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NMR and LC-MS assessment of compound variability of common bean (*Phaseolus vulgaris*) stored under controlled atmosphere

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ABSTRACT

As the chemical profiling of common beans (*Phaseolus vulgaris*) modify according to storage conditions, the aim of this study was to analyze the physicochemical parameters and composition variability of the cotyledon by NMR, and coat by LC-MS, all submitted to different storage atmosphere of O_2, CO_2, and humidity. Through NMR, chemometrics along with metabolite pathway analysis it is observed the overexpression of the saccharides sucrose (from 2.30 ± 0.32 to 2.71 ± 0.14 g/100 g), raffinose (from 0.86 ± 0.04 to 1.02 ± 0.07 g/100 g), and stachyose (from 3.37 ± 0.12 to 4.13 ± 0.28 g/100 g) in stored beans (regardless atmosphere conditions), indicating changing on galactose, and starch and sucrose pathways that are intrinsically linked to germination and sprouting processes. Those mechanisms seem to be partially activated after 6 months since amino acids or lipid metabolism were not affected. Therefore, storage conditions induce the role in regulating the germination and sprouting processes but do not affect the germination capability of the seeds (data not shown). The beans stored in low humidity showed less luminosity and chroma values, and relative reduction of proanthocyanidin B-type dimer, epi-catechin, and kaempferol, which indicate darkening when submitted to high humidity with controlled atmosphere.

1. Introduction

The chemical profile of common beans (*Phaseolus vulgaris*) can change according to storage conditions (Prolla et al., 2010). Storage under adverse conditions as high temperature and humidity induces the hard-to-cook (HTC), a textural defect that affects its quality (K. Liu, McWatters, & Phillips, 1992). The impermeability of the bean coat occurs due to oxidation of tannins and formation of protein-tannin complexes (Pirhayati, Soltanizadeh, & Kadivar, 2011). Furthermore, the environment during the storage may cause changes in seed metabolism and influence the physiological quality of seeds (Delouche & Baskin, 2016). Therefore, the use of reduced oxygen levels and increase of gases, such as carbon dioxide (CO_2) and nitrogen to control storage pests, can lead to inhibition of biochemical processes as sucrose, amino acids, and lipids biosynthesis (Geigenberger, 2003).

Modified atmosphere technologies are well-known methods and are alternative for preserving beans quality by reducing chemical changes (Vanier, Rupollo, Paraginski, de Oliveira, & Elias, 2014) and extending shelf life due to the decrease of microorganisms incidence (Freitas, Faroni, & Sousa, 2016). In normal atmosphere conditions, the concentrations of nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2) are 78%, 21%, and 0.04% respectively, along with small percentages of other gases. In order to increase the shelf life of products stored under controlled atmospheres, it is necessary to reduce O_2 concentrations to 1–3% while increase levels of CO_2 to 2–20% (de Souza Aguiar et al., 2015).

Therefore, the aim of this study was evaluate the composition variability of common bean under different storage conditions using the quantitative Nuclear Magnetic Resonance (*q*NMR), and Ultra-High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (UPLC-HRMS) to evaluate the chemical changes according to different controlled atmosphere conditions as storage period and levels of O_2, CO_2, and humidity.

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Table 1
Storage conditions of the common beans (*P. vulgaris*).

Experiment	% O_2 (kPa)	% CO_2 (kPa)	Storage period	Relative humidity
T1(HCLH)	1.5	9	6 months	40%
T2(LCLH)	21	0.04	6 months	40%
T3(HCHH)	1.5	9	6 months	70%
T4(LCHH)	21	0.04	6 months	70%
T5(HCLH.sl)	1.5	9	6 months +7 days sl	40%
T6(LCLH.sl)	21	0.04	6 months +7 days sl	40%
T7(HCHH.sl)	1.5	9	6 months +7 days sl	70%
T8(LCHH.sl)	21	0.04	6 months +7 days sl	70%

Legend: LCLH – low CO_2 level, low humidity; LCHH – low CO_2 level, high humidity; HCLH – high CO_2 level; HCHH – high CO_2 level, high humidity; sl - storage in normal atmosphere at 25 °C.

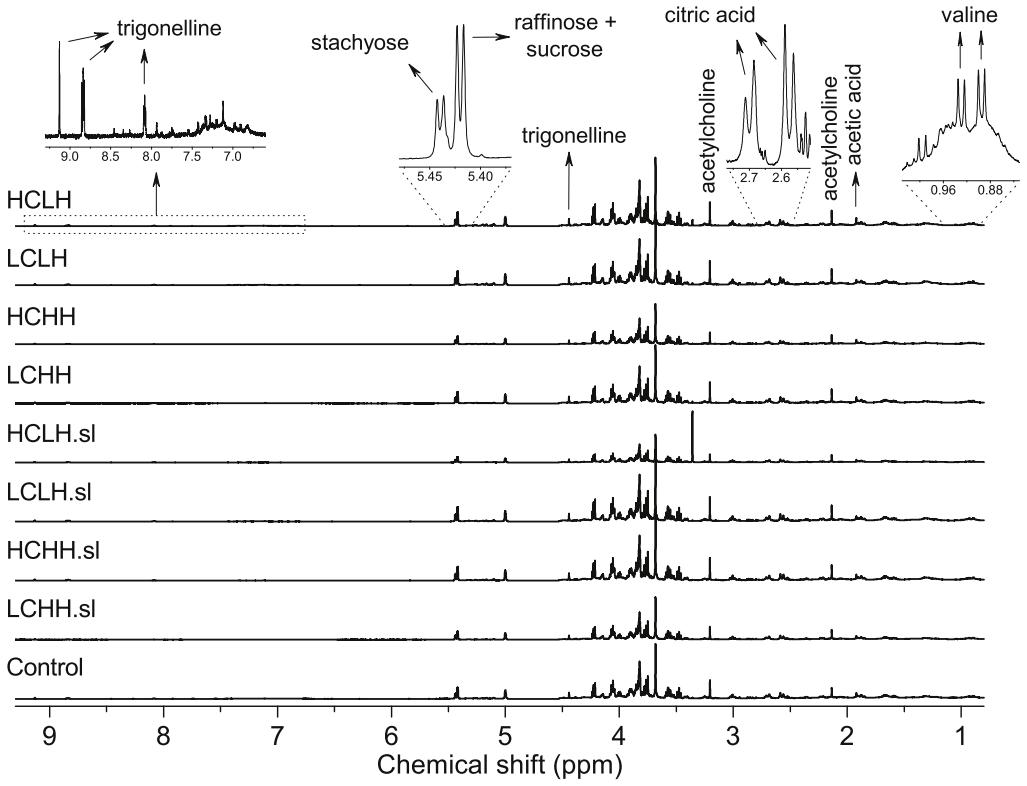

Fig. 1. Overview of the ^{1}H NMR spectra from aqueous extract of cotyledon of *Phaseoulus vulgaris*. Legend: LCLH – low CO_2 level, low humidity; LCHH – low CO_2 level, high humidity; HCLH – high CO_2 level; HCHH – high CO_2 level, high humidity; sl – storage at ambient condition.

2. Materials and methods

2.1. Samples

Phaseolus vulgaris type Pinto Bean (cv. IAC Imperador) was harvested on January 2016 in a farm located at Paraná State, Brazil – latitude 24°54′74″S, longitude 54°03′50″W. These beans were harvested, cleaned, and stored in raffia bags with moisture content of 11%.

2.2. Experimental layout

The experimental design was completely randomized with four replications. Approximately 350 g of dry beans were placed in different storage conditions of CO_2 (0.04 and 9%), O_2 (1.5 and 21%) and relative humidity (40 and 70%), as described in Table 1. In controlled atmosphere, 350 g were stored in raffia bags and placed in 15 L airtight vessels ($N = 3$). These conditions were obtained with nitrogen and carbon dioxide (White Martins Gases Industriais Ltda, Cascavel, Brazil). Both gases were mixed to obtain the oxygen (1.5 KPa) and carbon dioxide (9.0 KPa) levels and were monitored for six months using an electronic analyzer – Agri-Datalog™ Oxidox II Isocel (Rio Grande do Sul, Brazil). The relative humidity (40 and 60%) and temperature (20 °C) were adjusted and controlled by a dehumidifier and air conditioner, respectively. After six months a subset of samples were maintained under ambient atmosphere at 25 °C (stored at ambient condition – sl) to evaluate the capacity of metabolic restoration. At the end of the experiments, 100 g of beans were frozen at −25 °C for 24 h and freeze dried (Terroni, model Enterprise II, São Carlos, Brazil). A control sample before storage was obtained as described above.

2.3. NMR analysis

For NMR analysis, the bean coat was manually separated from the cotyledon. The cotyledon were powdered, and approximately 30.0 mg were soaked in a mixture of 600 μL of D_2O and 1 mM of TMSP-d_4 (sodium-3-trimethylsilylpropionate-2,2,3,3-d_4). The solutions were mixed for 2 min at room temperature and centrifuged at 804.6 *g* for 2 min. The supernatants were transferred to 5 mm NMR tubes.

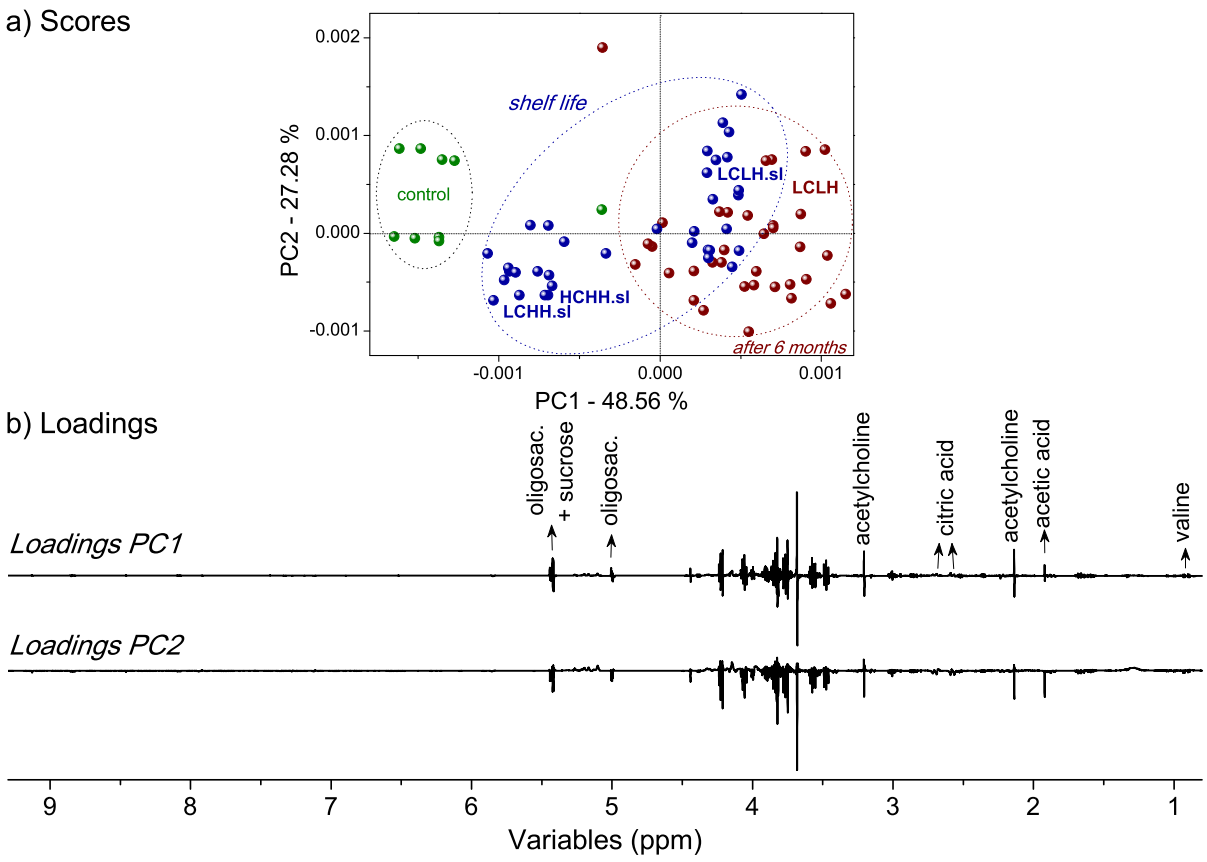

Fig. 2. PC1 × PC2 scores coordinate system for common bean stored under different conditions, with control samples (green), beans stored during 6 months (red), and beans stored during 6 months with subsequent storage at ambient condition (blue) (a); respective loadings plotted in lines form (b). Legend: LCLH – low CO_2 level, low humidity; LCHH – low CO_2 level, high humidity; HCLH – high CO_2 level; HCHH – high CO_2 level, high humidity; sl – storage at ambient condition. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The NMR experiments were performed on an Agilent 600-MHz spectrometer equipped with a 5 mm (H–F/^{15}N–^{31}P) inverse detection One Probe™ with actively shielded Z-gradient. The ^{1}H NMR spectra were acquired with 32 free induction decays (FID), 48 k of time domain points for a spectral window of 16 ppm, under quantitative parameters at 298 K: acquisition time of 5.0 s and a recycling delay of 25.0 s (determined by the inversion-recovery pulse sequence). The spectra were processed by applying exponential line broadening of 0.3 Hz, zero filling of 32 k points, and referenced to the TMSP-d_4 resonance at δ 0.0. The identification of the constituents was performed through ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, and ^{1}H–^{13}C HMBC experiments, supplementary open access database (http://www.hmdb.ca) and literature reports (Alves Filho, Silva, Teofilo, Larsen, & de Brito, 2017; Choze et al., 2013; Lião et al., 2011). Complete signaling is provided in Supporting Information (SI).

2.4. LC-MS analysis

The bean coat samples were grinded and 200 mg was sonicated (40 kHz, Eco-Sonic, Brazil) in 10 mL of acidified methanol: acetic acid (90/10, v/v) during 1 h at 25 °C. The supernatant was filtered on PTFE 0.2 μm membranes (Whatman, Merck, Germany) and 5 μL was injected into the UPLC system (Waters Co., Milford, MA, USA). The UPLC analysis was performed with Acquity UPLC BEH column (150 × 2.1 mm, 1.7 μm; Waters) on a Waters Acquity UPLC system. The column temperature was set at 40 °C. The binary gradient elution system consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with linear gradient from 2 to 95% B (0–15 min), with a flow rate of 0.4 mL min^{-1}. The profiling was obtained by a Xevo Q-TOF mass spectrometer (Waters) with electrospray ionization (ESI) interface operating in negative ionization mode in the range of 110–1180 Da with scan time of 0.1. The desolvation gas was nitrogen set at 350 °C with flow rate of 500 L h^{-1}. The capillary and cone voltages were adjusted to 2.6 kV and 0.5 V, respectively. 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 identification of the organic compounds was performed considering the respective *m/z* values, fragmentation profile, and literature reports.

2.5. Color measurement

Color was determined using a Minolta CR-410 chromameter (Minolta, Japan) with granular-materials attachment CRA50. A white porcelain plate (Y = 85.8; x = 0.3195 and y = 0.3369) was used for calibration, and an illuminat D65 (day light) to data collection for L* (luminosity), a*, and b* values. The hue angle (h*) and chroma (C*) values were determined based on a previous publication (Giusti, Caprioli, Ricciutelli, Vittori, & Sagratini, 2017).

2.6. Statistical analysis

2.6.1. Multivariate statistical analysis

The NMR and LC-MS dataset were evaluated by multivariate statistical analysis. Therefore, the ^{1}H NMR spectra and chromatograms were converted to American Standard Code for Information Interchange (ASCII) files to covariance matrices construction separately

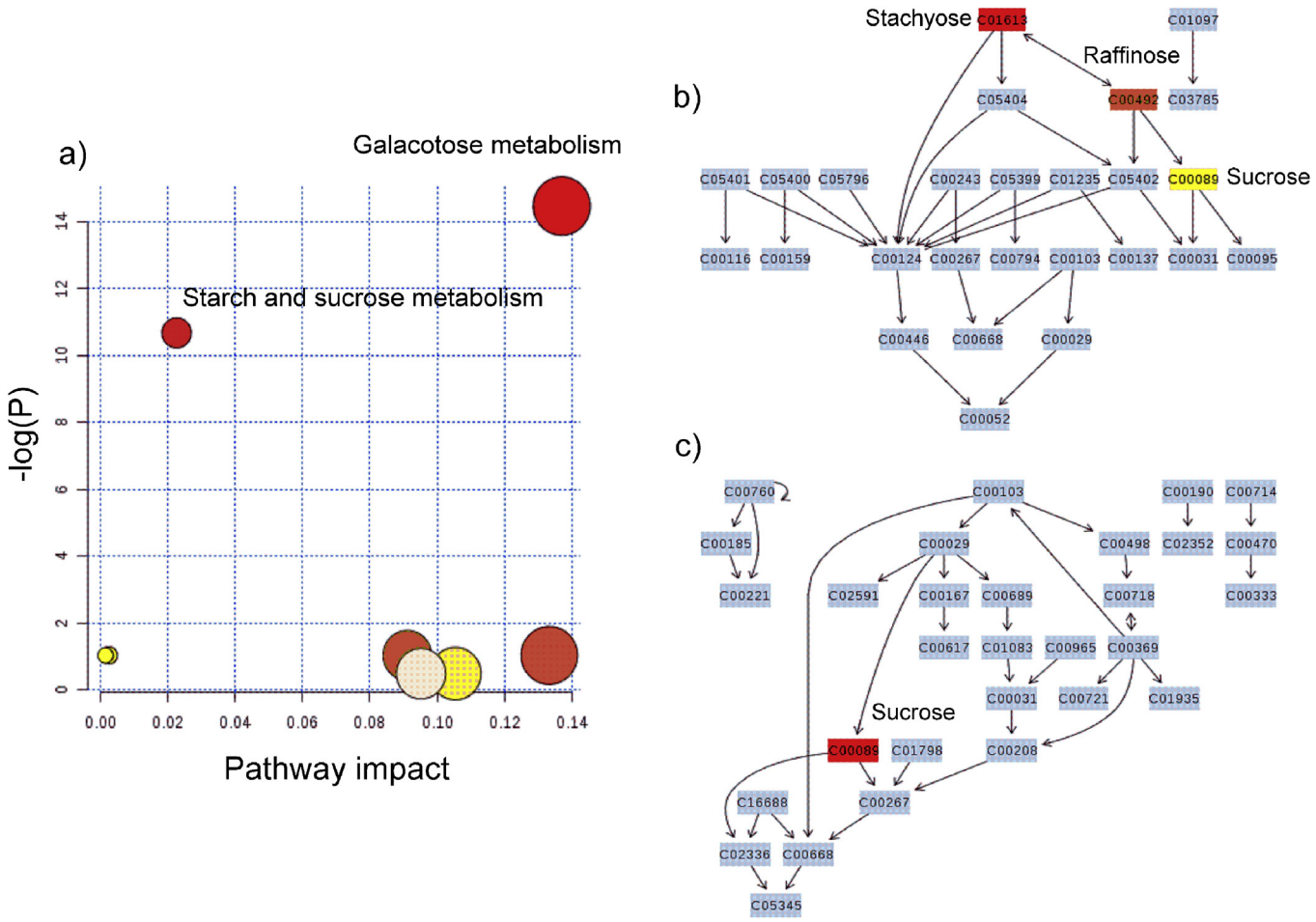

Fig. 3. Metabolic pathway analysis using MetaboAnalyst: (a) overview altered metabolic pathways; b) galactose metabolism pathway; c) starch and sucrose metabolism. The colour and size of each circle is based on the p value and the on value of pathways impact and the highlighted pathways were selected based on false discovery rate (FDR) less than 0.05. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

for each analytical method. For NMR dataset, unsupervised chemometric evaluation was performed by Principal Component Analysis (PCA) using The Unscrambler X™ program 10.4 (CAMO software, Woodbridge, NJ, USA). The area of non-deuterated water suppression (δ 4.70 to 4.90 – according to the saturation profile evaluation) was excluded. For LC-MS dataset, the chromatograms region between 0.5 and 8.0 min was used to perform PCA.

The alignment using COW (Correlation Optimized Warping) algorithm was applied over the NMR and LC-MS datasets. For HCA, the variables were mean-centered and Ward's method using Squared Euclidian distance was applied for distance metric. Singular Value Decomposition (SVD) algorithm was used for PCA, which were carried out after the baseline correction, normalization processing, and mean-centered processing over the variables (composition).

In order to evaluate the variables that posses higher impact on the beans composition after controlled atmosphere, the classification model using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) algorithm was applied using the PLS Toolbox™ program (version 8.6.2, Eigenvector Research Incorporated, Manson, WA, USA). It was performed pair comparison of control *versus* the treatments that leads to higher impact on the beans composition (as observed by PCA): control *versus* atmosphere with high humidity level (regardless the CO_2 level); control *versus* atmosphere with low humidity level (regardless the CO_2 level) at 6 months; control *versus* samples conditioned for 6 months (regardless the atmosphere condition); control *versus* samples conditioned for 6 months plus 7 days of shelf life (regardless the atmosphere condition). The loadings and coefficient plots were analyzed and the variables important for projection (VIP) with values higher than 1 were quantified and used as input for metabolic pathway analysis using MetaboAnalyst 4.0 (http://www.metaboanalyst.ca) (Chen et al., 2019; Liu et al., 2017, 2018; Xia, Psychogios, Young, & Wishart, 2009). The organic compounds were quantified by an external reference method provided by the software VnmJ™ (version 4.2, Agilent) (Freitas et al., 2018; Lião et al., 2011).

2.6.2. Univariate statistical analysis

The color data results were submitted to analysis of variance using the software Origin™ 9.4. The variables with significant differences according to F-test ($p < 0.05$) were submitted to mean comparison by Tukey's test ($p < 0.05$).

3. Results and discussion

3.1. Primary metabolites variability of the cotyledon by NMR

The composition of the *P. vulgaris* cotyledon was evaluated after 6 months of storage: with low CO_2 level and low humidity (LCLH); low CO_2 level and high humidity (LCHH); high CO_2 level and low humidity (HCLH); high CO_2 level and high humidity (HCHH); and after an additional 7 days storage under ambient condition. Fig. 1 presents a comparison among the ^{1}H NMR spectra from each experimental condition and control.

The spectra showed that the common bean comprised a high level of aliphatic, carbohydrates, and aromatic compounds (for complete

Table 2
Tentative assignment of the compounds from *P. vulgaris* bean coat ([M-H]$^-$ ion observed and calculated) with produced ions (MS/MS).

Peak	RT (min)	[M-H]$^-$ obs.	[M-H]$^-$ calc.	Product ion	Empirical formula	ppm error	Compounds	Ref.
1	0.72	500.9168	500.9168	386.9402, 272.9549, 158.9770	$C_{10}H_9N_4O_{12}P_4$	0.0	unknown	–
2	1.57	263.0693	263.0702	128.0348	$C_9H_{15}N_2O_3S$	3.4	γ-glutamyl-methylcystein	Morris and Thompson (1958)
3	2.07	865.1972	865.1980	739.1860, 577.1226, 451.1136, 425.0829, 289.0675	$C_{45}H_{37}O_{18}$	0.9	procyanidin B-type trimer	Suh et al. (2016)
4	2.37	451.1240	451.1254	289.0716, 245.0827	$C_{21}H_{22}O_{11}$	3.1	catechin-*O*-hexoside	Journi et al. (2015)
5	2.74	577.1339	577.1346	465.1107, 427.5090, 289.0721	$C_{30}H_{25}O_{12}$	1.2	procyanidin B-type dimer isomer	Zhang and Zhu (2015)
6	2.86	259.1290	259.1294	197.1280, 130.0868, 128.0371	$C_{11}H_{19}N_2O_5$	1.5	γ-glutamyl-leucine	Abu-Reidah, Ali-Shtayeh, Jamous, Arráez-Román, and Segura-Carretero (2015)
7	2.93	289.0720	289.0712	245.0795, 205.0546	$C_{15}H_{13}O_6$	2.8	catechin	Standard
8	3.31	1153.2596	1153.2614	865.2073, 577.1371, 425.0898, 289.0731	$C_{60}H_{49}O_{24}$	1.6	procyanidin B-type tetramer	Lv et al. (2015)
9	3.60	1153.2662	1153.2614	865.2056, 577.1348, 425.0901, 289.0701	$C_{60}H_{49}O_{24}$	4.2	procyanidin B-type tetramer	Lv et al. (2015)
10	3.71	1153.2598	1153.2614	865.2043, 577.1348, 425.0807, 289.0706	$C_{60}H_{49}O_{24}$	1.4	procyanidin B-type tetramer	Lv et al. (2015)
11	4.64	447.0915	447.0927	285.0386, 284.0323, 255.0302, 227.0315	$C_{21}H_{19}O_{11}$	2.7	kaempferol-*O*-hexoside	Abu-Reidah, Arráez-Román, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez (2013)
12	5.06	640.2941	640.2929	489.0965	$C_{26}H_{46}N_3O_{15}$	1.9	unknown	–
13	5.99	1105.5471	1105.5431	–	$C_{53}H_{85}O_{24}$	3.6	soyasaponin A2	Kitagawa et al. (1988)
14	6.30	1089.5520	1089.5575	–	$C_{53}H_{85}O_{23}$	3.5	unknown	–
15	6.69	285.0388	285.0399	151.0071, 133.0392	$C_{15}H_9O_6$	3.9	kaempferol	Standard
16	6.96	327.2167	327.2167	211.1352	$C_{18}H_{31}O_5$	1.2	tri-hydroxyoctadecadienoic acid	Simirgiotis et al. (2017)
17	8.20	957.5059	957.5018	–	$C_{48}H_{77}O_{19}$	4.0	soyasaponin V	Han (2011)
18	8.37	941.5061	941.5010	–	$C_{48}H_{77}O_{18}$	2.2	soyasaponin I	Shiraiwa, Harada, and Okubo (1991)
19	9.09	1067.5435	1067.5427	–	$C_{54}H_{83}O_{21}$	0.7	soyasaponin *Bg*	Suh et al. (2016)
20	9.47	921.4853	921.4848	–	$C_{48}H_{73}O_{17}$	0.5	soyasaponin γg	Noguez Martins et al. (2014)

signaling see Supplementary Information). The major compounds detected were oligosaccharides (confirmed by cross-link of anomeric proton from glucose units with quaternary carbon of the fructose at δ 106.7 observed by HMBC) and sucrose. Therefore, in order to understand the variability of the primary metabolites in common beans according to different storage conditions, an unsupervised chemometric analysis by PCA was performed, which exhibited a separation tendency of the samples along PC1 × PC2 with 75.84% of the total variance explained (Fig. 2).

The scores plot presented the main tendency of separation of the common bean under different storage conditions according to the PC1 axis. The examination of the loadings showed the most important metabolites that lead their discrimination. In general, the principal information was the cotyledon metabolism recovery by the storage at ambient condition after the controlled atmosphere storage, from positive to negative values of PC1. In particular, beans stored under high humidity level and submitted to subsequent storage at ambient condition (HCHH.sl and LCHH.sl) are located close to control, showing their capacity to restore the metabolism. The common beans stored with low CO_2 level and low humidity (LCLH and LCLH.sl) located at positive scores of both PC1 and PC2 axes, presenting the lower amount of the major compounds (negative PC2 loadings).

For comprehensive analysis of the metabolites, a supervised chemometric method by PLS with pair wise comparison (discriminant analysis of control *versus* the selected storage conditions) followed by the data orthogonalization was performed to assists the model interpretation (Izquierdo-García et al., 2011). The compounds with VIP scores higher than 1 were considered statistically significant for two classes discrimination. The analysis of those variables showed that regardless the used storage condition (atmosphere and time), the important metabolites were the same: raffinose (δ 5.42), sucrose (δ 5.42), stachyose (δ 5.46), verbascose (δ 5.46), acetylcholine (δ 3.21), citric acid (δ 2.58) and acetic acid (δ 1.92). This fact was in accordance with the PCA analysis, which showed the main variation with respect to PC1 axis. Therefore, the control samples *versus* 6 months of storage were used as model for metabolites analysis. In order to quantify the sucrose and oligosaccharides separately, some considerations were made. It was possible to obtain the value of sucrose and raffinose separately by considering the integration of the signal at δ 5.00 (hydrogen of anomeric linkage between glucoses) of the oligosaccharides, which is 1 for raffinose, 2 for stachyose, and 3 for verbascose (Alves Filho et al., 2017; Lião et al., 2011). In addition, the signal at δ 5.46 was considered solely from stachyose since the content of verbascose was nearly to zero in beans, especially in Pinto beans (Iyer, Salunkhe, Sathe, & Rockland, 1980; Moghaddam et al., 2018).

To identify the relevant pathways linked to metabolites variation after the storage conditions, the variables were submitted to MetaboAnalyst (Xia et al., 2009; Xia & Wishart, 2011). As shown in Fig. 3a, eight pathways were suggested to be associated with the metabolism response to storage conditions. However, pathways with false discovery rate ($P < 0.05$) were considered as affected by the storage conditions, which includes galactose metabolism (Fig. 3b), and starch and sucrose metabolism (Fig. 3c). The metabolites in deep red color to yellow indicates an increased concentration of metabolites in stored beans and also higher impact on pathway and –log(P).

In non-photosynthetic tissues as beans, sucrose is the material for several metabolic pathways, providing energy and carbon skeletons for the production of amino acids, nucleotides and structural carbohydrates (Stein & Granot, 2019). In addition, raffinose and stachyose are also all closely associated with plant energy metabolism (Eveland & Jackson, 2011). In the galactose metabolism pathway, it was observed that the overexpression of certain saccharides as sucrose, raffinose, and stachyose were induced by storage conditions. As consequence to sucrose increasing, the starch and sucrose metabolism was also affected. Starch, sucrose, and galactose metabolisms participate in the germination and sprouting processes (Chen et al., 2019). In the light of these data, it

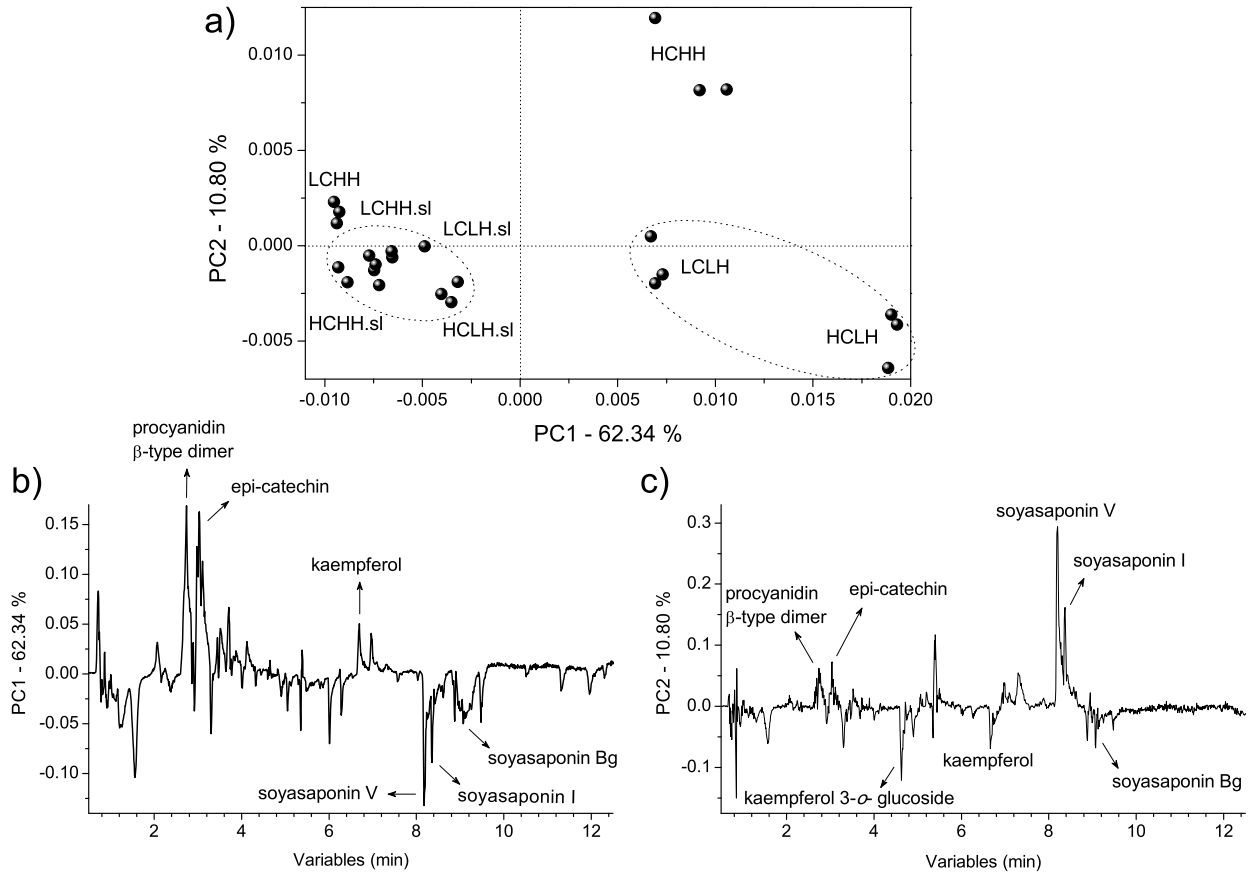

Fig. 4. PC1 × PC2 scores plot from bean coats submitted to different storage conditions (a); loadings of PC1 and PC2 plotted in lines form (b and c), respectively.

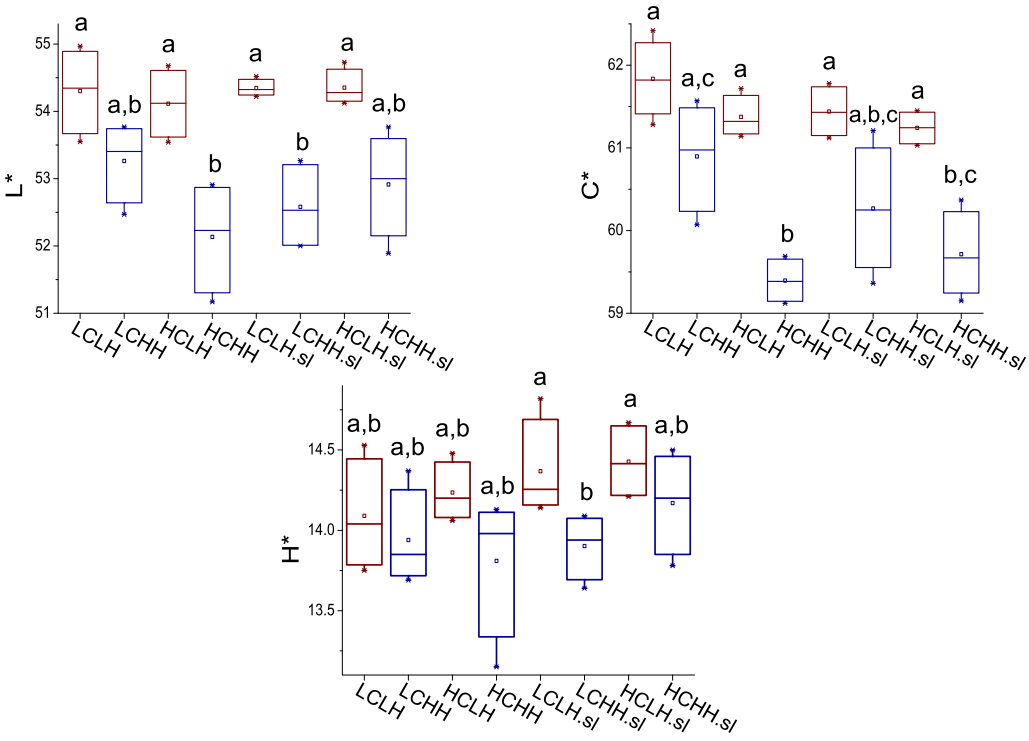

Fig. 5. Common beans coat color parameters of luminosity (L*), chroma angle (C*) and hue angle (H*) presented as box-and-whiskers graphs. Legend: LCLH – low CO_2 level, low humidity; LCHH – low CO_2 level, high humidity; HCLH – high CO_2 level; HCHH – high CO_2 level, high humidity; sl – storage at ambient condition. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

seems possible that storage conditions induce the role in regulating the germination and sprouting processes. However, it was not observed changing on amino acids or lipid metabolism that are also related to the aforementioned mechanisms. Therefore, those mechanisms seem to be partially activated after 6 months regardless the atmosphere conditions. Despite this, the germination capability of the seeds after the storage time and conditions were not compromised as observed in the germination experiments (data not shown).

3.2. Bean coat analysis

It was characterized some of the major compounds according to their *m/z* and fragmentation profiles (Table 2). A comparison among typical chromatographic profiles from bean coats are illustrated in Fig. 1SI.

The presence of procyanidin, catechin, kaempferol derivatives, and saponins was detected. The compounds **2** (γ-glutamyl-methylcystein) and **6** (γ-glutamyl-leucine) showed $[M-H]^-$ ion at *m/z* 263.0693 and 259.1290, with fragments at *m/z* 128, and 197, 130, 128, relatives to the loss of methylcysteine, glutamic acid, and leucine. The compound **4** showed a precursor ion at *m/z* 451.1240 and fragment product at *m/z* 289.0716, which is compatible to the loss of hexose in catechin and, therefore, it was characterized as catechin-*O*-hexoside (Journi, Hammouda, Trabelsi-Ayadi, & Chérif, 2015). The compounds **3, 5, 8–10** exhibited $[M-H]^-$ at *m/z* 577, 865, and 1153, respectively. These compound and fragments were compatible with procyanidin *B*-type dimer, trimer, and tetramer isomers (Zhang & Zhu, 2015). The compounds **7** and **15** exhibited precursor ion $[M-H]^-$ at *m/z* 289.0720 and 285.0388, which correspond to catechin and kaempferol, respectively, by comparison with standard. In *Phaseolus*, kaempferol is commonly detected in *O*-glycosidic form. The compound **11** showed a precursor ion $[M-H]^-$ at *m/z* 447.0915 and fragment at *m/z* 285.0386, compatible with kaempferol product ion. Accordingly, it was identified as kaempferol-*O*-hexoside (Abu-Reidah, Arráez-Román, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2013). The compounds **13**, **17–20** exhibited molecular ion $[M-H]^-$ at *m/z* 1105.5471, 957.5059, 941.5061, 1067.5435, and 921.4853, which were characterized as soyasaponin A2, V, I, Bg, and γg, respectively (Suh et al., 2016). The compound **16** with molecular ion $[M-H]^-$ at *m/z* 327.2167 was tentatively assigned as tri-hydroxyoctadecadienoic acid based on the fragmentation pattern (Simirgiotis et al., 2017). The compounds **1**, **12** and **14** were not identified.

The aforementioned compounds were detected in all samples in different intensities. Therefore, due to the elevate complexity of the data chemometric analysis was developed. Fig. 4 shows the PC1 × PC2 scores plot, discriminating samples regarding different storage conditions with 73% of the total variance explained. Fig. 4b and c show the corresponding loadings graph for both PC1 and PC2 axes, respectively.

Common bean stored under low humidity was located at positive scores of PC1 and negative of PC2 and furthermore, those beans stored under high CO_2 condition were located at higher positive scores of PC1 and PC2 concomitantly. This fact was related to appropriated storage, since low O_2 and high CO_2 atmosphere with reduced humidity lead to arthropods death during grain storage, preserving the bean quality (Freitas et al., 2016). At negative scores of PC1 are located the beans submitted to additional 7 days of storage at ambient condition, and the beans stored under high humidity with low CO_2 level (closest to storage at ambient condition), which indicate the reactivation of the metabolism after withdrawal of the beans from the controlled atmosphere.

According to loadings graph from PC1 (Fig. 4b), it was noticeable the reduction of procyanidin *B*-type dimer, epi-catechin, and kaempferol in beans submitted to 7 days of storage at ambient condition, as well as beans stored at low CO_2 and high relative humidity. In general, pigments responsible for bean coat color are flavonoids (Ranilla, Genovese, & Lajolo, 2007). 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 of the bean coat (Coutin et al., 2017). Therefore, the decrease of procyanidin *B*-type dimer, kaempferol; and epi-catechin can be associated to bean coat darkening after 7 days of storage at ambient condition as corroborate by color evaluation (Fig. 5). In addition, it also indicated that the beans submitted to low CO_2 and high relative humidity might present coat darkening. Often, kaempferol content reduces after darkening (Beninger et al., 2005).

For beans located in negative scores of PC1, an increase in content of the saponins soyasaponin *Bg*, soyasaponin *I*, soyasaponin *V* was observed. The soyasaponins are triterpenoid aglycones, and commonly found in *P. vulgaris* (Kitagawa et al., 1988). Therefore, higher content of saponins after 7 days of storage at ambient condition may indicate the restoration of the bean metabolism. This dynamics is more noticeable by observing the loadings graph of PC2 axis (Fig. 4c), in which beans submitted to high CO_2 and high relative humidity presented high amounts of the saponins. It is known that bean coat chemical pattern and color in *P. vulgaris* is controlled by a group of genes, which regulate the flavonol and anthocyanin biosynthetic pathways (McClean, Lee, Otto, Gepts, & Bassett, 2002). Therefore, alterations in storage conditions affected the biosynthesis of these compounds.

3.3. Bean color

Since the color of common bean is an important visual attribute and presents important influence on consumer's intention-to-buy and acceptability, the coat color was evaluated in order to correlate to storage conditions (Fig. 5). The common bean storage under low humidity (40%) showed highest luminosities, which indicate the preservation of the lighter color. However, the color was not influenced by different CO_2 levels or 7 days of storage condition after controlled atmosphere storage because no significant differences were observed (95% of reliability). It is known that common bean stored under high temperature and/or humidity are susceptible to development of hardening and darkening phenomena (Coelho, Prudencio, Christ, Sampaio, & Schoeninger, 2013). Therefore, beans stored in lower humidity presented higher L* (luminosity) and C* (chroma) values, as well as increases in amounts of proanthocyanidin *B*-type dimer, epi-catechin, and kaempferol contents (Fig. 4b). The low procyanidin *B*-type dimer content was coherent with darkening of the bean coat due to proanthocyanidin oxidation to reactive quinones (Ranilla et al., 2007). Hue angles (H*) from stored beans were similar and ranged within the 90° region, suggesting an apparent reddish yellow color.

4. Conclusion

The NMR spectroscopy coupled to chemometrics enables the study of effected pathway of the beans submitted to controlled atmosphere. It was observed changing on galactose, starch and sucrose pathways that are closely associated to regulation on the germination and sprouting processes. However, it was not observed changing on amino acids or lipid metabolism, and metabolites also linked to aforementioned mechanisms, indicating that those mechanisms seems to be partially activated after 6 months regardless the atmosphere conditions. For LC-MS analysis, it was observed that common beans stored under low relative humidity presented highest luminosity and chroma values, which was corroborated by the reduction of proanthocyanidin *B*-type dimer, epi-catechin, and kaempferol contents indicating correlations between color and chemical parameters. These findings showed bean coat darkening when submitted to high humidity during the controlled atmosphere storage. Through the LC-MS analysis, it was also observed higher content of saponins after 7 days of storage at ambient condition, as well as in the beans stored at low CO_2 and high relative humidity, indicating the restoration of bean metabolism. In general, seeds presented viability for germination after storage time regardless the atmosphere condition,

despite of the darkening process observed.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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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.2019.108673.

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