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Phenolic and saponin profile in grains of carioca beans during storage

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ABSTRACT

The objective of this study was to evaluate the behavior of slow and fast-darkening carioca beans during storage, and the profile of phenolics and saponins, aiming to identify which compounds may be involved in the browning process of the integument of these grains. Cultivars Madreperola and Dama presented the lowest total color difference (ΔE) and the highest luminosity (L*) values during storage time indicating to be slow-darkening cultivars. In contrast, the cultivars Notavel and Imperador rapidly darkened with storage time, evidenced by the highest ΔE and lowest L*. Regarding the content of phenolic during storage, it was observed a reduction for free phenolics in fast-darkening beans and the maintenance/increase in slow-darkening beans. In the PCA of the phenolic profile, Notavel and Imperador were similar, differing from Dama and Madreperola, indicating that fast-darkening grains have similar phenolic profile. The OPLS/DA indicated that aged grains from bright beans had more kaempferol than darker beans. For saponins, the cultivars Imperador and Notavel presented higher content of Soyasaponin Bd, which might be related to the darkening process. Besides, kaempferol was found at higher concentrations in slow-darkening aged grains and can be a chemical marker of darkening in carioca beans.

1. Introduction

Common beans (*Phaseolus vulgaris* L.) are a valuable food source for humans around the world providing an important source of protein, dietary fiber, complex carbohydrates, vitamins, minerals, and phenolics. Phenolics, which include phenolic acids, flavonoids, and proanthocyanidins, are particularly notable because of their potent antioxidant properties (Chen, Tang, et al., 2015). Their presence gives common bean grains diverse colors, and are found in both the cotyledons and seed coats, but most are concentrated in the seed coats (Yang, Gan, Ge, Zhang, & Corke, 2018). Moreover, these compounds have been presumed as prebiotic compounds in the colon and as protective factors against cariogenic bacteria (*Streptococcus mutans*) in the mouth and ulcerogenic *Helicobacter pylori* in the stomach (Cires, Wong, Carrasco-Pozo, & Gotteland, 2017).

Among different bioactive components present in common beans, saponins have attracted a considerable interest owing to their diverse biological activities (Singh, Singh, Singh, & Kaur, 2017). Among these, saponins demonstrate health functionalities with antioxidant, anti-inflammatory and anti-diabetic properties (Luo, Cai, Wu, & Xu, 2016), immune-modulatory activities (Sun, Yan, Guo, & Zhao, 2014), suppress tumor progression, as a colon cancer prevention and as a cure for Alzheimer's disease (Chitisankul et al., 2018; Singh et al., 2017).

In the post-harvest darkening of pinto beans, the involvement of phenolics, specifically the flavonoids catechin and kaempferol, has also been suggested (Beninger et al., 2005). Seed coat darkening has a negative impact on the marketability of dry beans because this color change is believed to be associated with a hard-to-cook trait and hence, requires extended cooking times (Bento et al., 2020). Bean darkening is prevalent in cultivars with a high phenolic content in the seed coat, and the degree of this phenomenon is proportional to the loss of phenolics during storage (Beninger et al., 2005; Luthria & Pastor-Corrales, 2006; Nasar-Abbas et al., 2009).

Given the importance of phenolic compounds and saponins present

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in beans and the darkening process of their grains, the objective of this study was to evaluate the behavior of slow and fast-darkening carioca bean cultivars during storage, as well as the profile of phenolic compounds and saponins of some contrasting cultivars, aiming to identify which compounds may be involved in the process of browning the tegument of those genotypes.

2. Material and methods

2.1. Solvents and reagents

Methanol, ethyl acetate, and acetonitrile were HPLC and LC-MS grade and purchased from Merck (Darmstadt, Germany). Formic acid was HPLC grade and purchased from CNW Technologies GmbH. The gallic acid standard and the Folin–Ciocalteu's phenol reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). The others chemicals used in the experiments, as ethanol, sodium carbonate, NaOH and HCl were of analytical grade, and deionized water was used.

2.2. Plant materials preparation

Beans from commercial carioca group (beige integument): cultivars TAA Dama, BRSMG Madreperola, IAC Imperador, and BRS Notavel were used, called as Dama, Madreperola, Imperador, and Notavel throughout the article, respectively. Beans were produced at the experimental field of Capivara farm of Embrapa Rice and Beans, located in the municipality of Santo Antônio de Goiás, State of Goiás, Brazil, using a randomized block design, with 6 rows of 6 m each, and three replicates (crop at august 2018). After harvesting, the beans were processed (dried and insect disinfested) and conditioned with a moisture content of approximately 10% in 10 kg cotton sacks. After quartering for sample homogenization, grain random aliquots of 1 kg were separated for evaluation (recently harvested beans), that is, approximately 20 days after processing. The rest of the beans of each genotype were kept in the polyethylene sacks and stored under ambient conditions (27.5 ± 1.6 °C/56.9 ± 10.0% RH) for 3 months and 6 months. The dry beans were ground in a mill, cyclone type (CT 193 Cyclotec™, FOSS, Denmark), to a fine powder (<200 μm) for chemical analysis.

2.3. Instrumental color

The color of samples was determined by direct reading in ColorQuest XE colorimeter (Hunter Lab, USA), which considers the Cielab system (L* a* b*), responsible for luminosity and intensity of red and yellow color, respectively. Equipment was previously calibrated with a ceramic plate according to standards established by the manufacturer (Y = 85.8 x = 0.3195; y = 0.3369) using the illuminant D65 which represents the average daylight. Results obtained were presented in terms of luminosity (L*), chromaticity a* and b*, and total color difference (equation (1)). The difference between each parameter (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 (ΔE* = total color difference).

$$\Delta E^{*} = \sqrt{(n L*)\hat{2} + (n a^{*})\hat{2} + (n b^{*})\hat{2}} \quad (1)$$

2.4. Determination of bean resistance to cooking by Mattson cooker (RMC)

Classification of bean resistance to cooking was obtained after cooking beans in the Mattson cooker apparatus with 25 plungers of 90 g and 1.0 mm of diameter of pin tip, interrupting the test run after the drop of the 13th rods (Bento et al., 2020). The time (t13) recorded until the drop of the 13th rods converted into a rank (RMC), according to following scale: very susceptible for cooking (t13 < 16 min); moderately susceptible (16 min < t13 < 20 min); normally resistant (21 min < t13 < 29 min); moderately resistant (29 min < t13 < 32 min); resistant (33 min < t13 < 36 min); and very resistant for cooking (t13 > 36 min).

2.5. Total anthocyanin content (TAC)

Anthocyanins in bean powder were extracted according to the method described by Abdel-Aal, Young, and Rabalski (2006); Prior et al. (2010) with modifications: 1.5 g of the powder was weighed into a 50 mL conical tube. 12 mL of the acidified methanol (methanol with HCl 1 M, 85:15) was added to the samples. The samples were vortexed for 30s followed by sonication at room temperature (25 °C) for 30 min. Samples were then placed on an orbital shaker for 1 h and subsequently centrifuged at 10,000 g at 25 °C for 10 min. The extraction was made twice, and the supernatant was collected for analysis. TAC was determined using the spectrophotometric method previously described (Abdel-Aal et al., 2006). The supernatant of the crude extracts was poured into a 25 mL volumetric flask and made up to volume with acidified methanol. Absorbance was measured on a UV–Vis spectrophotometer (FEMTO, 700 Plus, SP, Brazil) at 535 nm, using acidified methanol as blank. The results were expressed as μg 3-glycoside cyanidin per g of samples.

2.6. Phenolics extraction

The free, soluble conjugate and insoluble-bound phenolic acids from beans were prepared using the procedure explained in the literature (Wang et al., 2016) with a slight modification: 15 mL of 40% ethanol was added to 1.0 g of the powder, and then samples were sonicated for 10 min at room temperature (25 °C). The mixture was centrifuged at 4000 *g* for 5 min at room temperature (25 °C) (Eppendorf, Centrifuge 5804 R, Germany) to obtain free phenolic acids. The residue was extracted twice, and the supernatants were combined. The supernatants were evaporated at 50 °C under reduced pressure until the remaining 5 mL. Then, it was extracted three times with ethyl acetate at 1:1 (v/v) solvent to supernatant ratio. The combined extracts (organic phase) were evaporated to dryness at 50 °C under reduced pressure, and subsequently dissolved in 50% methanol (5 mL) to obtain free phenolic acid fraction.

The supernatant containing soluble conjugates (water phase) was subsequently hydrolyzed with 20 mL of 4 M NaOH for 4 h at room temperature. The resultant hydrolysate was acidified to pH 2 using 6 M HCl followed by extraction with ethyl acetate (three times). The extracts (organic phase) were combined and evaporated to dryness at 50 °C under reduced pressure and subsequently dissolved in 50% methanol (5 mL) to obtain soluble conjugate fraction.

The solid residues were treated with 20 mL of 4 M NaOH and hydrolyzed for 4 h at room temperature and acidified to pH 2 with 6 M HCl. Then, it was centrifuged at 4000 *g* for 5 min. The supernatant was extracted with ethyl acetate (three times). The combined extracts (organic phase) were evaporated to dryness at 50 °C under reduced pressure and subsequently dissolved in 50% methanol (5 mL) to obtain insoluble bound phenolics.

2.7. Determination of total phenolic contents

Total phenolic contents were determined by using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). The reaction was initiated by the addition of 2.5 mL of Folin–Ciocalteu phenol reagent (10-fold dilution) to 1 mL of the diluted extract. Following 5 min incubation at room temperature, the reaction was terminated by the addition of 2 mL of 7.5% sodium carbonate (w/v). After incubation at room temperature for 120 min, the absorbance of the mixture was measured at 760 nm using the respective solvent as blank. Gallic acid was used as the standard for a calibration curve (5–50 mg L^{-1}), and the results were expressed as mg of gallic acid equivalents per wet weight of beans.

2.8. UPLC-QTOF-MS analysis: phenolics and saponins profile

The analyses of free phenolics, conjugated and bound phenolics extracts were performed using an Acquity UPLC (Water, Milford, MA, USA) system coupled to a Xevo Quadrupole and Time-of-Flight mass system (Q-TOF) for phenolic and saponins identification. A Waters Acquity BEH C18 column for separation condition (150 mm × 2.1 mm, 1.7 μm) set at 40 °C. An injection volume of 5-μL aliquot of the phenolic extract was subjected to an exploratory gradient with the mobile phase composed of deionized water (A) and acetonitrile (B), both containing formic acid (0.1% v/v). The extracts were subjected to exploratory gradient as follows: 2–95% B (15.0 min), 100% B (15.1–17.0 min), 2% B (17.1–19.0) with a flow rate of 400 μL min^{-1}.

Ionization was 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 and the optimized instrumental parameters were as follows to negative: capillary voltage at 2.8 kV, cone voltage at 50 V, source temperature at 120 °C, desolvation temperature at 350 °C, flow cone gas at 20 L h^{-1}, desolvation gas flow at 500 L h^{-1}. The mode of acquisition was MS^E. The system was controlled using Mass Lynx 4.1 software (Waters Corporation, MA, USA).

2.9. Chemometrics analysis

The UPLC-MS data of all samples were pre-processed using the MarkerLynx XS software for peak retention and alignment (Waters Corporation, Manchester, UK). The following set parameters were retention time (t_R) 2.2–5.0 min, extracted ion chromatograms windows mass 0.05, mass range 110–1200 Da. After, the data sets were imported into SIMCA (Umetrics, Umea, Sweden) to identify potential discriminatory chemical markers using center scaling. An arbitrary ID was assigned to each t_R-*m/z* pair based on their order elution from the UPLC system. A list with the identities of the detected peaks was generated using pairs (retention time/mass data) as the identifier for each peak. The resulting three-dimensional data comprising peak number (t_R-*m/z* pairs), sample name, and ion intensity were analyzed by PCA and OPLS-DA.

2.10. Statistical analysis

All analyzes were performed in three repetitions. The data were expressed as mean ± standard deviation. The results were subjected to analysis of variance (one-way ANOVA) by using the Statistic 10.0® software (StatSoft®, SOUTH AMERICA). Differences among means were evaluated by the Tukey test and the significance was accepted at $p <$ 0.05 level.

3. Results and discussion

3.1. Technological properties of bean sample

Darkening occurred during storage can also be noted by parameters of instrumental color, where the values of L* had decreased, and ΔE (color total difference) had increased (Table 1). The color of carioca beans seed coat is a characteristic that influences the final consumer's purchase, while browning is often associated with longer storage and cooking time (Bento et al., 2020). Cultivars Madreperola and Dama presented the lowest ΔE_3, ΔE_6, and the highest luminosity values during storage time (Table 1), close to 55, a value that according to Arns et al. (2018) has a higher market value. This characteristic was expected since the cultivar Madreperola and Dama are cultivars of slow darkening.

On the other hand, the cultivars Notavel and Imperador rapidly darkened with storage time, evidenced by the highest ΔE_3, ΔE_6, and the lowest luminosity values (Table 1). According to Bento et al. (2020), beans with color differences (ΔE) of less than four units are generally not visible to or perceived by the majority of observers. Therefore, the consumers would probably see no difference in the color of the Dama and Madeperola and a small alteration in the colors of the Notavel and Imperador after three storage months. However, the consumers would be able to detect changes in the color of these genotypes at 180 days of storage (Table 1).

In the evaluation of bean resistance to cooking in the Mattson apparatus, it was verified that only cultivar Madreperola recently harvested showed normal resistance to cooking. However, in the third month of storage, this cultivar, and all other cultivars, presented an increase in cooking time (Table 1). Cultivars Dama and Imperador presented the longest cooking time and the highest increase of percentage value during the entire storage period, 58.1% and 65.6% respectively. In the sixth month, cultivar Notavel showed a 48.5% increase in cooking time, reaching 64.9 min of cooking (very resistant to cooking). These results indicate that the rate of increase in cooking time varied as a function of storage time, corroborating with the results encountered by other authors which also observed that cooking time (bean resistance to cooking) gets longer as the storage period increases (Bento et al., 2020; Silochi et al., 2016).

Moreover, in evaluation of bean resistance to cooking in Mattson apparatus (RCM), it was verified that almost all cultivars had a profile of very resistance to cooking. Only the cultivars Madreperola freshly harvested and at 3 moths of storage presented RMC corresponding to normally and resistant classes, respectively (Table 1). Finally, it should be

Table 1
Instrumental color (Luminosity, a*and b* chromaticity and total color difference - ΔE) and bean resistance to cooking by Mattson in three periods (freshly harvested, at 3 months and 6 months) of different carioca common bean cultivars.

Storage	Parameters	Common bean carioca cultivar			
		Dama	Madreperola	Notável	Imperador
Freshly harvested	L *	53.86 ± 1.52^{A}	53.60 ± 1.73^{A}	48.63 ± 2.53^{A}	50.75 ± 2.05^{A}
	a *	7.06 ± 0.89^{B}	7.00 ± 0.98^{B}	10.03 ± 0.68^{A}	9.08 ± 0.78^{A}
	b *	20.25 ± 1.22^{A}	20.56 ± 2.01^{A}	21.46 ± 2.08^{A}	21.75 ± 1.98^{A}
	Mattson[1]	54.9 ± 2.2^{A} (very resistant)	28.6 ± 0.3 (normal)	43.7 ± 5.9^{B} (very resistant)	58.3 ± 2.1^{A} (very resistant)
3 months	L *	51.43 ± 1.43^{A}	51.40 ± 1.40^{A}	44.39 ± 1.93^{B}	46.42 ± 1.46^{B}
	a *	7.84 ± 0.48^{B}	8.81 ± 0.88^{B}	11.12 ± 0.99^{A}	10.11 ± 0.89^{A}
	b *	20.35 ± 0.88^{A}	20.98 ± 0.89^{A}	21.20 ± 0.98^{A}	21.20 ± 0.99^{A}
	ΔE_3	1.63 ± 0.48^{B}	2.21 ± 0.23^{B}	4.38 ± 0.62^{A}	4.48 ± 0.53^{A}
	Mattson[1]	78.9 ± 3.1^{A} (very resistant)	35.5 ± 1.2^{C} (resistant)	51.6 ± 4.2^{B} (very resistant)	72.6 ± 4.1^{A} (very resistant)
6 months	L *	50.36 ± 1.63^{A}	50.40 ± 1.40^{A}	39.33 ± 1.99^{B}	42.58 ± 1.85^{B}
	a *	8.43 ± 0.84^{B}	9.13 ± 0.91^{B}	12.19 ± 0.91^{A}	11.52 ± 0.51^{A}
	b *	20.73 ± 0.37^{A}	21.90 ± 0.91^{A}	20.24 ± 0.42^{A}	20.97 ± 0.79^{A}
	ΔE_6	2.89 ± 0.43^{C}	3.61 ± 0.22^{C}	14.47 ± 0.89^{A}	11.23 ± 0.98^{B}
	Mattson[1]	86.8 ± 3.6^{B} (very resistant)	48.7 ± 0.7^{D} (very resistant)	64.9 ± 1.1^{C} (very resistant)	96.40 ± 1.9^{A} (very resistant)

[1] Time in minutes for dropping off 13 rods and classification of Resistance to cooking by Mattson Coker; Simple arithmetic mean of three determinations ± standard deviation. Different letters on the same line present statistical differences ($p < 0.05$).

noted that the high resistance to cooking observed for cultivars Dama, Notavel and Imperador may be related to the environmental conditions in the cultivation, as well as to the grain genotype (Bento et al., 2020; Siqueira et al., 2014, 2016).

3.2. Chemical characterization of beans samples

The content of free phenolics showed no significant variation ($p < 0.05$) only for the cultivar Dama. On the other hand, the cultivar Madreperola showed an increase of 19%, while the cultivars Notavel and Imperador showed a reduction of 18% and 20%, respectively (Fig. 1A). The higher content of phenolic compounds in the freshly harvested grains of the cultivars Notavel and Imperador, as well as their reduction in the course of storage, can be associated with the greater color variation (Table 1) in the stored grains (fast-darkening grains). A substantial reduction in free phenolic compounds was also observed in fava bean and was associated with postharvest color darkening (Nasar-Abbas et al., 2009). In other studies, Ferreira et al. (2017) and Kibar and Kibar (2019) stated that the total phenolic contents of black and carioca beans, stored during 12 months, decreased after storage when compared to freshly harvested beans. The reduction in total free phenolics is probably due to the polymerization of existing polyphenolic compounds, resulting in insoluble, high molecular weight polymers.

The increase in free phenolics observed in cultivar Madreperola is justified by its composition, since cultivars present differences in the phenolic profile (Yang et al., 2018). Taking this fact into account, the increase in free phenolics content during storage may be related to the content of proanthocyanidins (also known as condensed tannins), present in phenolic extracts (Chen, Bozzo, et al., 2015; Wang et al., 2016). The results of Mariotto-Cezar, Coelho, Christ, Schoeninger, and Almeida (2013) were consistent with this finding and showed that the proanthocyanidins increased with storage time and reached the highest concentration at 180 days. Beninger et al. (2005) reported a more specific conclusion that the high molecular-weight procyanidins decreased after storage, while that of low molecular-weight procyanidins increased.

For soluble conjugated-phenolics, only the cultivar Notavel showed an increase (10%) over storage, while the other cultivars did not show significant variation over time ($p < 0.05$) (Fig. 1B). The insoluble-bound phenolics did not show significant variation during storage ($p < 0.05$) for the cultivars Dama and Madreperola, while Notavel and Imperador showed an increase of 6.9% and 18%, respectively (Fig. 1C). Bound phenolics are non-extractable phenolics remaining in the residue following the initial extraction. These phenolic compounds are esterified to cell wall polysaccharides but can also be covalently linked to lignin monomers via an ether linkage. These bonds utilize hydrophobic aromatic ring and hydrophilic groups such as carboxylic acid and hydroxyl groups present in phenolic acids (Chen, Tang, et al., 2015). Bound phenolics released upon alkaline hydrolysis contained ferulic aldaric acids and were found in bean cultivars studied (Table 2). Regarding anthocyanins, there was a significant increase ($p < 0.05$) in content for all cultivars studied (Fig. 1D).

The total ion current chromatograms of Dama, Madreperola, Notavel, and Imperador are shown in Supplementary Figure 1, and Table 2 summarizes the thirty compounds identified considering retention time values, mass spectral, error, and comparison with authentic samples.

3.3. Identification of flavonoids

Flavonoids share a common structure consisting of two aromatic rings that are linked through three carbons, forming an oxygenated heterocycle. The main flavonoids contained in beans are catechin, kaempferol, quercetin, myricetin, and procyanidin. These, along with phenolic acids and tannins, confer to food a superior antioxidant capacity (Chávez-Mendoza & Sánchez, 2017; Yang et al., 2018). In general, flavonoids are pigments responsible for the bean coat color (Coelho et al., 2020).

Compounds **3–6, 8, 10–13** exhibited, in MS^2, a predominant fragment ion at *m/z* 289 ($C_{15}H_{13}O_6$) revealing (iso)catechin aglycone (Fig. 2). In comparison with standard, peaks **10** and **11** were identified as catechin and epicatechin (Table 2). Peaks **3** and **4** display the same

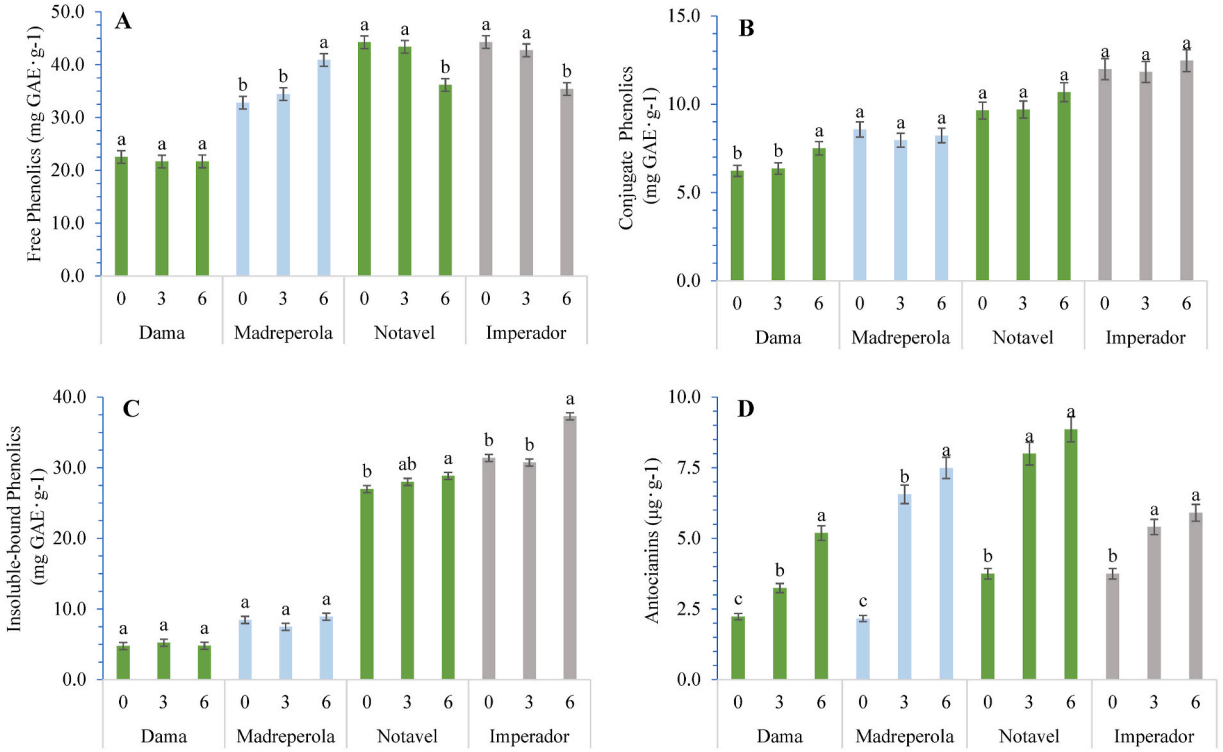

Fig. 1. Free phenolic content (A), Conjugated phenolic content (B), Insoluble-bound phenolic content (C), and anthocyanins content (D) in Dama, Madreperola, Notavel and Imperador carioca beans stored at room temperature for zero (0), three (3) and six (6) months.

Table 2
Compounds tentatively identified in Dama, Madreperola, Imperador, and Notavel carioca beans freshly harvest and aged.

Peak	t_R[a]min	[M-H]$^-$	Product Ions	Molecular ion	Error	Tentative identification	Reference
1	1.20	191.0188	111.0090	$C_6H_7O_7$	−2.1	Citricacid[b]	Spinola, Pinto, and Castilho (2015)
2	1.56	263.0675	128.0375	$C_{12}H_{11}N_2O_5$	2.7	Unknown	-
3	2.35	451.1235	289.0810, 245.0765	$C_{21}H_{23}O_{11}$	−1.1	Catechin-*O*-hexoside	(Journi, Hammouda, trabelsi ayadi, & Cherif, 2015)
4	2.52	451.1251	289.0688, 245.0889	$C_{21}H_{23}O_{11}$	2.4	Catechin-*O*-hexoside	Journi et al. (2015)
5	2.55	577.1343	451.0957, 425.0760, 407.0713, 289.0655	$C_{30}H_{25}O_{12}$	−0.5	Procyanidin B dimer	Chen, Tang, et al. (2015)
6	2.70	577.1339	451.0892, 425.0921, 407.0763, 289.0727	$C_{30}H_{25}O_{12}$	−1.2	Procyanidin B dimer	Chen, Tang, et al. (2015)
7	2.72	259.1300	197.1252, 130.0865	$C_{11}H_{19}N_2O_5$	2.3	*γ*-Glutamyl-leucine	Abu-Reidah, Ali-Shtayeh, Jamous, Arráez-Román, and Segura-Carretero (2015)
8	2.88	577.1356	451.1038, 425.0757, 407.0821, 289.0734	$C_{30}H_{25}O_{12}$	1.7	Procyanidin B dimer[b]	Chen, Tang, et al. (2015)
9	2.98	385.0773	209.0292, 191.0174, 147.0318	$C_{16}H_{17}O_{11}$	0.5	Feruloyl aldaricacid	Mecha et al. (2019)
10	3.02	289.0707	245.0844, 205.0496	$C_{15}H_{13}O_6$	−1.7	Catechin[b]	Zhang and Zhu (2015)
11	3.24	289.0711	245.0871, 205.0505	$C_{15}H_{13}O_6$	−0.3	Epicatechin[b]	Zhang and Zhu (2015)
12	3.40	865.1954	577.1297, 425.4070, 289.0737	$C_{45}H_{37}O_{18}$	−3.0	Procyanidin B trimer	Chen, Tang, et al. (2015)
13	3.66	1153.2637	865.1878, 577.1303, 289.0663, 287.0480, 245.0795	$C_{60}H_{49}O_{24}$	2.0	Procyanidin B tetramer	Yan, Hu, Wang, Hong, and Jia (2014)
14	4.59	447.0914	285.0406, 284.0309, 255.0276, 227.0290	$C_{21}H_{19}O_{11}$	−2.9	Kaempferol-*O*-hexoside	Journi et al. (2015)
15	4.84	447.0921	285.00396, 284.0317, 255.0296, 227.0363	$C_{21}H_{19}O_{11}$	−1.3	Kaempferol-*O*-hexoside	Journi et al. (2015)
16	4.99	640.2980	221.0535	$C_{31}H_{46}NO_{13}$	1.7	Unknown	-
17	5.27	625.2879	221.0632, 101.0261	$C_{31}H_{45}O_{13}$	3.0	Unknown	-
18	5.70	625.2856	221.0616, 101.0244	$C_{31}H_{45}O_{13}$	−0.6	Unknown	-
19	5.93	1105.5486	–	$C_{46}H_{89}O_{29}$	−0.4	SoyasaponinA0-ag	Chitisankul et al. (2018)
20	6.07	1105.5487	–	$C_{46}H_{89}O_{29}$	−0.3	SoyasaponinA0-ag	Chitisankul et al. (2018)
21	6.14	285.0394	255.0271	$C_{15}H_9O_6$	−1.8	Kaempferol[b]	Coelho et al. (2020)
22	6.25	1089.5575	-	$C_{46}H_{89}O_{28}$	3.2	Soyasaponin A0-bg	Chitisankul et al. (2018)
23	6.33	1089.5536	-	$C_{46}H_{89}O_{28}$	−0.4	Soyasaponin A0-bg	Chitisankul et al. (2018)
24	8.15	957.5060	–	$C_{48}H_{77}O_{19}$	0.1	Soyasaponin Ba	Peng et al. (2017)
25	8.29	941.5101	–	$C_{48}H_{77}O_{18}$	−1.0	Soyasaponin Bb	Peng et al. (2017)
26	8.38	955.4927	–	$C_{48}H_{75}O_{19}$	2.5	Soyasaponin Bd	Lee et al. (2014)
27	8.68	925.5175	–	$C_{48}H_{77}O_{17}$	1.5	Soyasapogenol E	Pollier, Morreel, Geelen, and Goossens (2011)
28	8.85	1083.5382	–	$C_{54}H_{83}O_{22}$	0.6	Soyasaponin αg	Peng et al. (2017)
29	8.96	1067.5413	–	$C_{54}H_{83}O_{21}$	−1.3	Soyasaponin βg	Peng et al. (2017)
30	9.29	921.4825	–	$C_{48}H_{73}O_{17}$	−2.5	Soyasaponin γg	Peng et al. (2017)

[a] Retention time.
[b] Comparison with standard.

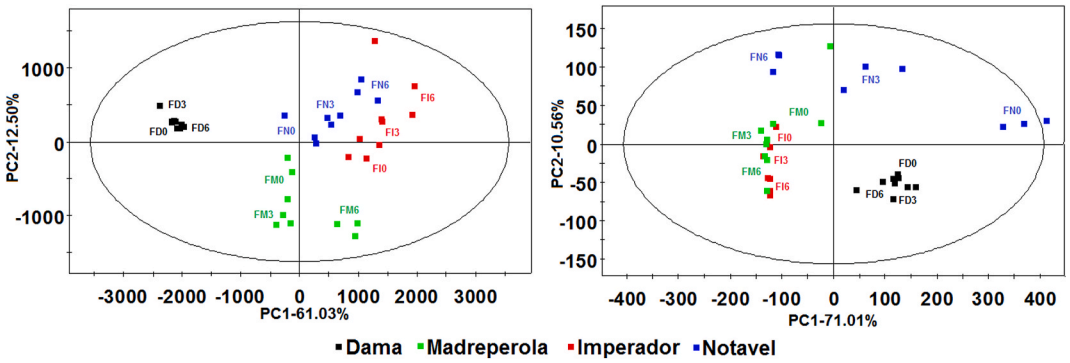

Fig. 2. Data analyzed by PCA comparative of groups: (FD0, FD3 and FD6) Dama storage time zero, tree and six months; (FI0, FI3 and FI6) Imperador at storage time zero, three and six months; (FM0, FM3 and FM6) Madre perola at storage time zero, three and six months; (FN0, FN3 and FN6) Notavel at storage time zero, three and six months: left, phenolic; right, saponins.

molecular ion at *m/z* 451 ($C_{21}H_{23}O_{11}$) with a production at *m/z* 289 resulted from the loss of hexose (162 Da) and were identified as Catechin-*O*-hexoside isomers. Based in the monomeric unit of (iso)catechin, the peaks **5**, **6** and **8** with molecular ion at *m/z* 577 ($C_{30}H_{25}O_{12}$) were identified as procyanidin B-type dimer based in the fragmentation pattern as heterocyclic ring fission (HRF, *m/z* 451) and retro-Diels Alder (RDA, *m/z* 425). Likewise, the peaks **12** and **13** showed [M-H]$^-$ ion at *m/z* 865 (three monomeric units of (epi)catechin, $C_{45}H_{37}O_{18}$) and 1153 (four monomeric units of (epi)catechin, $C_{60}H_{49}O_{24}$) and were identified as procyanidin B-type trimer and tetramer, respectively. The procyanidin B-type dimer contributes to the darkening process of the bean coat due to proanthocyanidin oxidation to reactive quinones (brown compound) (Ranilla, Genovese, & Lajolo, 2007). The presence of catechin and proanthocyanidin compounds in seeds, like beans, has been associated with embryo protection against pathogens, ensuring seed germination (Mecha et al., 2019).

Peaks **14** and **15** showed the same molecular ion at *m/z* 447 ($C_{21}H_{19}O_{11}$) with a prominent product ion at *m/z* 285 and 255 typical of

kaempferol aglycone resulted from the loss of hexose (162 Da) (Table 2). Thus, they were identified as kaempferol-*O*-hexoside. Current research highlighted the role of kaempferol as an antioxidant and immune-modulator agent with possible anti-carcinogenic effect in 5-fluorouracil resistant LS174 colon cancer cells (Riahi-Chebbi et al., 2019). Such beneficial health impact supports the recommendation of regular consumption of common bean, including the coat fraction, as a natural rich source of kaempferol aglycone (Dueñas, Martínez-Villaluenga, Limón, Peñas, & Frias, 2015). Peak **21** showed a molecular ion at *m/z* 285 and identified as kaempferol by standard comparison.

3.4. Identification of saponins

Saponins in negative mode showed the presence of an intense $[M-H]^-$ molecular negative ion with the absence of product ions (Table 2). According to the literature reported, soyasaponin and its derivatives are found in *Phaseolus* (Chávez-Mendoza & Sánchez, 2017; Coelho et al., 2020; Peng, Li, Li, Deng, & Zhang, 2017). Saponins are composed of two triterpenoid aglycones that are stored as multi-glycosidic forms in the plant tissues. Saponins are classified as groups A saponins (gr.A) and DDMP saponins (DDMPs), based on their aglycones structures. Group A saponins are soyasapogenol Aglycosides (SAGs) with two sugar chains attached at the C-3 and C-22 positions of the aglycone. All hydroxyl groups of the terminal sugar of the sugar chain attached to the C-22 position are fully acetylated. DDMP saponins (DDMPs) are 2,3-dihydro-2,5-dihydroxy-6-methyl-4Hpyran-4-one (DDMP)-conjugated soyasapogenol B glycosides (SBGs) at the C-22 position. DDMPs degrade to group B saponins (gr.B) and group E saponins (gr.E), which have soyasapogenol B and soyasapogenol E as the aglycone, respectively (Chávez-Mendoza & Sánchez, 2017; Chitisankul et al., 2018). The saponins identified were: soyasaponin A0-ag (**19**, $C_{53}H_{85}O_{24}$) and soyasaponin A0-bg (**22**, $C_{46}H_{89}O_{28}$) from group A; soyasaponin Ba (**24**, $C_{48}H_{77}O_{19}$) and soyasaponin Bb (**25**, $C_{48}H_{77}O_{18}$) from group B; soyasaponin Bd (**26**, $C_{48}H_{75}O_{19}$), and soyasaponin E (**27**, $C_{48}H_{77}O_{17}$) from group E; soyasaponin αg (**28**, $C_{54}H_{83}O_{22}$), soyasaponin βg (**29**, $C_{54}H_{83}O_{21}$), and soyasaponin γg (**30**, $C_{48}H_{73}O_{17}$) from group DDMP.

Their bioavailability and bioactivity also depend on the hydrolysis of the conjugated forms to the aglycone forms (Berhow et al., 2020). DDMPs, including DDMP saponins, gr.B, and gr.E saponins have health benefits. Within gr.A, fully-acetylated SAGs (FSAGs) are the major cause of undesirable taste characteristics including bitterness, astringency, roughness, dry mouthfeel, and green beany flavors (Chitisankul et al., 2018). Saponins were considered as anti-nutrients, but nowadays they have been reported as beneficial for health. Dietary saponins from grains, like beans, were therapeutic to control obesity pathogenicity, since contribute to bodyweight management, waist circumference, and decrease blood pressure (Jeepipalli, Du, Sabitaliyevich, & Xu, 2020; Pascale et al., 2018). Moreover, they have shown strong cytotoxic effects against cancer cell lines. However, more epidemiological, and clinical studies are required for the proper validation of these health-promoting activities.

3.5. Other compounds identified

Peak **1** displays a molecular ion at *m/z* 191 ($C_6H_7O_7$) with a product ion at *m/z* 111 resulted in the loss of CO and two H_2O molecules and was identified as citric acid (Table 2). Peak **7** was identified as γ-Glutamyl-leucine by the precursor ion at *m/z* 259 ($C_{11}H_{19}N_2O_5$) and the fragment at *m/z* 130 of leucine. Peak **9** showed a [M-H]- ion at *m/z* 385 ($C_{16}H_{17}O_{11}$) with loss of feruloyl given an intense fragment at *m/z* 209 of aldaric acid and was identified as feruloyl aldaric acid. As described by Dueñas et al. (2015), the aldaric derivatives of hydroxyciannamic acids represent the most typical hydroxyciannamic acids in common beans. The compounds **2**, **16**, **17**, and **18** were no identified.

3.6. Chemometric analysis

Firstly, it was verified that the cultivars presented different compounds and can be separated at different groups (Fig. 2). For phenolics, the cultivars Notavel and Imperador showed a similar profile, differing from the cultivars Dama and Madreperola. Several factors, such as genotype, agronomic practices, climatic conditions, or harvest conditions (e.g., maturity state), may influence the phenolic composition of common beans (Luthria & Pastor-Corrales, 2006; Mecha et al., 2019). Besides, the profile of phenolic compounds may be related to the color of the tegument of the bean grain, contributing or not to its browning during storage (Beninger et al., 2005). Therefore, the results obtained are in line with expectations, since Dama and Madreperola are slow-darkening cultivars, while Notavel and Imperador darken faster (greater ΔE_3 and ΔE_6) (Table 1), which justifies the separation observed in the PCA, with the two first principal components explaining 73.5% of the variance for phenolics. The saponins observed resulted in a new PCA created to describe differences among the beans with a total variance of 81.6% (Fig. 2).

No significant differences were observed in the grains stored for three months in relation to the freshly and stored for six months, but the comparison between freshly and stored grains for six months was statistically significant (Supplementary figure 2). Therefore, further chemometric analysis was performed only between the freshly harvested grains and those stored for six months. The results (Supplementary figure 3) confirm a reduction of kaempferol content during storage time in grains with fast darkening; and a high content of procyanidin B-type dimer in aged grains from the cultivar Madreperola. As for the phenolic compounds evaluated, only free phenolics presented significant differences, therefore, they were evaluated chemometrically. Also, the chemometric evaluation was divided into two blocks: retention time from 2.00 to 5.00 min (phenolic compounds); and retention time from 7.00 to 10.00 min (saponins).

3.7. OPLS-DA of phenolic compounds

The cultivars Dama and Imperador can be separated into two distinct groups with an explained variance of R2Y- 0.98 and predicted variance Q2 - 0.97. The cultivars differ in relative ion/intensity from phenolic compounds. The cultivar Dama (slow darkening) presented as difference kaempferol-*O*- hexoside (**14**, t_R 4.59, *m/z* 447.0914) when compared to the cultivar Imperador (rapid darkening) (Fig. 3A–D). The difference in kaempferol content, during storage, between slow and fast-darkening genotypes was also verified by Beninger et al. (2005) who reported that the kaempferol content in the tegument of the slow-darkening cultivar (1533-15, an F4-derived line from the cross CDC Pintium x SC11743-3) was higher than the kaempferol content in the tegument of the stored CDC Pentium cultivar (fast-darkening grains).

The cultivars Madreperola and Imperador can also be separated into two distinct groups with an explained variance of R2Y- 0.93 and predicted variance Q2 - 0.80 (Fig. 4A–C). As seen in Fig. 4, the slow-darkening grains (Madreperola) had the kaempferol-*O*-hexoside as marker (**14**, t_R 4.59, *m/z* 447.0914) (Fig. 4) when compared to the fast-darkening cultivar (Imperador) with predominant relative ion intensity. However, the relative ion intensity of the beans analyzed presented for Dama and Notavel (Table 3) showed the same proportion among kaempferol-*O*-hexoside. Therefore, the relative area of kaempferol when compared among the beans showed higher for Madreperola, suggesting a conversion of kaempferol-*O*-hexoside in kaempferol for broken of *O*-glucoside ligation.

Procyanidin B-type (**8**, t_R 2.88, *m/z* 577.1356) (Fig. 4A–C), as a marker, in the Imperador cultivar suggested that this cultivar tend to darken faster than cultivar Madreperola. Since the proanthocyanidins are oligomeric flavonoids originated from the condensation of catechin, epicatechin, and gallic acid esters. This class of compounds oxidizes to reactive quinones that interact with proteins, resulting in the darkening

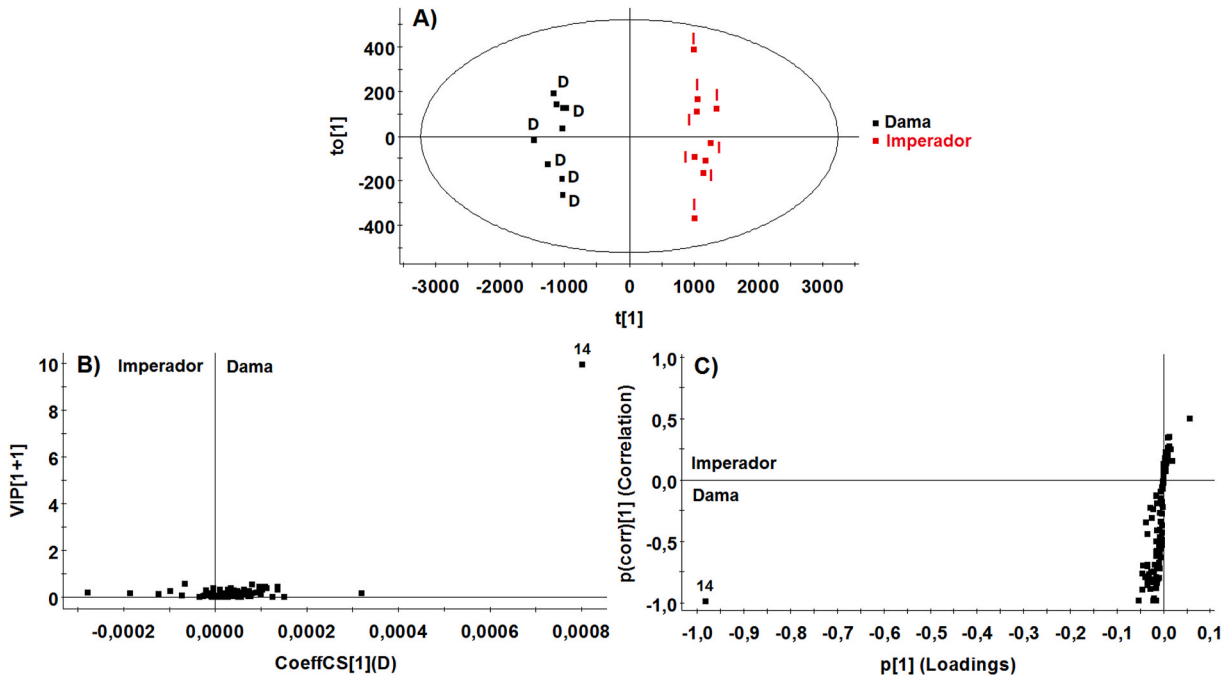

Fig. 3. Data analyzed by OPLS/Da comparative of groups of stored carioca beans: (FD) Dama; (FI) Imperador. A) OPLS (Orthogonal) graph; B) Variable importance in projection graph; C) S-Plot graph. Markers: **14**, t_R 4.59, *m/z* 447.0914.

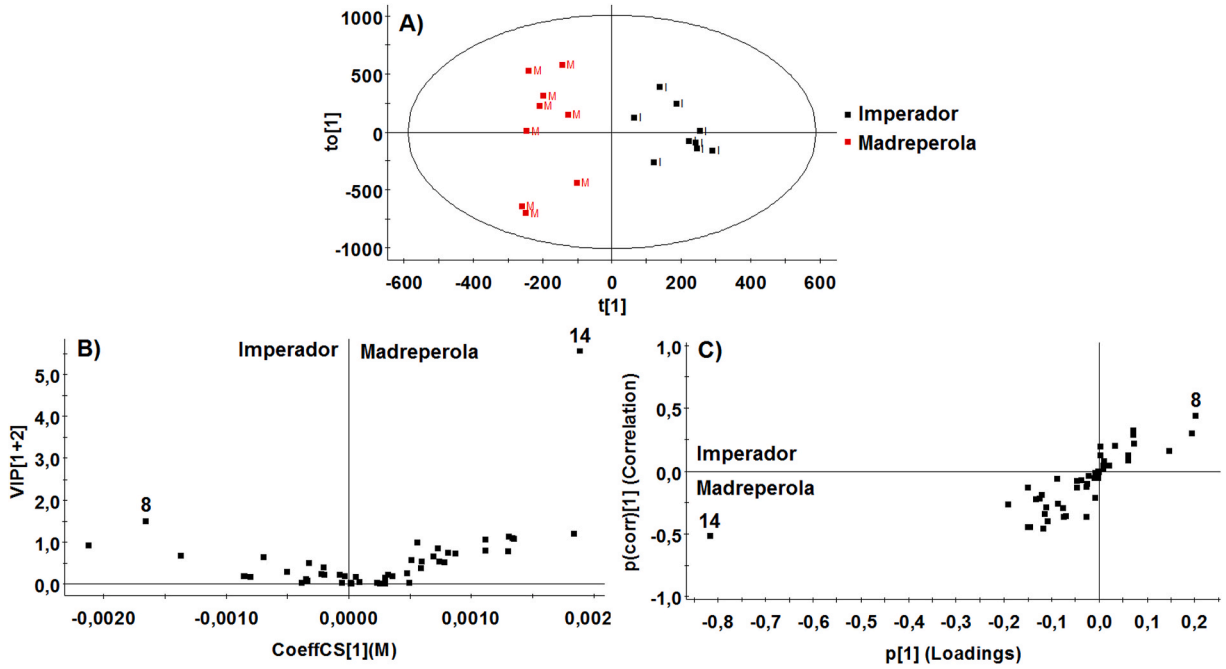

Fig. 4. Data analyzed by OPLS/DA comparative of groups of stored carioca beans: (FM) Madreperola; (FI) Imperador.A) OPLS (Orthogonal) graph; B) Variable importance in projection graph; C) S-Plot graph. Markers: **8**, t_R 2.88, *m/z* 577.1356; **14**, t_R 4.59, *m/z* 447.0914.

of the bean coat (Coutin et al., 2017).

Based on the presented results (Table 3), we propose that kaempferol glucosides might be a marker to differ carioca bean with fast-darkening behavior from those with slow-darkening profile, since the fast-darkening bean presents a reduction in kaempferol content during storage time, and the aged bean also presents a low content of this compound when compared with the slow-darkening bean (Beninger et al., 2005).

3.8. OPLS-DA of saponins

The cultivars Dama and Imperador can be separated into two distinct groups with an explained variance of R2Y- 0.94 and predicted variance Q2 - 0.89 (Fig. 5A and B). The cultivars differ in saponins, in which the cultivar Dama presented as marker saponins from group DDMP (**30**, t_R 9.29, *m/z* 921.4825), Soyasaponin Ba (**24**, t_R 8.15, *m/z* 957.5060) and Soyasaponin Bb (**25**, t_R 8.29, *m/z* 941.5101) than cultivar Imperador. On the other hand, the compounds in cultivar Imperador do not present

Table 3
Markers identified in stored carioca beans: Dama (D), Madreperola (M), Imperador (I) and Notavel (N).

Peak	t_R[a] min	Tentative identification	Beans[b]								VIP[c]	*p*-value[d]
			Dama		Madreperola		Imperador		Notavel			
			t0	t6	t0	t6	t0	t6	t0	t6		
8	2.88	Procyanidin B dimer	+	+	+	+	++	++	++	++	2.51	3.30×10^{-2}
14	4.59	Kaempferol-*O*- hexoside	++	++	++	++	+	+	++	++	3.64	1.22×10^{-3}
21	6.14	Kaempferol	++	++	++	++	+	+	+	+	–	–
24	8.15	Soyasaponin Ba	++	++	+	+	+	+	+++	++	4.22	2.40×10^{-2}
25	8.29	Soyasaponin Bb	+	+	+	+	++	++	+++	++	4.41	2.65×10^{-2}
26	8.38	Soyasaponin Bd	++	++	+	+	+	+	+++	++	5.98	8.48×10^{-6}
30	9.29	Soyasaponin γg	++	++	+	+	+	+	++	++	7.49	1.95×10^{-9}

[a] Retention time.
[b] Markers at storage conditions: +, ++ relative intensity.
[C] Variable of importance in projection.
[d] Probability value.

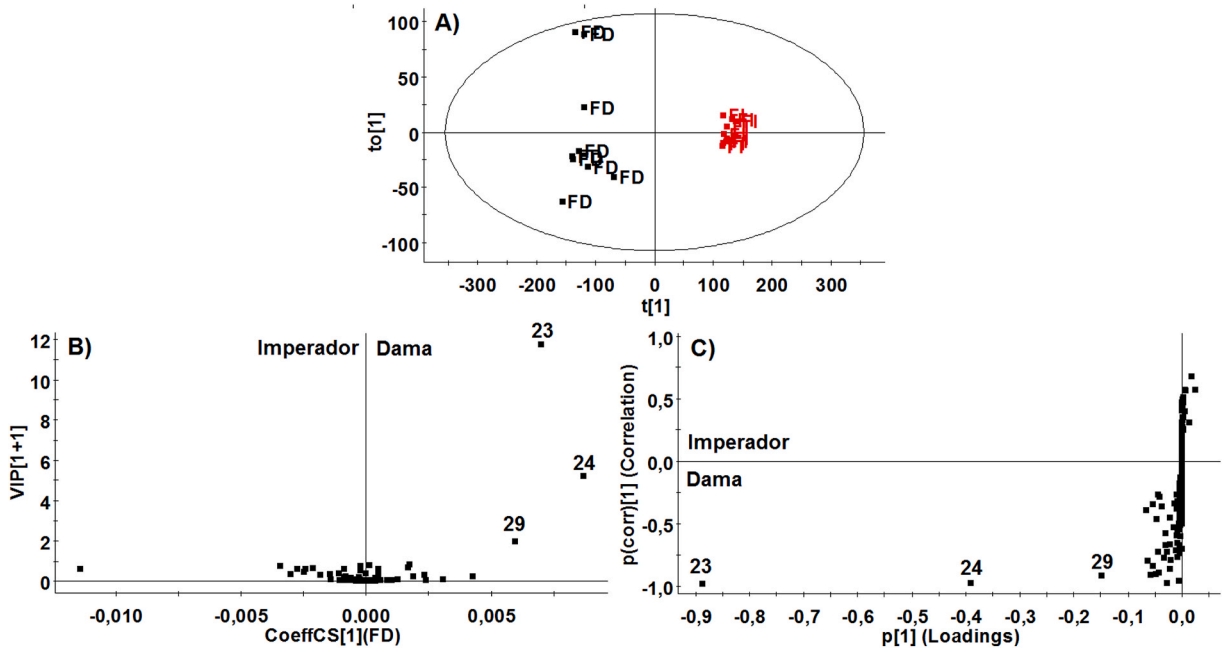

Fig. 5. Data analyzed by OPLS/DA comparative of groups of stored carioca beans: (FD) Dama; (FI) Imperador. A) OPLS (Ortogonal) graph; B) Variable importance in projection graph; C) Relative ion/intensity; D) S-Plot graph. Markers: **24**, t_R8.15, *m/z* 957.5060; **25**, t_R8.29, *m/z* 941.5101; **30**, t_R9.29, *m/z* 921.4825.

a significant marker in variance importance of projection (VIP>1, Fig. 6B).

As seen in Table 3, the cultivar Madreperola, when compared to the other cultivar, showed a smaller relative ion/intensity for saponins. Imperador presented the high relative ion/intensity for saponins from Soyasaponin Bb (**25**, t_R 8.29, *m/z* 941.5101), when compared to Madreperola (Table 3).

The cultivars Dama and Notavel were separated into two distinct groups with an explained variance of R2Y- 0.88 and predicted variance Q2 - 0.63 (Fig. 6A–C). The cultivar Notavel presented the highest relative ion/intensity for soyasaponin Ba (**24**, t_R8.15, *m/z* 957.5060) and soyasaponin Bb (**25**, t_R8.29, *m/z* 941.5101) from group B; soyasaponin Bd (**26**, t_R 8.38, *m/z* 955.4927) from group E and soyasaponin γg (**30**, t_R9.29, *m/z* 921.4825) from group DDMP. Moreover, the cultivar Notavel showed a reduction of saponins during storage time which can be observed by the shift in PC1 from the positive to the negative region (Fig. 2, Table 3).

These results can be explained by the fact that different cultivars present distinct saponins profile and the soil moisture modifies the chemical composition of crop seeds, wherein the mild and severe water restriction exacerbates the accumulation of saponins in common bean seeds (Herrera, Acosta-Gallegos, Reynoso-Camacho, & Pérez-Ramírez, 2019). The presence of high content from gr.E saponins (Soyasaponin Bd) in the cultivars Notavel (Table 3) could be related with the fast-darkening process, since the gr.E saponins are known to be photo-oxidation products of gr.B saponins (Singh et al., 2017).

4. Conclusion

The cultivars Madreperola and Dama presented characteristics of slow-darkening beans since they had the lowest total color difference and the highest luminosity values during storage time. On the other hand, the cultivars Notavel and Imperador rapidly darkened with storage time. Regarding the content of phenolic compounds during storage, it was observed a reduction in their content for fast-darkening beans and the maintenance/increase for slow-darkening ones. These results were in accordance with the change browning of the beans tegument during storage. Regarding the phenolic profile from aged grains, Notavel and Imperador were similar, differing from Dama and Madreperola, where Dama and Madreperola had a higher relative intensity/ion kaempferol when compared with Imperador and Notavel. With these results, we conclude that kaempferol might be a marker to differ carioca bean with

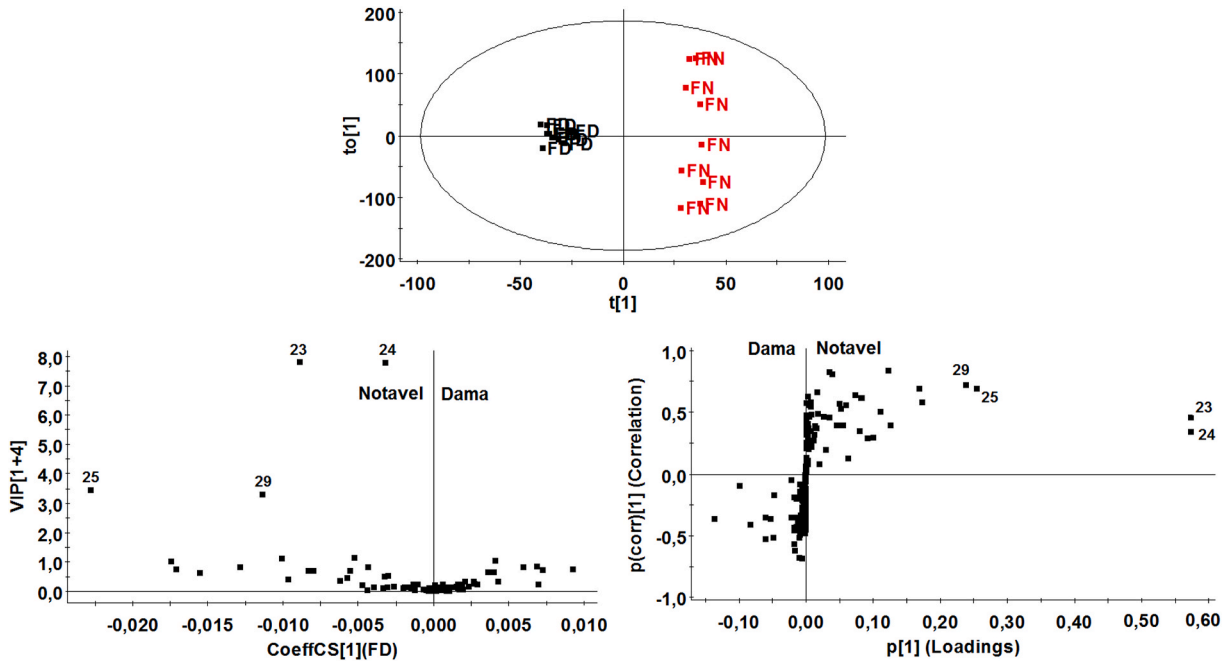

Fig. 6. Data analyzed by OPLS/DA comparative of groups of stored carioca beans: (FD) Dama (FN) Notavel. A) OPLS (Ortogonal) graph; B) Variable importance in projection graph; C) S-Plot graph. Markers: **24**, t_R 8.15, *m/z* 957.5060; **25**, t_R 8.29, *m/z* 941.5101; **26**, t_R 8.38, *m/z* 955.4927; **30**, t_R 9.29, *m/z* 921.4825.

fast-darkening characteristic from those with slow-darkening trend, since the fast-darkening bean presented a reduction in kaempferol content during storage time, and the aged bean also presented a low content of this compound when compared with the slow-darkening bean. Regarding the content of saponins, the cultivars Imperador and Notavel presented higher content of Soyasaponin Bd, which might be related to the fast-darkening process of these cultivars. Therefore, the content as well as the profile of phenolic compounds and saponins are different between slow and fast-darkening carioca beans.

CRediT authorship contribution statement

Juliana Aparecida Correia Bento: Conceptualization, Investigation, Validation, Visualization, Formal analysis, Writing - original draft, Writing - review & editing. **Paulo Riceli Vasconcelos Ribeiro:** Investigation, Validation, Formal analysis, Writing - review & editing. **Priscila Zaczuk Bassinello:** Conceptualization, Resources, Writing - review & editing, Visualization, Supervision. **Edy Sousa de Brito:** Resources, Writing - review & editing, Supervision. **Guilherme Juliao Zocollo:** Resources. **Márcio Caliari:** Resources. **Manoel Soares Soares Júnior:** Writing - review & editing, Supervision.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110599.

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