Meyer, Berhow, and de Mejía (2015) also evaluated the coat of raw carioca beans, including BRS Pontal, for the phenolic composition. These previous studies demonstrated that carioca beans present high levels of kaempferol glycosides (Dias et al., 2018; Ranilla et al., 2007), in addition to other flavonol glycosides, such as quercetin 3-O-glucoside and myricetin 3-O-glucoside (Mojica et al., 2015; Dias et al., 2018).

In the present study, stored and fresh cooked whole carioca beans presented higher antioxidant capacity (251.67 ± 11.40 mmol TE/g of dried flour, on average) than seed coats of raw and fresh BRS Pontal (209.9 ± 28.2 mmol TE/g), evaluated by the same method (Mojica et al., 2015).

Some market class of dry beans, such as pinto, reds and carioca, present postharvest seed coat darkening. It results in considerable economic loss due to undesirable decline in the visual quality, which consumers associate with prolonged cooking time (Siqueira et al., 2014). It is suggested that the hardening and/or darkening phenomenon is initiated by complex reactions activated inside the grains, which involve different cell components, such as cell wall polymers, phenolics, starch, protein and enzymes. In addition, environmental (climate, crop season and soil) conditions and intrinsic characteristics contribute to

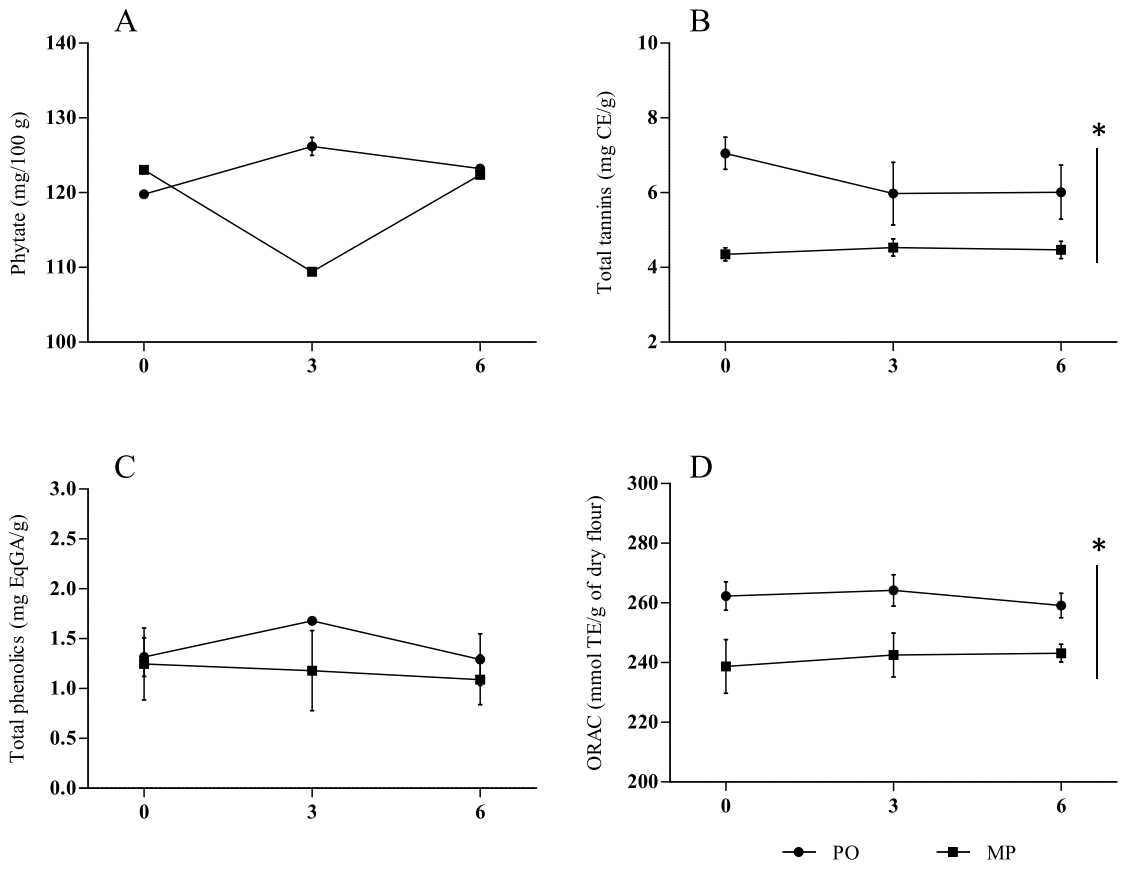

Fig. 3. Phytochemical and antioxidant capacity of carioca beans stored up to six months. PO: BRS Pontal bean; MP: BRSMG Madreperola bean; GAE: gallic acid equivalents; EC: equivalent of catechin; ORAC: oxygen radical absorbance capacity; TE: trolox equivalent. Numbers 0, 3 and 6 indicate months of storage. * Indicate significant differences between the types of beans ($p < 0.05$).

the degree and speed at which grains develop these phenomena (Ribeiro et al., 2005). Postharvest darkening includes a combination of environmental, genetic and chemical changes. Darkening is accelerated by exposure to light, high temperature (> 25 °C) and humidity (> 65%), during postharvest carioca bean storage.

Besides the nutritional importance of worldwide consumption of beans, carioca beans can provide antihypertensive, antioxidative and anti-inflammatory peptides, thus contributing to health maintenance. Therefore, the assessment of time and commercial postharvest storage conditions are important to ensure bioactive and sensory quality. The present study demonstrates that storage up to six months, under commercial postharvest storage conditions (uncontrolled relative humidity and room temperature), was able to prevent physicochemical alterations and preserve important nutrients, phytochemicals and bioactive compounds, as well as the antioxidant capacity of PO and MP carioca beans. We also showed that the commercial postharvest storage did not necessarily increase cooking time. Despite the phenotypic change that often causes rejection by the consumer or grain processing industry, the physicochemical and functional properties of beans can be preserved.

4. Conclusions

The commercial storage time (at room temperature and uncontrolled relative humidity up to six months) and conventional cooking method (under pressure) are able to maintain adequate physicochemical and functional properties of carioca beans. The presented study is useful for guide the production and commercialization of carioca beans, since six month-storage is time enough to change the color of fast darkening beans, which is one of the main reasons of Brazilian market rejection. We emphasize that despite technological changes, it was not observed physicochemical and functional alterations along time and glycosylated kaempferol was suggested as a potential chemical marker to differentiate the evaluated cultivars throughout storage time.

CRediT authorship contribution statement

Natália E. G. Alves: Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing. **Mariana J. C. Gomes:** Formal analysis, Visualization, Writing - review & editing. **Christiane M. Vasconcelos:** Methodology, Formal analysis. **Ana C. Lima:** Methodology, Formal analysis. **Sâmara L. S. Lima:** Methodology, Formal analysis. **Edy S. Brito:** Formal analysis, Investigation. **Priscila Z. Bassinello:** Formal analysis, Visualization, Writing - review & editing. **Hércia S. D. Martino:** Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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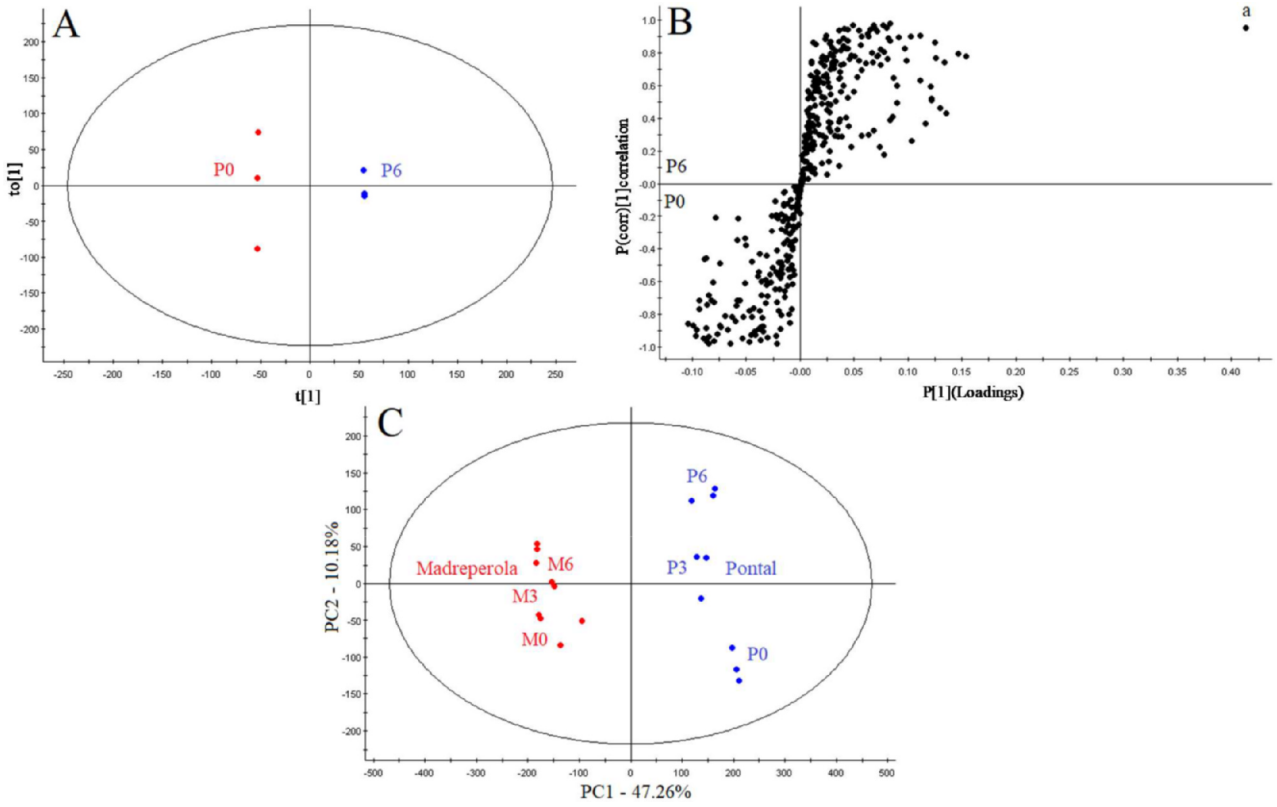

Fig. 4. Principal component analysis (PCA) showing phytochemical composition and the effect of storage time on carioca beans. A: effect of six months of storage on BRS Pontal bean phytochemicals; B: representative chemical marker; C: effect of the storage time. PO: BRS Pontal bean; MP: BRSMG Madreperola bean. [a] kaempferol 3-hexoside. Numbers 0, 3 and 6 indicate months of storage.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128390.

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