Ciência Rural, Santa Maria, v.53:9, e20220342, 2023
http://doi.org/10.1590/0103-8478cr20220342
ISSNe 1678-4596
FOOD TECHNOLOGY

Functional properties and chemical profile of aged carioca beans and cooked under thesteam of autoclave

Juliana Aparecida Correia Bento[1*] **Paulo Riceli Vasconcelos Ribeiro**[2] **Priscila Zaczuk Bassinello**[3] **Menandes Alves de Souza Neto**[1] **Rosângela Nunes Carvalho**[3] **Edy Sousa de Brito**[2] **Márcio Caliari**[1] **Manoel Soares Soares Júnior**[1]

[1]Escola de Agronomia, Universidade Federal de Goiás (UFG), 74690-900, Goiânia, GO, Brasil. E-mail: julianaap.ufg@gmail.com. [*]Corresponding author.
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Agroindústria tropical, Fortaleza, CE, Brasil.
[3]EMBRAPA Arroz e Feijão, Santo Antônio de Goiás, GO, Brasil.

ABSTRACT: This study evaluated the changes caused by cooking presoaked aged carioca beans in the autoclave steam, focusing on its bioactive components, antioxidant activity, and nutritional compounds. Additionally, to identify which carioca bean cultivar could preserve the most quantity of bioactive compounds in cooked flour. The cooked flours from Imperador had the highest antioxidant activity (DDPH: 10.58 µmolTrolox·g^{-1}, ABTS: 18.71 µmolTrolox·g^{-1}), anthocyanins (8.08 µg·g^{-1}), and total phenolic content (TPC) (36.69 mg·g^{-1}). The cultivar Gol also retained part of these compounds after cooking. The phenolic and saponin profiles of cooked flours revealed a reduction in phenolic compounds such as catechin, epicatechin, and kaempferol and an increase in soyasaponin-Ba and Bb. The samples Notavel, Dama, and Madreperola, presented the highest amount of soyasaponin-A0. Thus, the cooked flours from Imperador and Gol stood out due to their retention of part of their bioactive compounds, such as polyphenols and group B saponins.
Key words: antioxidant activity, phenolic profile, phytates, saponins, tannins.

Propriedades funcionais e perfil químico de feijão carioca envelhecido e cozido no vapor da autoclave

RESUMO: Este estudo avalia as alterações causadas pelo cozimento do feijão carioca, envelhecido e pré-embebido, no vapor da autoclave, com foco em seus componentes bioativos, atividade antioxidante e compostos nutricionais. Além disso, identifica qual cultivar de feijão carioca preserva a maior quantidade de compostos bioativos na farinha de feijão cozida. As farinhas cozidas do Imperador apresentaram a maior atividade antioxidante (DDPH: 10.58 µmolTrolox·g^{-1}, ABTS: 18.71 µmolTrolox·g^{-1}), antocianinas (8.08 µg·g^{-1}) e fenólicos totais (TPC) (36.69 mg·g^{-1}). A cultivar Gol também reteve parte desses compostos após o cozimento. Os perfis fenólicos e saponínicos das farinhas cozidas revelaram redução de compostos fenólicos, como a catequina, epi-catequina e kaempferol e aumento de soyasaponin-Ba e Bb. As amostras Notável, Dama e Madrepérola, apresentaram a maior quantidade de sojasaponina-A0. Assim, as farinhas cozidas do Imperador e Gol se destacaram pela retenção de parte de seus compostos bioativos, como polifenóis e saponinas do grupo B.
Palavras-chave: atividade antioxidante, perfil de fenólicos, fitatos, saponinas, taninos.

INTRODUCTION

The carioca beans (*Phaseolus vulgaris* L) are the most cultivated and eaten in Brazil (around 70% of the Brazilian bean market), followed by black beans (approximately 15% of the Brazilian bean market). The common bean is rich in essential nutrients such as high protein contents, with high lysine content (essential amino acid), and may be used as a complementary protein to the cereal. It has a high complex carbohydrate content, and dietary fibers with recognized hypocholesterolemic and low glycemic effects. It also has minerals (Ca, Fe, Cu, Zn, P, K, and Mg), vitamins (mainly the B complex), and bioactive compounds with antioxidant effects, such as saponins and phenolics (CELMELI et al., 2018; BENTO et al., 2021a; BENTO et al., 2021b). Therefore, regular consumption of beans may contribute to improvement in health and well-being. However, the integument of some carioca bean cultivars darkens very quickly, and the bean itself also hardens rapidly, economically depreciating the product (BENTO et al., 2020; BENTO et al., 2021b).

Bean flour could be a way of using dark and hard common beans, which can contribute to the sustainability of the food industries and still aligned with new trends and consumption habits based on sensory quality, diversity, and healthiness. So, the beans flours could be an ingredient with high potential to distribute the benefits of common beans since pulse flours may be added to foods such as baked goods, meat, pasta, and snacks. Nevertheless,

Received 06.13.22 Approved 11.02.22 Returned by the author 01.17.23
CR-2022-0342.R1
Editors: Leandro Souza da Silva Melissa Walter

the direct addition of raw pulse flours is limited by their green bean and other off-flavors associated with several flavor compounds. Thermal treatment of legumes (as cooking) makes the consumption of these foods possible. It could decrease the off-flavors and improve sensory quality before adding to food products (SIMONS & HALL III, 2018). Although, the thermal treatment of beans can cause considerable changes in nutrients, anti-nutritional compounds, and chemical profiles (LIU et al., 2020; BENTO et al., 2021a). Some studies have shown the impact of traditional cooking (i.e., boiling in water) on the chemical composition of common beans (LINSBERGER-MARTIN et al., 2013; BELMIRO et al., 2020). Still, little has been reported about the effect of non-traditional cooking methods on carioca beans.

Studies that propose the development of aged carioca bean flour from cooked grains and their chemical evaluation are justified in this context. They would provide information about changes in the chemical compounds and help industries improve the nutritional quality of processed foods, while still attending to specific consumers' demands (e.g., vegan, and vegetarian groups). Thus, this research proposes a method of non-traditional cooking with an autoclave. In this method, the water vapor generated in the autoclave directly connects with the product, which is not immersed in a solution (BENTO et al., 2022). Thus, this study evaluated the changes caused by cooking presoakedaged carioca beans in the steam of an autoclave, focusing on its bioactive components, antioxidant activity, and nutritional compounds. Additionally, to identify which carioca 0bean cultivar could preserve the most quantity of bioactive compounds in cooked flour.

MATERIALS AND METHODS

Plant materials

The common bean (*Phaseolus vulgaris* L) cultivars: Madreperola (BRSMG Madrepérola), Dama (TAA Dama), Notavel (BRS Notável), Imperador (IAC Imperador), Gol (TAA Gol) and Bola Cheia (TAA Bola Cheia) from commercial carioca group were grown at the Capivara Farm (16°29́46.7̋ S, 49°17́41.5̋ W), located in the municipality of Santo Antônio de Goiás (GO, Brazil). The local soil is classified as red dystrophic latosol. The soil was fertilized with 200 kg/ha of nitrogen, phosphorus, and potassium, at 11:52:00 concentrations. All genotypes were submitted to the same procedure during handling and post-harvesting. The beans were harvested in September 2018, and dried in an oven with air circulation (40 °C) up to 10 % of moisture for insect disinfestation and later storage. The grains were packed in the polyethylene sacks and stored at 27.5 ± 1.6 °C and 56.9 ± 10.0 % R.H. for three months. After quartering for sample homogenization, random aliquots of 1 kg of the aged samples were separated for flour processing.

Flour preparation and experimental design

The flours were prepared as described by BENTO et al. (2022), following a 2 x 6 factorial design arrangement (raw bean flour and cooked bean flour x 6 carioca bean cultivars), with three original replicates. The flours (raw and cooked) were obtained in triplicate and were stored at -20 °C until analysis.

Total phenolic content

Phenolic compounds were extracted according to the method of FAN & BETA (2017) with some modifications, as we previously described in BENTO et al. (2021a). The extracts were stored at - 4 °C and used for total phenolic content (TPC) and LC-MS analyses. Extraction and all analysis were performed in triplicate. The TPC was determined method reported by SINGLETON et al. (1999). Gallic acid (Sigma-Aldrich, St. Louis, MO, USA) solution was prepared and used as standard at concentrations ranging from 5 to 50 mg·L^{-1}. TPC was expressed as mg GAE·g^{-1} (milligrams of gallic acid equivalent per gram) of bean flour.

Anthocyanin content (TAC)

The extraction of anthocyanins was performed and determined as described by and PRIOR et al. (2010) with modifications. The bean flour was weighed (1.5 g) into a 50 mL conical tube, and 12 mL of the acidified methanol (methanol with HCl 1M, 85:15) was added. After that, the samples were vortexed for 30 seconds, sonicated at 25 °C for 30 min, placed on a shaker for 1 h, and finally centrifuged at 12,857 *g* at 25 °C for 10 min. The extraction was performed twice, and the supernatant was saved for analysis. The samples (supernatant of the crude extracts) were adjusted into a volumetric flask (25 mL) and made up to volume with acidified methanol, and then the absorbance was measured at 535 nm, using acidified methanol as blank.

Antioxidant activity by ABTS and DPPH methods

The extracts for analysis of antioxidant activity were obtained as described by BENTO et al. (2021a). Antioxidant capacity based on the method of

ABTS radical [2,2' -azinobis (3- ethylbenzothiazoline -6- sulfonic acid)] (Sigma-Aldrich, St. Louis, MO, USA) reduction was made as described by RE et al. (1999). Antioxidant potential determined by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, St. Louis, MO, USA) was according to BRAND-WILLIAMS et al. (1995). Both antioxidant determinations were calculated based on the standard Trolox curve and the results were expressed in an antioxidant activity equivalent to Trolox (μM of Trolox·g^{-1} sample).

Condensed tannins

The tannin content was determined according to the methodology described by PRICE et al. (1980), with modifications. The extracts were dissolved in methanol, and stirred for 20 min on a shaking table, followed by centrifugation for 20 min at 2,057 *g*. The reading was performed on an absorbance spectrophotometer at 500 nm. The results were expressed in g of catechin100 g^{-1} of bean flour.

Phytates

The flour bean samples (1 g) were dissolved in 50 mL of 0.2 N HCl solution, followed by shaking for 2 h at 150 rpm. After filtering on filter paper (14 to 18 μm pore), 1 mL of ferric solution was added to 0.5 mL of extract, kept in a water bath for 30 min with boiling water, and centrifuged at 2,447 *g* at 25 ºC, for 30 min. A volume of 1.5 mL of bipyridine solution was added to 1.0 mL of the supernatant, followed by a reading on a spectrophotometer (519 nm) (HAUG & LANTZSCH, 1983).

Saponin and flavonoids profile by LC-MS

An Acquity UPLC system (Waters Co., Milford, MA, USA) was used for phenolic and saponins identification. The UPLC analysis was performed with the Acquity UPLC BEH column (150 × 2.1 mm, 1.7 μm; Waters) with the temperature set at 40 °C. The MS analyzes were performed using a QTOF mass spectrometer (Water, Milford, MA, USA) with an electrospray ionization source in negative mode (ESI^-), acquired in the range of 110–1200 Da. The HPLC injection conditions, as well as the QTOF instrumental parameters, were used as we previously described in BENTO et al. (2021b). The MS conditions were as follows: negative ionization mode (ESI); acquisition range: 110–1200 Da; source temperature: 120 °C; desolvation gas temperature: 350 °C; desolvation gas flow: 500 L h^{-1}; extract cone voltage: 50 V; capillary voltage: 2.8 kV; The mode of acquisition was MSE. The mass accuracy and reproducibility were maintained by infusing lock mass (leucine-enkephalin, 0.2 ng μL^{-1}; [M-H]− ion at m/z 556.2771) and molecular formula assignments were obtained by MassLynx 4.1 software (Waters Corporation). The compound identification was performed considering the respective *m/z* values, fragmentation profile, and literature reports.

Multivariate and univariate statistical analysis

The LC-MS chromatograms (range 2.00 to 9.20 min) were pre-processed using Masslynx version 4.1. The dataset (36x2335) was imported for Matlab, version 2020a, and Icoshift was used for alignment. The singular Value Decomposition (SVD) algorithm was used for PCA after smoothing, baseline correction, normalization (area), and mean-centered processing applied over the variables. The other data were evaluated by the analysis of variance and the Tukey test ($P < 0.05$), and the Levene test applied to verify the variance homogeneity. Pearson's correlation was used, and a heatmap was created in ClusVis (https://biit.cs.ut.ee/clustvis/) using clustering distance and method for the rows Euclidian and Ward, and the tree was ordered by tightest cluster first.

RESULTS AND DISCUSSION

Total phenolic content, anthocyanin, phytates, tannin content, and antioxidant activity

The TPC showed significant variation $P < 0.05$) between cultivar's flours. The cultivar Madreperola raw flour presented the highest value (around 42 mg·g^{-1}) (Table 1). The TPC values for all raw flours were within the reported range by BENTO et al. (2021b) for different carioca bean genotypes (22.0 – 45.0 mg·g^{-1}). The thermal treatment promoted a significant ($P < 0.05$) reduction in TPC, except for the Notavel and Imperador cultivars, since they did not present a significant difference between raw and cooked flours. Its polyphenol profile may cause the stability of TPC in cooked flours of the cultivars Notavel and Imperador since the phenolic acids are more thermally stable (CHÁVEZ-MENDOZA & SÁNCHEZ, 2017). The cultivars Madreperola and Dama presented the most significant decrease for TPC, more than 52% (Table 1). DÍAZ-BATALLA et al. (2006) also reported, a reduction of phenolic compounds in cooked beans from 12.4 to 44.5%.

The cultivars presented significant variation ($P < 0.05$) for TAC, and Notavel and Imperador showed the highest values (around 8.0 μg·g^{-1}) (Table 1). DZOMBA et al. (2013) reported

Table 1 - Total phenolic content (TPC), total anthocyanin content (TAC), antioxidant activity by DPPH and ABTS of different carioca bean cultivars flours (raw and cooked).

Cultivar[1]	Treatment	TPC ($mg \cdot g^{-1}$)	TAC ($\mu g \cdot g^{-1}$)	DPPH (μmol $Trolox \cdot g^{-1}$)	ABTS (μmol $Trolox \cdot g^{-1}$)	Phytate ($mg \cdot g^{-1}$)	Tannins ($mg \cdot g^{-1}$)
Dama	Raw	40.16 ± 1.22^{AB}	4.99 ± 0.17^{DE}	7.59 ± 0.24^{F}	16.33 ± 0.66^{DE}	0.92 ± 0.01^{C}	ND[2]
	Cooked	17.21 ± 0.77^{F}	5.83 ± 0.36^{BCD}	6.45 ± 0.15^{G}	12.18 ± 0.91^{G}	1.20 ± 0.01^{A}	ND
Notavel	Raw	26.44 ± 0.29^{D}	7.88 ± 0.17^{A}	11.11 ± 0.20^{BC}	17.14 ± 0.57^{CD}	0.82 ± 0.02^{D}	1.20 ± 0.06^{B}
	Cooked	28.31 ± 0.13^{CD}	6.64 ± 0.42^{B}	9.09 ± 0.27^{E}	14.67 ± 0.25^{EF}	1.13 ± 0.01^{B}	0.01 ± 0.00^{D}
Madreperola	Raw	42.70 ± 1.30^{A}	5.57 ± 0.17^{CDE}	9.32 ± 0.10^{E}	16.94 ± 0.53^{CD}	0.84 ± 0.01^{D}	ND
	Cooked	20.28 ± 0.32^{EF}	4.83 ± 0.07^{DE}	7.46 ± 0.13^{F}	13.09 ± 1.00^{FG}	1.12 ± 0.01^{B}	ND
Imperador	Raw	38.37 ± 1.47^{B}	7.78 ± 0.29^{A}	13.25 ± 0.26^{A}	21.37 ± 0.30^{A}	0.70 ± 0.02^{E}	1.56 ± 0.04^{A}
	Cooked	36.69 ± 2.64^{B}	8.08 ± 0.74^{A}	10.58 ± 0.27^{CD}	18.71 ± 0.73^{BC}	1.13 ± 0.03^{B}	0.02 ± 0.01^{D}
Gol	Raw	31.42 ± 0.37^{C}	6.72 ± 0.17^{B}	11.74 ± 0.32^{B}	19.65 ± 0.72^{AB}	0.69 ± 0.00^{E}	1.26 ± 0.06^{B}
	Cooked	27.11 ± 0.10^{D}	4.61 ± 0.43^{E}	10.83 ± 0.38^{CD}	15.36 ± 1.01^{DE}	0.91 ± 0.01^{C}	0.01 ± 0.01^{D}
Bola Cheia	Raw	38.99 ± 0.04^{B}	6.15 ± 0.17^{BC}	10.30 ± 0.13^{D}	18.81 ± 0.31^{BC}	0.83 ± 0.02^{E}	0.33 ± 0.04^{C}
	Cooked	21.27 ± 2.05^{E}	6.70 ± 0.50^{B}	9.62 ± 0.02^{E}	15.20 ± 0.27^{DE}	1.15 ± 0.03^{AB}	0.01 ± 0.00^{D}

[1]Means of three determinations ± standard deviation.
[2] ND: not detected. Different letters on the same column represent a statistical difference ($P < 0.05$).

higher anthocyanin contents in brown, spotted black, and pinto beans (0.45-0.59 $mg \cdot g^{-1}$). The anthocyanins are compounds associated with the bean coat color, so the lower values found were expected since the carioca beans present a pale color (light brown). For example, colorful beans, such as black beans, present higher TAC (1.94 to 3.47 $mg \cdot g^{-1}$) (AQUINO-BOLAÑOS et al., 2016). The heat treatment after soaking the grains promoted a decrease in TAC ($P < 0.05$) only in flours made with the cultivars Gol and Notavel (Table 1). The other cultivars did not observe significant variation ($P > 0.05$), which suggested that cooking with the steam in an autoclave could preserve some pigments.

The antioxidant activity measured by DPPH and ABTS radicals was significantly affected by the cultivars ($P < 0.05$). This effect indicated that this variable depends on flavonoids and condensed tannins in the respective common bean (CHÁVEZ-MENDOZA & SÁNCHEZ, 2017). Regarding DPPH and ABTS analyses, the raw flour of cultivar Imperador presented the highest values (13.25 and 21.37 μmol $Trolox \cdot g^{-1}$, respectively), and the lowest value was reportedin the raw flour of cultivar Dama (7.59 and 16.33 μmol $Trolox \cdot g^{-1}$, respectively) (Table 1). Similar results of DPPH (13.4 μmol $Trolox \cdot g^{-1}$) were found by (AQUINO-BOLAÑOS et al., 2016) for common Mexican beans (cream beans). The antioxidant activity presented a positive Pearson's correlation with TAC (DPPH: $r = 0.58$, $P < 0.001$; ABTS: $r = 0.55$, $P < 0.001$) and TPC (ABTS: $r = 0.71$, $P < 0.001$). Results confirmed that anthocyanins and polyphenols confer high antioxidant activity to bean grains. Similar correlations were found by DZOMBA et al. (2013) and AQUINO-BOLAÑOS et al. (2016). The antioxidant potential measured by DPPH presented a decrease between 6.6% (Gol) and 20.15% (Imperador) in the flours of cooked beans (Table 1). The cooked flours also reduced the ABTS radical's antioxidant capacity, and the ratio was between 12.4% (Imperador) and 25.4% (Dama). As the cultivars present different profiles of chemical compounds, the difference in the proportion of decrease is justified.

The phytate content exhibited significant variation ($P < 0.05$) between bean cultivars. The Imperador (Im) and Gol cultivars presented the lowest values (around 0.70%). Similar results were reported by DÍAZ-BATALLA et al. (2006), who reported the phytic acid content of domesticated and wild-type varieties of the common Mexican bean within 0.78–1.76% (raw grains). The phytates presented a negative Pearson's correlation with the antioxidant capacity of the flours (DPPH: $r = -0.71$, $P < 0.001$; ABTS: $r = -0.76$, $P < 0.001$), which suggested that increase of phytate content corroborates for antioxidant activity reduction in cooked flours (Table 1). The phytate content of the common beans increased ($P < 0.05$) with the cooking with the steam in an autoclave after soaking, with the highest values (around 1.2 %) observed for the flours of cooked beans of cultivars Dama (Da_C) and Bola

Cheia (BC_C) (Table 1). These results are contrary to those detected by LINSBERGER-MARTIN et al. (2013), who reported that phytic acid was decreased by high pressure up to 11% in beans and 36% in peas. Still, BELMIRO et al. (2020) showed that phytate content in common beans was not affected by cooking with high pressure.

The decrease of the phytate content occurs because, during the soaking, there are changes in the membrane permeability of the grains, increasing water absorption. Therefore, the intrinsic phosphatase is activated, causing hydrolysis and the increase of phytate releasedinto the soaking water. This process is dependent on the soaking time and the bean genotype. Furthermore, the leaching of phytate in water during the traditional cooking process (boiling in water) occurs due to the greater gradient concentration, which causes the diffusion of this nutrient into the water (VALDÉS et al., 2011). Thus, the time used in the soaking step (6 h) may not be enough for phytate reduction. Moreover, as in this study, the beans were cooked in the autoclaving steam (without broth formation), and the leaching of phytate during the cooking step was not viable. The heating process changes the solubility and consequently the bioaccessibility of phytates (DE OLIVEIRA et al., 2018), and also reduced the amount of inositol hexaphosphate (IP6) and increases other inositol phosphates (inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), and inositol triphosphate (IP3) (RAMÍREZ-CÁRDENASI et al., 2008), thus increasing the results obtained by the methodology used, which could justify the results. Furthermore, phytates have been recognized as antinutrients because of their ability to decrease the bioavailability of protein- or starch-bound divalent and trivalent cations, for example, Fe^{3+} and Zn^{3+} (CHÁVEZ-MENDOZA & SÁNCHEZ, 2017). The IP6 and IP5 reduce the bioavailability of zinc and iron while IP4 and IP3 do not have this characteristic (RAMÍREZ-CÁRDENASI et al., 2008). Besides, phytates can reduce triglycerides, serum cholesterol, and iron-mediated oxidation. They also present anti-cancer, antioxidant, anti-diabetics, and kidney stone decalcification properties (BEPARY et al., 2017).

Tannin was not detected in the cultivars Dama and Madreperola (Table 1). These results were expected because these present cultivars characteristics of slow-darkening beans (BENTO et al., 2020; BENTO et al., 2021b), and the color of the bean seed coat is attributed to the presence and the concentration of anthocyanins and condensed tannins (CHÁVEZ-MENDOZA & SÁNCHEZ, 2017). Condensed tannins are polymeric flavonoids that comprise a small part of the widely diverse group of phenolic compounds. They are considered anti-nutrients because they affect nutrient bioavailability for the consumer. The condensed tannin content presented a positive Pearson's correlation with the antioxidant capacity of the flours (DPPH: $r = 0.75$, $P < 0.001$; ABTS: $r = 0.69$, $P < 0.001$). These results followed the line that tannins also present health benefits since they are antioxidants and potentially anti-carcinogenic (CHÁVEZ-MENDOZA & SÁNCHEZ, 2017).The condensed tannin content of cooked flours presented a significant reduction ($P < 0.05$). Other researchers also reported that some cooking methods promote reductions in tannin content, which can be explained by the rupture of the cell wall promoted by cooking with pressure, initiating fluid migration from the cell to the extracellular medium, and extracting the tannin together. Furthermore, the heat treatment may cause changes in their structure or solubility (CHÁVEZ-MENDOZA & SÁNCHEZ, 2017; BELMIRO et al., 2020).

The TPC, TAC, phytates, condensed tannin content, and antioxidant activity and their correlation are shown in the cluster tree (Figure 1) created to describe differences among the flours with a total variance of 83.25%. The flours were clustered by cultivars and treatment, indicating that both cultivars and heat treatment significantly influence flours properties. The cultivars Gol, Imperador, and Notavel, huddled in an individual group, presented a higher distance from those flours of cooked beans (Figure 1). These results reflected that these cultivars' dissimilarities compared with the others, especially the highest amount of condensed tannin and TAC. The cluster tree also allows seeing that the flours of cooked beans from cultivar Imperador have the highest antioxidant activity (DPPH and ABTS), TAC, and TPC. The other cultivar that retains part of these characteristics before cooking was Gol.

Flavonoids and saponins profile by UPLC-QToF-MS^E: Unsupervised chemometric evaluation

The chromatogram of the ethanolic extract from carioca beans exhibited phenol and saponins as the main compounds. Their tentative identification resulted in thirty compounds and is presented by BENTO et al. (2021b). To evaluate the effect of the heat treatment on the phenolic and saponins profile, principal component analysis (PCA) was applied to highlight specific changes

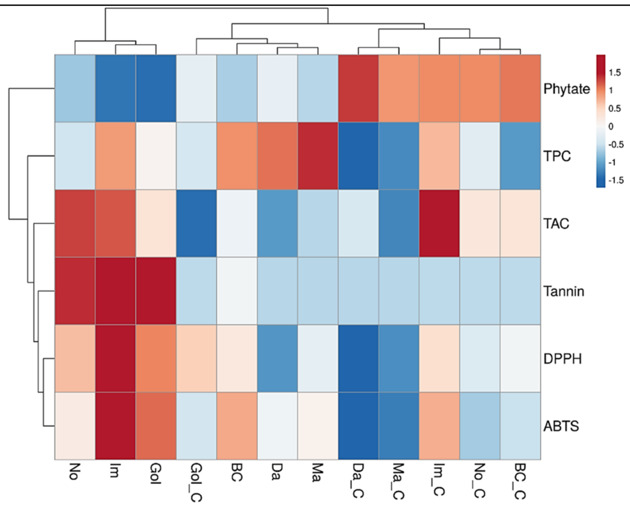

Figure 1 - Heatmap visualization showing compounds identified by sum of squared differences from points to centroids where red and blue color represent the highest and the lowest concentration, respectively. Beans are described as No and No_C (Notavel), Im and Im_C (Imperador), Gol and Gol_C (Gol), B.C. and BC_C (Bola Cheia), Da and Da_C (Dama), and Ma and Ma_C (Madreperola), where the 'C' indicates cooked flours.

and sample grouping. The score plot PC1 vs PC2 explained the variance of 71.31 % and 10.35%, respectively, resulting in a two-dimension total conflict of 81.66%. The PC1 scores clustered the samples in two groups: flours of cooked beans pressed on the left (negative values of PC1) and raw beans flours on the right (positive values of PC1) (Figure 2a).

The PC1 showed phenolic compounds like catechin, epicatechin, kaempferol, soyasaponins αg and βg in positive and soyasaponin Ba and Bb in the negative (Figure 2b). These results were following the previous report for carioca beans since the group of phenolic compounds involves various sorts of molecules, and the most abundant in common beans are flavonoids, such as catechin, kaempferol, and procyanidin (SANTOS et al., 2020; BENTO et al., 2021b). Moreover, kaempferol also was predicted to be a marker for identifying carioca beans genotype sensible to the browning process during storage at ambient conditions (BENTO et al., 2021b). Regarding health benefits, polyphenolic compounds present in beans act as antioxidant agents due to their ability to scavenge free radicals and bind to free molecules forming chelates, which reduce oxidative stress processes in the cell. As a result, they also can reduce inflammatory processes (SANTOS et al., 2020).

Regarding the effect of the heat treatment, the cooked bean flours presented a reduction in flavonoid compounds and an increase in soyasaponins from group B (Figure 2b). Degradation of phenolic compounds is common when the samples are subjected to a cooking procedure resulting in decreased concentration (BENTO et al., 2021a). These reductions in phenolic compounds observed in cooked flours justified the reduction in antioxidant activity, TPC as well in tannins content (Table 1). Furthermore, thermal treatment affords a conversion of soyasaponin αg and βg in Ba and Bb by the loss of DDMP linkage, which resulted in an increase of soyasaponin Ba and Bb in cooked flours (BENTO et al., 2021a), as can be observed in the negative value of PC1. The saponins present functional benefits, such as antioxidant activity, resulting in health benefits (e.g., saponins from group DDMPs) (JEEPIPALLI et al., 2020; BENTO et al., 2021b).

The PC2 clustered the bean flours regarding their chemical similarities presenting cultivars Bola Cheia, Imperador, and Gol on the top (positive) and cultivars Notavel, Dama, and Madreperola on the bottom (negative) (Figure 2c). The cultivars Gol, Imperador, and Bola Cheia flours showed the highest concentration of phenolic compounds as catechin-*O*-hexoside, procyanidin

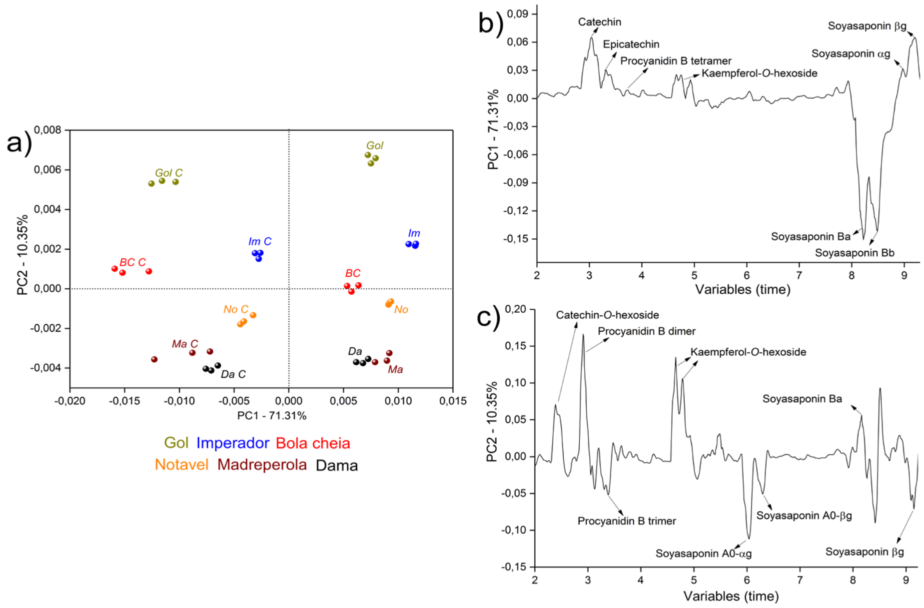

Figure 2 - PC1 x PC2 scores plot on the beans in different heat times (a). Loading in line with scores (b and c) for PC1 and PC2, respectively. Beans are described as: No and No_C (Notavel), Im and Im_C (Imperador), Gol and Gol_C (Gol), B.C. and BC_C (Bola Cheia), Da and Da_C (Dama), and Ma and Ma_C (Madreperola), where the ‘C’ indicates cooked flours.

B dimer, kaempferol-*O*-hexoside, and the soyasaponin Ba. These compounds may contribute to the most increased antioxidant activity and TPC of these bean genotypes. Finally, the flours of Notavel, Dama, and Madreperola cultivars presented the highest amount of procyanidin B trimer, soyasaponin A0-αg, A0-βg, and βg (Figure 2c). The highest concentration of soyasaponin A0 (FSAGs) is the primary cause of undesirable bitter taste and astringent flavor in soybean/bean food products (CHITISANKUL et al., 2018).

The variations of the content of soyasaponin A0-αg, A0-βg were evaluated by semi-quantification (Figure 3) to statistically certify the compound’s variation among the flours of Notavel, Dama, and Madreperola. This cultivar presented the highest amount of soyasaponin A0-αg, and the cultivar Notavel showed the highest amount of soyasaponin A0-βg. The heat treatment was not able to reduce these compounds on the cooked bean flours. Thus, these flours might not be suitable for food development since they may present an undesirable taste and flavor, and the heat treatment could not reduce it. It is essential to say that saponins were considered antinutrients. Still, recent studies have shown that dietary saponins from grains, like beans, present health benefits as therapeutic to obesity pathogenicity control since they contribute to bodyweight management, waist circumference, and decrease blood pressure (JEEPIPALLI et al., 2020).

CONCLUSION

Cooking the beans in the autoclave steam affected the chemical profile of carioca bean flours, and these changes were dependent on the bean cultivars. The LC-MS analysis of phenolic and saponins of the cooked flours showed a reduction in phenolic compounds and an increase in soyasaponin Ba and Bb, soyasaponins from group B. Concerning the cultivars, Notavel, Dama, and Madreperola presented the highest amount of soyasaponin A0. Thus, the flours of these cultivars might not be suitable for food development, at least in the native form (raw flours) since they may present an undesirable taste and flavor. Conversely, cultivars Gol, Imperador, and Bola Cheia showed the highest concentration of phenolic compounds as kaempferol-*O*-hexoside and the soyasaponin Ba. Thus, the

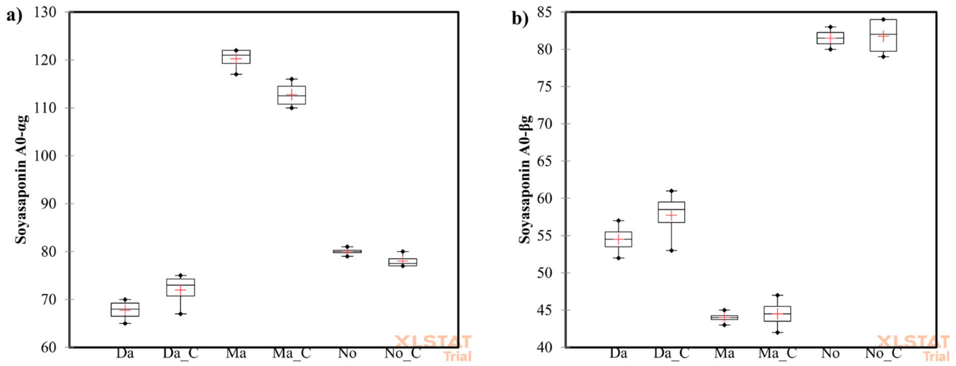

Figure 3 - Box plot of semi-quantitative (relative peak area) evaluation from soyasaponin A of the beans in different heat times: (a) Soyasaponin A0-αg and (b) Soyasaponin A0-βg. Beans are described as No and No_C (Notavel), Da and Da_C (Dama), and Ma and Ma_C (Madreperola), where the 'C' indicates cooked flours.

cultivars Imperador and Gol showed that they would be suitable to produce cooked flours by autoclave steaming after soaking, as food ingredients, and retain part of their antioxidant activity and bioactive compounds, such as polyphenols and saponins.

ACKNOWLEDGMENTS

The authors thank EMBRAPA for the research financial of the project Dark bean approved at the Call 07/2011 – Macroprograma 2 – code 02.11.07.010.00.00. Andwasfinanced in partbythe Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Financecode 001.

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

REFERENCES

AQUINO-BOLAÑOS, E. N., et al. Anthocyanins, polyphenols, flavonoids and antioxidant activity in common bean (*Phaseolus vulgaris L.*) landraces. **Emirates Journal of Food and Agriculture**, p.581-588. 2016. Available from: <https://www.ejfa.me/index.php/journal/article/view/1178>. Accessed: Nov. 12, 2021. doi: 10.9755/ejfa.2016-02-147.

BELMIRO, R. H., et al. Effects of high processing on common beans (*Phaseolus vulgaris l.*): cotyledon structure, starch characteristics, and phytates and tannins contents. **Starch - Stärke**, v.72, n.3-4, p.1900212. 2020. Available from: <https://doi.org/10.1002/star.201900212>. Accessed: Nov. 12, 2021. doi: 10.1002/star.201900212.

BENTO, J. A. C., et al. Convenience of non-conventional methods for evaluation of the culinary quality of beans. **Research, Society and Development**, v.9, n.11, p.e44491110103. 2020. Available from: <https://rsdjournal.org/index.php/rsd/article/view/10103>. Accessed: Nov. 2, 2021. doi: 10.33448/rsd-v9i11.10103.

BENTO, J. A. C., et al. Functional, thermal, and pasting properties of cooked carioca bean (Phaseolus vulgaris L.) flours. **Applied Food Research**, v.2, n.1, p.100027. 2022. Available from: <https://www.sciencedirect.com/science/article/pii/S2772502221000275>. Accessed: Jan. 12, 2022. doi: 10.1016/j.afres.2021.100027.

BENTO, J. A. C., et al. Chemical profile of colorful bean (Phaseolus vulgaris L) flours: Changes influenced by the cooking method. **Food Chemistry**, v.356, p.129718. 2021a. Available from: <https://www.sciencedirect.com/science/article/pii/S030881462100724X>. Accessed: Jan. 12, 2022. doi: 10.1016/j.foodchem.2021.129718.

BENTO, J. A. C., et al. Phenolic and saponin profile in grains of carioca beans during storage. **LWT-Food Science Technology**, v.139, p.110599. 2021b. Available from: <http://www.sciencedirect.com/science/article/pii/S0023643820315875>. Accessed: Jan. 12, 2022. doi: 10.1016/j.lwt.2020.110599.

BEPARY, R. H., et al. Studies on physico-chemical and cooking characteristics of rice bean varieties grown in NE region of India. **Journal of food science and technology**, v.54, n.4, p.973-986. 2017. Available from: <https://doi.org/10.1007/s13197-016-2400-z>. Accessed: Jan. 12, 2022. doi: 10.1007/s13197-016-2400-z.

BRAND-WILLIAMS, W., et al. Use of a free radical method to evaluate antioxidant activity. **LWT - Food Science and Technology**, v.28, n.1, p.25-30. 1995. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0023643895800085>. Accessed: Jan. 12, 2022. doi: 10.1016/s0023-6438(95)80008-5.

CELMELI, T., et al. The Nutritional Content of Common Bean (Phaseolus vulgaris L.) Landraces in Comparison to Modern Varieties. **Agronomy**, v.8, n.9, p.1-9. 2018. Available from: <https://www.researchgate.net/publication/327256826_The_Nutritional_Content_of_Common_Bean_Phaseolus_vulgaris_L_

Landraces_in_Comparison_to_Modern_Varieties>. Accessed: Jan. 12, 2022. doi: 10.3390/agronomy8090166.

CHÁVEZ-MENDOZA, C.; E. SÁNCHEZ. Bioactive compounds from Mexican varieties of the common bean (*Phaseolus vulgaris*): Implications for health. **Molecules**, v.22, n.8, p.1360. 2017. Available from: <https://pubmed.ncbi.nlm.nih.gov/28817105>. Accessed: Jan. 12, 2022. doi: 10.3390/molecules22081360.

CHITISANKUL, W. T., et al. Saponin composition complexities in hypocotyls and cotyledons of nine soybean varieties. **LWT - Food Science and Technology**, v.89, p.93-103. 2018. Available from: <http://www.sciencedirect.com/science/article/pii/S0023643817307545>. Accessed: Jan. 12, 2022. doi: 10.1016/j.lwt.2017.10.016.

DE OLIVEIRA, A. P., et al. Effect of cooking on the bioaccessibility of essential elements in different varieties of beans (*Phaseolus vulgaris L.*). **Journal of Food Composition and Analysis**, v.67, p.135-140. 2018. Available from: <http://www.sciencedirect.com/science/article/pii/S0889157518300127>. Accessed: Jan. 12, 2022. doi: 10.1016/j.jfca.2018.01.012.

DÍAZ-BATALLA, L., et al. Chemical components with Health Implications in Wild and Cultivated Mexican Common Bean Seeds (*Phaseolus vulgaris L* .). **Journal of Agricultural and Food Chemistry**, v.54, p.2045-52. 2006. Available from: <https://pubmed.ncbi.nlm.nih.gov/16536573>. Accessed: May 12, 2022. doi: 10.1021/jf051706l.

DZOMBA, P., et al. Anthocyanin content and antioxidant activities of common bean species (*Phaseolus vulgaris* L.) grown in Mashonaland Central, Zimbabwe. **African Journal of Agricultural Research**, v.8, p.3330-3333. 2013. Available from: <https://academicjournals.org/journal/AJAR/article-full-text-pdf/F4BC4AF36155>. Accessed: May 12, 2021. doi: 10.5897/AJAR12.225.

FAN, G.; T. BETA. Discrimination of geographical origin of Napirira bean (*Phaseolus vulgaris* L.) based on phenolic profiles and antioxidant activity. **Journal of Food Composition and Analysis**, v.62, p.217-222. 2017. Available from: <http://www.sciencedirect.com/science/article/pii/S0889157517301552>. Accessed: Feb. 12, 2022. doi: 10.1016/j.jfca.2017.07.001.

HAUG, W.; H.-J. LANTZSCH. Sensitive method for the rapid determination of phytate in cereals and cereal products. **Journal of the Science of Food and Agriculture**, v.34, n.12, p.1423-1426. 1983. doi: 10.1002/jsfa.2740341217.

JEEPIPALLI, S. P. K., et al. New insights into potential nutritional effects of dietary saponins in protecting against the development of obesity. **Food Chemistry**, v.318, p.126474. 2020. Available from: <http://www.sciencedirect.com/science/article/pii/S0308814620303368>. Accessed: Feb. 12, 2022. doi: 10.1016/j.foodchem.2020.126474.

LINSBERGER-MARTIN, G., et al. High hydrostatic pressure influences antinutritional factors and in vitro protein digestibility of split peas and whole white beans. **LWT - Food Science and Technology**, v.51, n.1, p.331-336. 2013. Available from: <http://www.sciencedirect.com/science/article/pii/S0023643812004562>. Accessed: Feb. 12, 2022. doi: 10.1016/j.lwt.2012.11.008.

LIU, Y., et al. Effect of different cooking methods and heating solutions on nutritionally important starch fractions and flatus oligosaccharides in selected pulses. **Cereal Chemistry**, v.n/a, n.n/a. 2020. Available from: <https://doi.org/10.1002/cche.10344>. Accessed: Nov. 12, 2020. doi: 10.1002/cche.10344.

PRICE, M. L., et al. Tannin content of cowpeas, chickpeas, pigeon peas, and mung beans. **J Agric Food Chem**, v.28, n.2, p.459-61. 1980. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/7391382>. Accessed: Feb. 12, 2020. doi: 10.1021/jf60228a047.

PRIOR, R. L., et al. multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. **Journal of the Science of Food Agriculture**, v.90, n.9, p.1473-8. 2010. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20549799>. Accessed: Feb. 12, 2020. doi: 10.1002/jsfa.3966.

RAMÍREZ-CÁRDENASI, L., et al. Effect of domestic processing on nutrient and antinutritional factor content in different cultivars of common beans. **Food Science and Technology**, v.28, n.1, p.200-213. 2008. doi: 10.1590/S0101-20612008000100029.

RE, R., et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radic Biol Med**, v.26, n.9-10, p.1231-7. 1999. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/10381194>. Accessed: Apr. 12, 2022. doi: 10.1016/s0891-5849(98)00315-3.

SANTOS, E.; MARQUES, G.; LINO-NETO, T. Chapter 29 - Phaseolus vulgaris L. as a functional food for aging protection. In: PREEDY, V. R. e PATEL, V. B. (Ed.). **Aging (Second Edition)**: Academic Pressv. 2020. p.289. ISBN 978-0-12-818698-5. Accessed: Feb. 12, 2020. doi: 10.1016/B978-0-12-818698-5.00029-8.

SIMONS, C. W.; C. HALL III. Consumer acceptability of gluten-free cookies containing raw cooked and germinated pinto bean flours. **Food Science & Nutrition**, v.6, n.1, p.77-84. 2018. Available from: <https://doi.org/10.1002/fsn3.531>. Accessed: Feb. 12, 2020. doi: 10.1002/fsn3.531.

SINGLETON, V. L., et al. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: (Ed.). **Oxidants and Antioxidants Part A**: Academic Press, v.299, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, p.152-178. (Methods in Enzymology). Accessed: Feb. 12, 2020.

VALDÉS, S. T., et al. Association of genotype and preparation methods on the antioxidant activity, and antinutrients in common beans (Phaseolus vulgaris L.). **LWT - Food Science and Technology**, v.44, n.10, p.2104-2111. 2011. Available from: <http://www.sciencedirect.com/science/article/pii/S0023643811001976>. Accessed: Feb. 12, 2020. doi: 10.1016/j.lwt.2011.06.014.