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Genotype evaluation of cowpea seeds (*Vigna unguiculata*) using ^{1}H *q*NMR combined with exploratory tools and solid-state NMR

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ABSTRACT

The ultimate aim of this study was to apply a non-targeted chemometric analysis (principal component analysis and hierarchical clustering analysis using the heat map approach) of NMR data to investigate the variability of organic compounds in nine genotype cowpea seeds, without any complex pre-treatment. In general, both exploratory tools show that Tvu 233, CE-584, and Setentão genotypes presented higher amount mainly of raffinose and Tvu 382 presented the highest content of choline and least content of raffinose. The evaluation of the aromatic region showed the Setentão genotype with highest content of niacin/vitamin B3 whereas Tvu 382 with lowest amount. To investigate rigid and mobile components in the seeds cotyledon, ^{13}C CP and SP/MAS solid-state NMR experiments were performed. The cotyledon of the cowpea comprised a rigid part consisting of starch as well as a soft portion made of starch, fatty acids, and protein. The variable contact time experiment suggests the presence of lipid-amylose complexes.

1. Introduction

Phenotypic variation of a crop species may occur due to the environment and genetics, which is the focus for improvement of food. Therefore, the construction of a germplasm bank is important for breeding programs of plants (Diouf, 2013; Singh, 2002; Singh, Chambliss, & Sharma, 1997; Singh, Ehlers, Sharma, & Freire Filho, 2002). Cowpea (*Vigna unguiculata*) is an important and inexpensive source of proteins, complex carbohydrates, dietary fiber, iron, zinc, vitamins, and low amount of lipids (less than 2%) for millions of people in the world (Devi, Kushwaha, & Kumar, 2015; Ojwang, Yang, Dykes, & Awika, 2013). Additionally, bioactive compounds such as carotenoids and phenolics were found in cowpea (Dueñas, Fernández, Hernández, Estrella, & Muñoz, 2005; Ha et al., 2010). The carbohydrates are by far the most abundant nutrients in cowpea (Madodé et al., 2013). Despite the high nutritional potential, cowpea seeds contain indigestible substances, such as the oligosaccharides: raffinose, stachyose, and verbascose (Lião et al., 2011). The absence of α-galactosidase in humans, which digest these oligosaccharides, induces the anaerobic fermentation of the oligosaccharides by microorganisms in the intestines and, therefore, production of carbon dioxide, hydrogen and methane.

In the attempt to enhance the quality of cowpea for food applications a substantial focus has been dedicated to the study of physicochemical and nutritional attributes (Ávila et al., 2015; Ba, Pasquet, & Gepts, 2004; Ojwang, Dykes, & Awika, 2012). Consequently, to find cowpea cultivars that produce these oligosaccharides in lower concentrations, minimizing anti-nutritional effects could be an important strategy to improve seeds consumption. Moreover, cultivar type and cultivation conditions are, therefore, major factors that affect the concentration of organic compounds and the general quality of legumes consumed (Plahar, Annan, & Nti, 1997).

The ultimate aim of this study was to identify and investigate the range of organic compounds in different cowpea seed genotypes by non-targeted chemometric analysis of NMR spectra of cowpea extracts. Furthermore, an additional analysis was performed to evaluate the same cowpea seeds employing ^{13}C solid-state NMR in order to provide information on chemical structure and molecular dynamics of the intact seeds.

2. Material and methods

2.1. Plant materials

Nine genotypes of cowpea (*Vigna unguiculata*) acquired under the same conditions and planting season (harvest year: 2011; 2012; and 2013) were obtained from the germplasm bank of the Center of

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Agricultural Science at Federal University of Ceará (CCA/UFC), Brazil, and the respective genotypes are: Sempre Verde; Pitiuba; Novato; Tvu 233; CE-584; Setentão; Epace 10; Pingo de Ouro; and Tvu 382.

2.2. Sample preparation and liquid-state NMR spectroscopy

For liquid-state NMR analysis, three biological groups (for each genotype) that contain five seeds each were organized. Those seeds were peeled, grounded into fine powder in liquid nitrogen, and homogenized. From this powder, it was weight 15 mg and soaked in a mixture of 400 μL of D_2O and 200 μL phosphate buffer pH 4.3 (0.4 mM Na_2HPO_4/$NaHPO_4$; and 1.0 mM of TMSP-d_4 – sodium-3-trimethylsilylpropionate-2,2,3,3-d_4 as internal standard). The solutions were automatic mixed (Thermomixer Comfort) during 5 min at room temperature and centrifuged at 6000 rpm. The supernatants were transferred to 5 mm NMR tubes. The pH was *ca.* 6.5 for all the samples and for this reason all the compounds identified in this study are reported based on their neutral form.

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 performed using the PRESAT pulse sequence for water suppression. This pulse sequence presented the best irradiation profile for quantitative determination of the signals near of the water suppression region (Bharti & Roy, 2012). The data were acquired with the RF pulse calibrated to 90° and 128 scans, 64 k of time domain points for a spectral window of 15 ppm, acquisition time of 6.7 s and a relaxation delay of 15.0 s. The temperature was 298 K. The spectra were processed by applying exponential Lorentzian broadening of 0.3 Hz and zero filling to 64 k points before Fourier transformation. Phase correction was performed manually for each spectrum and the baseline correction was applied over the entire spectral range. All spectra were referenced to the TMSP-d_4 resonance at 0.0 ppm.

2.3. Molecular identification and quantification analysis

The identification of the constituents within the cowpea samples was performed through ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC experiments as well as supplementary existing open access databases and literature reports (Alves Filho et al., 2016; Boffo, Tavares, Ferreira, & Ferreira, 2009; Choze et al., 2013; Koda, Furihata, Wei, Miyakawa, & Tanokura, 2012; Nord, Vaag, & Duus, 2004; Sucupira et al., 2017; Wishart et al., 2007).

The oligosaccharides raffinose and stachyose, and niacin were quantified by an external reference. In this procedure, the absolute integral of the anomeric proton (at 5.42 ppm in ^{1}H spectrum) of a standard solution of sucrose solubilized in deuterium oxide (5.0 $mg \cdot mL^{-1}$) was used as a reference of quantification adapted for *Phaseolus* beans (Wider & Dreier, 2006). The analysis of variance ANOVA single factor was used to statistically certify the comparison between the concentrations using Tukey Test with significance level of 0.05 and Levene to test the homogeneity of variance.

2.4. Chemometric analysis

The ^{1}H NMR spectra were processed in Topspin 2.1 and utilized as input data for the PLStoolbox (version 7.8) in Matlab 2014a (version 8.3.0.532) to perform Principal Component Analysis (PCA), to obtain an overview showing trends, groupings and outliers with a confidence level of 95% (Larsen, van den Berg, & Engelsen, 2006; Soares et al., 2017). To overcome possible chemical shifts due to pH change, before the analysis the ^{1}H NMR spectra were aligned using interval-based Icoshift (Savorani, Tomasi, & Engelsen, 2010). The data was mean centered and the whole NMR spectrum was used for the chemometrics analysis, which was performed using a nine replicates of 9 cowpea samples. PCA's were performed using two sets of ^{1}H NMR spectra: total spectra (0.70 to 9.22 ppm), and aromatic region (6.00 to 9.22 ppm).

For the Hierarchical Clustering Analysis (HCA) using the heat map analysis, each spectrum was divided into 0.04 ppm wide buckets, using simple rectangular bucket, sum of intensities in integration mode and scaled to total intensity in scaling process, resulting 213 buckets per spectrum. The area around the non-deuterated water (4.71 to 5.15 ppm) was excluded from the bucketing process. The chemical shift values refer to the central position of each bucket. The bucket table from ^{1}H NMR data was imported into GENE-E program (http://www.broadinstitute.org/cancer/software/GENE-E/index.html) for pattern recognition and the classification was performed on the rows and columns. The Euclidean distance method was used to measure the proximity between samples (columns) and the one minus Pearson correlation method was performed to agglomerate the chemical shifts (rows). The results are visualized as a 2-D dendrogram (heat map) where the deeper red colour represents the higher relative intensity on the line (chemical shift), the deeper blue colour the lower relative intensity and the intermediary intensity in white colour.

2.5. Solid-state NMR (ssNMR)

2.5.1. Sample preparation

The cowpea seeds were peeled and the cotyledons were grounded into fine powder in liquid nitrogen, homogenized, and 50 to 55 mg of the powder was inserted in the Kel-F NMR rotor of 5 mm (o.d.) for the NMR analysis. It was also run hydration experiments. For this, 22 mg of the powder was loaded into the rotor, and 25 μL of D_2O was added to hydration studies. Previously to the analysis, the rotor was spun at 6 kHz for 2 h to guarantee the homogeneous hydration of the system. After this, the rotor was spun at 14 kHz to run the experiments. To analyse the truly rigid portion of the seed, ~130 mg of the endosperm was mixed with 3 mL of water two times. The supernatant was removed; the precipitate was dried, and only the precipitate was analysed through ^{13}C ssNMR experiments.

2.5.2. ssNMR experiment

The solid-state NMR experiments were performed on an Agilent 600-MHz spectrometer operating at 150.87 and 599.93 MHz for ^{13}C and ^{1}H, respectively. The ^{13}C cross polarization magic angle spinning (CP-MAS) experiment was performed for the cowpea powder and hydration experiments. For this a 80–100% ramp with a contact time of 1 ms, high power composite pulse decoupling (Spinal 64) with 2 K of data points and a spectra width of 487.37 ppm, 5 s of delay between each acquisition, and 2000 scans for the cowpea powder and 4000 scans for the hydration experiments was used. For the contact time variable in CP-MAS (Metz, Wu, & Smith, 1994) experiments, 80–100% ramp with contact time ranging from 0.1 to 10.0 ms with 256 scans, 2 K of data points, and 3 s of delay between each acquisition was used. The single pulse magic angle spinning experiments (SP-MAS) were performed with 350 scans, 2 K of data points, and 120 s of delay between each acquisition. All the experiments were performed with 14 kHz of spinning and the VNMRJ™ 4.0 software was used for the experiments acquisition and processing. Adamantane was used as external chemical shift reference.

3. Results and discussion

The ^{1}H NMR spectra in Fig. 1 showed that the cowpea samples comprise a high level of aliphatic, carbohydrates, and aromatic compounds. For the oligosaccharides characterization, the chemical shift of the anomeric proton of the glucose unit was confirmed by cross-link with quaternary carbon (^{13}C NMR chemical shift of 106.4 ppm) of the fructose unit in HMBC experiment. The doublet at 5.42 ppm was attributed to anomeric proton of raffinose, at 5.44 ppm to anomeric proton of

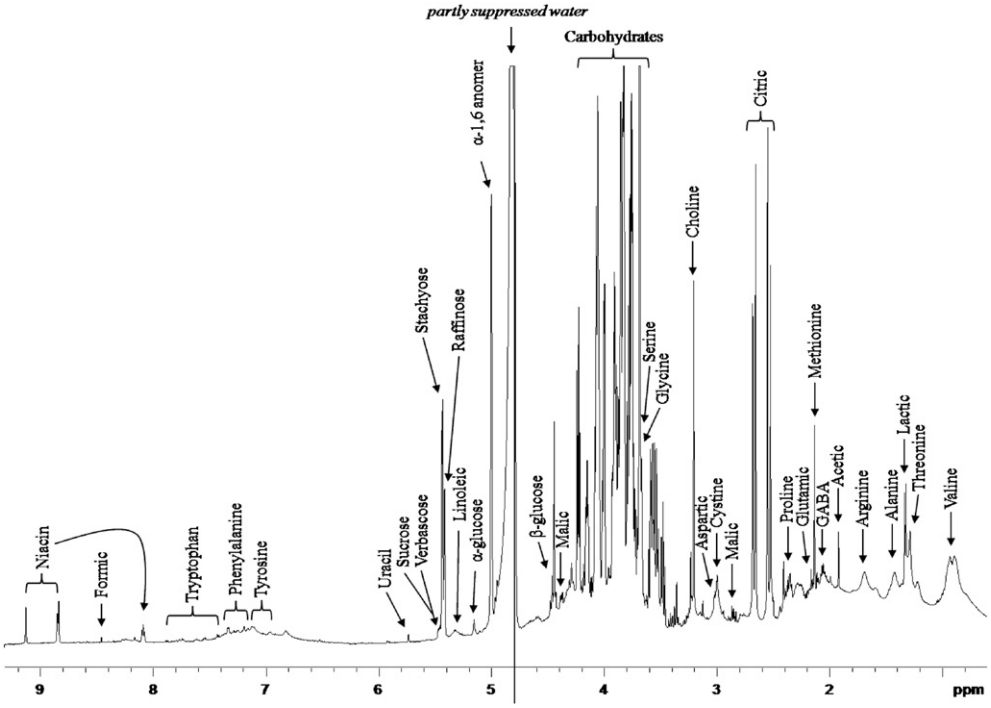

Fig. 1. Representative ^{1}H NMR spectrum of aqueous cowpea supernatant.

stachyose, and at 5.46 ppm to anomeric protons of sucrose and verbascose.

In order to obtain an overview and understand the variability of the primary metabolites in cowpea seeds, chemometric analyses were performed in the collected data (whole ^{1}H NMR spectra). Initially, the unsupervised agglomerative method HCA was applied and all similarities between the cowpea samples (columns) and organic constituents (chemical shifts in rows) are shown in the 2-D dendrogram (heat map) in Fig. 2. According to the columns in the dendrogram three samples detached from the majority group: Tvu 233 with highest amount of oligosaccharides; Pitiuba with highest amount of niacin, citric and lactic acids; and Tvu 382 with highest amount of choline and lowest amount of oligosaccharides.

To reduce the dimensionality of the original data and to assist the modelling and interpretation of multivariate data, PCA was applied to further explore the data. The grouping observed in HCA (Fig. 2) was very important to interpret the scores plot obtained by PCA, which exhibits a separation tendency of the samples with respect to PC1 and PC2. Models using two principal components were built and 81.15% of the total variance analysing the whole spectra (Fig. 3a), and 85.63% of the total variance analysing just the aromatic region (Fig. 3b) were explained.

The examination of the scores and loadings in PCA analysis was useful to understand the main markers responsible for the discrimination of the cowpea seeds from the different varieties. The application of PCA using the entire spectra (Fig. 3a), in general, resulted in the grouping of the samples Tvu 233, CE-584, and Setentão in positive scores of PC1 (68.99%) and, in negative scores, Epace 10 and Tvu 382. For the positive values of PC1 the dominating resonances of the loadings can roughly be described by a sum of oligosaccharides (raffinose and stachyose), choline, citric acid, methionine, acetic acid, lactic acid, and niacin. However, the variable raffinose stood out as the main variable (Fig. 3a) for distinction of the data as can be corroborated by the quantification (Table 1), which shows the seeds Tvu 233, CE-584, and Setentão with a higher content. For PC2 (12.16%) the seeds Tvu 382 and Tvu 233 were located at the extremes (same tendency observed in PC1) with Tvu 382 and Setentão at positive scores and Tvu 233 at negative scores. A close inspection of the loadings reveals that the

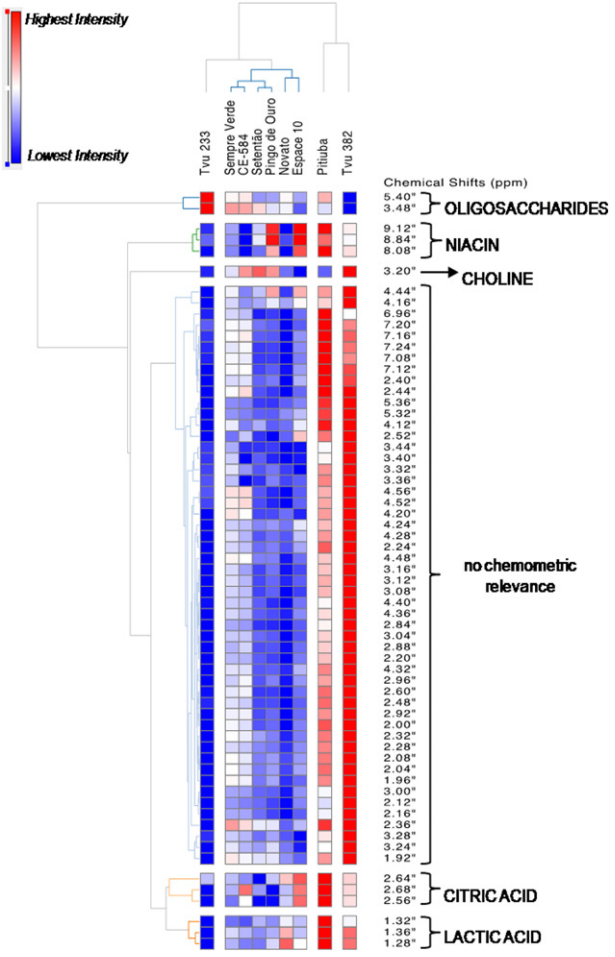

Fig. 2. Dendrogram representing chemical composition similarity relationships among cowpea seeds.

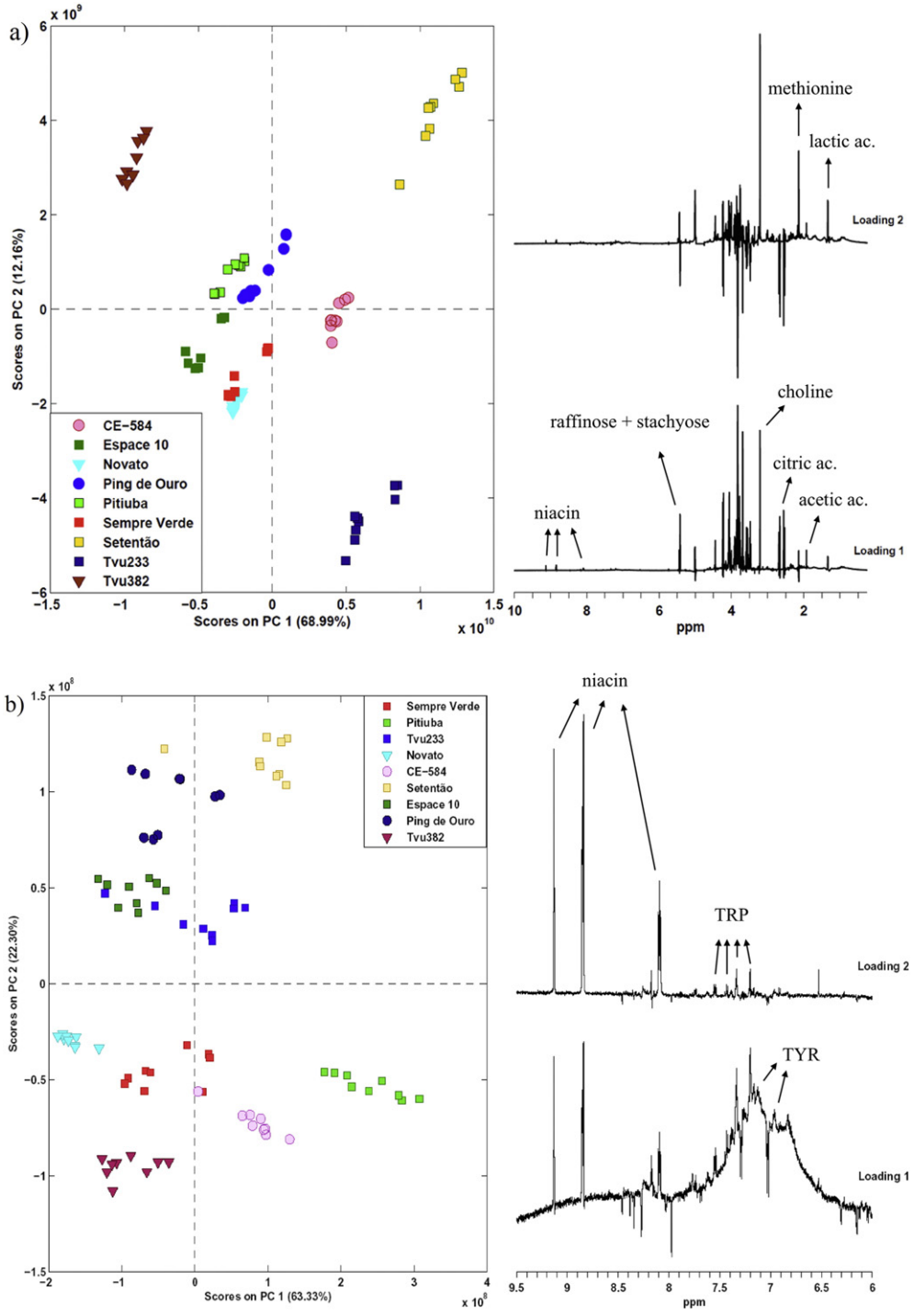

Fig. 3. PC1 *vs.* PC2 scores (left side) and loadings (right side) coordinate system for different cultivars of cowpea using: (A) whole spectrum; (B) only aromatic region with TRP to tryptophan and TYR to tyrosine.

Table 1
Means and standard deviation of the concentrations of niacin, raffinose, and stachyose (mg·g^{-1} of cotyledon) in the different genotypes of cowpea seeds. The values within the lines of concentrations with different superscript letters were significantly different at 0.05 of significance level as identified using a one-way ANOVA and Tukey Test.

	Genotype								
Compounds	Sempre Verde	Pitiuba	Novato	Tvu 233	CE-584	Setentão	Epace 10	Pingo de Ouro	Tvu 382
Niacin	$1.0^{a} \pm 0.1$	$1.2^{b} \pm 0.1$	$0.9^{a} \pm 0.0$	$1.2^{b} \pm 0.0$	$1.0^{a} \pm 0.0$	$1.5^{c} \pm 0.0$	$1.2^{b} \pm 0.0$	$1.3^{d} \pm 0.0$	$0.8^{e} \pm 0.0$
Raffinose	$26.0^{a} \pm 0.5$	$21.8^{b} \pm 0.5$	$24.4^{c} \pm 0.5$	$38.6^{d} \pm 0.9$	$32.1^{e} \pm 0.2$	$34.2^{f} \pm 0.7$	$19.8^{g} \pm 0.4$	$24.5^{c} \pm 0.6$	$11.4^{h} \pm 0.8$
Stachyose	$41.9^{a} \pm 0.5$	$47.0^{b} \pm 0.8$	$45.6^{c} \pm 0.6$	$45.0^{c} \pm 1.2$	$45.8^{bc} \pm 0.5$	$56.9^{d} \pm 0.9$	$46.5^{bc} \pm 0.6$	$47.4^{bc} \pm 1.4$	$44.6^{bc} \pm 1.3$
Total Oligosac.	67.9 ± 1.0	68.8 ± 1.3	70.0 ± 1.1	83.6 ± 2.1	77.9 ± 0.7	91.1 ± 1.6	66.3 ± 1.0	71.9 ± 2.0	56.0 ± 2.1

seeds at positive scores of PC2 (Setentão and Tvu 382) presented higher content of lactic acid, methionine, and choline. The former was the most important variable for the PC2 axis. In addition, the seed Tvu 233 presented a lower amount of the aforementioned compounds and higher content of citric acid.

In order to observe more clearly the aromatic compounds within the samples, PCA analysis was performed to the spectral region between 6.0 and 9.5 ppm (Fig. 3b). Overall, the samples presented a remarkable tendency of discrimination over PC2 with the seeds Setentão, Pingo de Ouro, Epace 10, and Tvu 233 at positive score of PC2 and Novato, Sempre Verde, CE-584, Pitiuba, and Tvu 382 at negative scores of PC2. In addition, both positive loadings (PC1 and PC2) were dominated by niacin/Vitamin B3 besides the slight influence of tyrosine (TYR) and tryptophan (TRP). Therefore, the highest content of niacin/Vitamin B3 was found in Setentão seed whereas the lowest content was observed in Tvu 382 (lower PC2 scores). Niacin in cowpea seeds is important because its presence in human body reduces total cholesterol, lipoprotein, and triglycerides (Pieper, 2003). According to the PC1 *vs.* PC3 analysis from the same region (see corresponding Data in Brief), Tvu 233 located in positive values of PC3 presented as the unique seed with high content of tryptophan. In particular, high tryptophan content is an advantage since it is one of the essential amino acids at limited concentration in foodstuffs (Yust et al., 2004).

Quantitative NMR (*q*NMR) is a powerful tool to provide simultaneous access to both the qualitative (chemical structure) and the quantitative information. Most quantitative NMR assay methods are based on using an internal standard, for particular analysis and improvement of the results (Alves Filho et al., 2015). Presently a sucrose spectrum was used as an external concentration standard because it is a very stable compound, its resonances are not overlapping with the partly suppressed water signal, and did not affect the concentration of the organic compounds in the sample, since it is not added to the sample (Wider & Dreier, 2006). The compounds that had a high variation in concentration and did not exhibit overlapping resonances were quantified. ^{1}H NMR analysis allowed the choice of signals in aromatic region and anomeric signals to be used in the quantification procedure. Each one of the oligosaccharides raffinose and stachyose has only one glucose unit that may be used for quantification, and niacin was quantified by the singlet well separated from others resonances. Therefore, the quantification was carried out using the known sucrose concentration related to proton signal of niacin (9.13 ppm) and H-1 signals of raffinose (5.42 ppm) and of stachyose (5.44 ppm) peaks areas. The results of the liquid-state ^{1}H NMR technique applied to the determination of niacin, raffinose, stachyose and the total of oligosaccharides concentrations in cowpea seeds are described in mg.g^{-1} of cotyledon in Table 1 and the comparison of the results was statistically certified based on the analysis of variance ANOVA single factor.

According to Table 1, raffinose had higher variation than stachyose among the cultivars. Further Tvu 233, CE-584, and Setentão cultivars had the highest content of total of oligosaccharides whereas Tvu 382 had the lowest content as was also seen in Fig. 3a. Previous studies presented the following concentrations of raffinose and stachyose (mg per g of cowpea – *Vigna unguiculata*), respectively: 7.8 and 35.3 mg·g^{-1} (Egounlety & Aworh, 2003); 7–8 and 42–52 mg·g^{-1} (Madodé et al., 2013); 12.4 and 33.4 mg·g^{-1} (Prinyawiwatkul, Beuchat, McWatters, & Phillips, 1996); 5.6 and 17.6 mg·g^{-1} (Kalidass & Mohan, 2012). In general, the contents of raffinose related in this work were 3–4 times higher than found in the previously published (by HPLC and UV-spectrophotometer analyses) due to the different cowpea genotypes studied and methodology. In addition, the samples Pitiuba, Setentão and Pingo de Ouro presented higher concentrations of niacin, corroborating manly the dendrogram in HCA results. The information of the variation between the oligosaccharides (integral values) into each sample is corroborated by the visualization of the anomeric region of ^{1}H NMR spectra (Fig. 4) and it is in agreement with NMR quantification.

The visualization of the anomeric signals in the ^{1}H NMR spectra emphasizes the seeds Setentão, Tvu 233, and CE-584 as the most raffinose (5.42 ppm) and stachyose (5.44 ppm) contents. Furthermore, Tvu 382 presented as the seed with less raffinose content, which corroborate the chemometric and *q*NMR analyses.

3.1. ssNMR

The ^{13}C SP-MAS NMR experiments were performed in order to characterize the entire cowpea cotyledon as both mobile and immobile components are observed, whereas only the immobile components are observed by the ^{13}C CP-MAS NMR experiments. In the ^{13}C CP-MAS experiments, magnetization is transferred from protons to the carbons

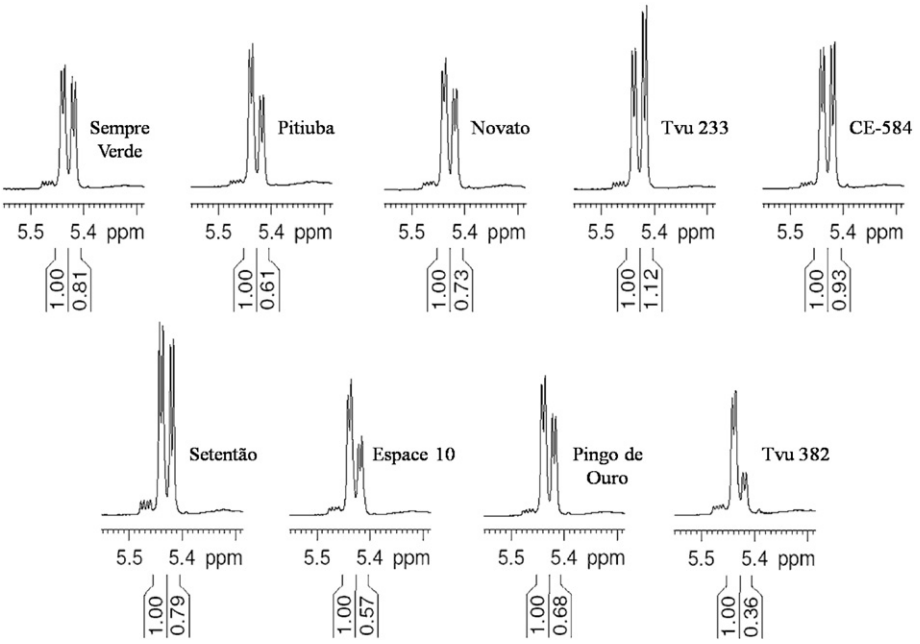

Fig. 4. Anomeric proton region highlighted to raffinose (5.42 ppm) and stachyose (5.44 ppm). The integral values are related internally for each spectrum.

via heteronuclear dipolar couplings. Consequently, the CP is not effective for mobile systems such as solutions and gels. In other words, the CP provides information of components with the most solid-like character. In general, the seeds presented similar composition for both rigid and non-rigid portions. In Fig. 5b the ^{13}C SP-MAS spectrum of a selected seed (CE-584) is presented. Despite the high heterogeneity of the system, it was performed a roughly spectral assignments of the main components of the seed as shown on Table 2. The ^{13}C CP-MAS NMR spectrum of the coatless grain of cowpea is shown in the Fig. 5a. The Fig. 5c displays the spectrum of the hydrated powder; Fig. 5d shows the precipitated (powder) resulting from the extraction with water. In order to get more information regarding the protein portion of the seed, the difference spectra between the ^{13}C SP-MAS of the entire seed (Fig. 5b) and dried precipitate (Fig. 5d), was performed and normalized with respect to the anomeric signal of the starch.

In the Fig. 5c, is shown the ^{13}C NMR spectrum of the hydrated powder. The amorphous phase of the starch is composed of truly rigid amorphous and/or crystalline components (Cheetham & Tao, 1998). In addition, the C6-resonances at ~63 ppm and *C*4 resonances at ~83 ppm were modified upon hydration, which indicates the mobility gain of these sites. This shows that these carbons are less obstructed and, therefore, easily accessible for water (Larsen et al., 2013). The Fig. 5d shows the spectra of the true rigid solid present at the system since the aqueous soluble part was removed after the partition with water. According to the spectrum, this portion is mainly composed of starch. It is also observed that the carbonyl resonance arising from either proteins or solid lipids remained in the spectrum, and on opposite the resonances between 14 and 45 ppm from protein reduced. This finding might be corroborated with the difference spectrum (Fig. 5e) between (b) and (d) showing the removal of the mobile components (mainly protein) with water. In the ^{1}H liquid-state NMR spectrum (Fig. 1) protein was also observed as a broad underlying spectral feature (from 0.5 to 2.5 ppm) as well as in the loadings for the PCA of the spectral region 6.0–9.5 ppm (Fig. 3b). The comparison of the seeds was also performed by ^{13}C CP-MAS and the ^{13}C SP-MAS. Comparison of the two spectra revealed that all the seed contained unsaturated fatty acids and similar composition.

The variable contact time (VCT) of the cross polarization mechanism for the seeds Sempre Verde, Pitiuba, Tvu 233, Novato, and Tvu 382 that presented high differences in PCA (from liquid samples) was performed (Fig. 6) for the integral of the range at ~90 to 105 ppm. This resonance contains contributions from *C*1 in starch as well as the anomeric carbons in the oligosaccharides. The data was fitted according to the Eq. (1) (Kolodziejski & Klinowski, 2002) where: I_0 is the absolute amplitude

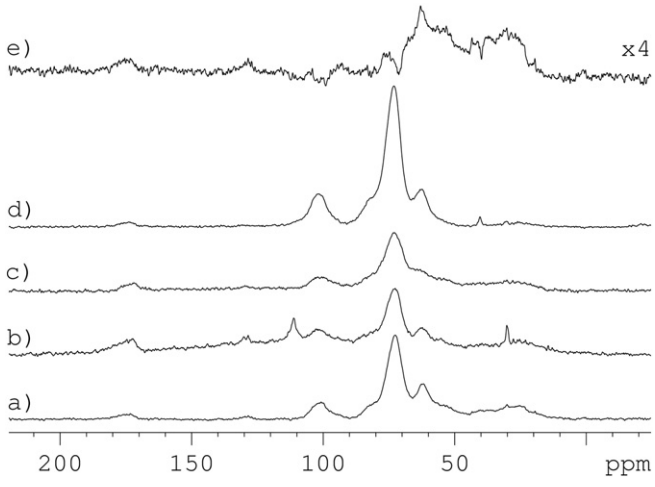

Fig. 5. ^{13}C NMR spectra of CE-584: a) corresponding ^{13}C CP-MAS spectrum of the coatless grain; b) ^{13}C SP-MAS spectra of the coatless grain; c) ^{13}C CP-MAS spectra of hydrated powder and d) dried precipitate; e) difference spectrum between (b) and (d).

Table 2
^{13}C NMR residues of compounds from cowpea seeds (Bardet, Foray, Bourguignon, & Krajewski, 2001; Lam et al., 2014).

Residue	δ ^{13}C (ppm)
Fatty acids	
—CH_3	15
—$(CH_2)_n$—	22 to 35
—C═ C—	125 to 132
—C═ O	169.0 to 178.0
Protein	
—CH_2—	15 to 40
—CH/—CH_2—	50 to 70
Aromatic residue	128–133
—C═ O	170 to 185
Starch	
C1 (—CH—)	90 to 105
C2, C3, C5 (—CH—)	65 to 78
C4 (—CH—)	77 to 88
C6 (—CH_2—)	59 to 64
PTFE/Teflon	
—$(CF_2)_n$—	111.3 ppm

of the NMR signal; T_{CH} is a cross polarization time constant, $T^{H}_{1\rho}$ is the spin-lattice time in the rotating frame and; *t* is the contact time.

$$I_0(t) = I_0\left(1-\frac{T_{CH}}{T^{H}_{1\rho}}\right)^{-1} \times \left[e^{\left(\frac{-t}{T^{H}_{1\rho}}\right)} - e^{\left(\frac{-t}{T_{CH}}\right)}\right] \quad (1)$$

This equation describes the double exponential behaviour of the cross-polarization mechanism. Initially the intensity increases steeply (with the contact time) by the magnetization transfer from proton to the nearest carbon. At longer contact times, the intensity decays due to the relaxation times of the proton (Kolodziejski & Klinowski, 2002). The evolution times for the mechanism of fast cross polarization regime for all the seeds were similar (T_{CH}) (Table 3). However, the ^{1}H $T_{1\rho}$ values was different, which is the ^{13}C decay governed by the rate of the ^{1}H relaxation time during spin-lock at longer contact times. The ^{1}H $T_{1\rho}$ are sensitive to the motion of the proton system in short distances (intermolecular interactions) reflecting the motion of the segments in the multiphase system (Du Prez, Goethals, Adriaensens, Gelan, & Vanderzande, 1996). Therefore, the value of the ^{1}H $T_{1\rho}$ increases with the increase of the molecular mobility of the phases as observed in the study of tropical seeds starches (de Miranda Costa, Bruno Tavares, Bathista, da Silva, & Nogueira, 2007; Maciel & Tavares, 2010; Tavares, Bathista, Silva, Filho, & Nogueira, 2003). The seeds Sempre Verde, Pitiuba, and Novato had similar patterns with the faster ^{1}H $T_{1\rho}$ decay while the Tvu 382 seed presented longer ^{1}H $T_{1\rho}$ decay. For the seed

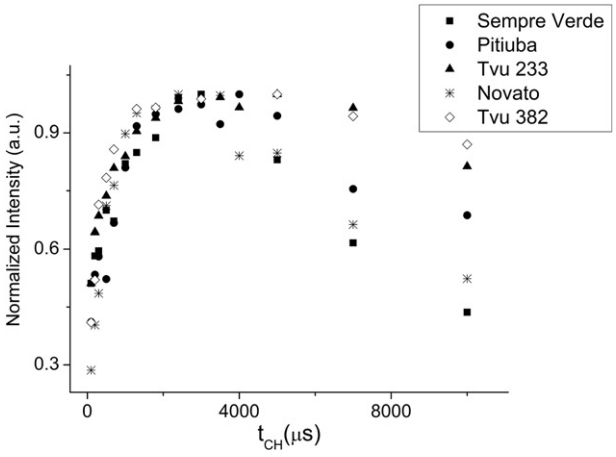

Fig. 6. CP curves for the selected cowpea seeds.

Table 3
T_{CH} and ^1H $T_{1\rho}$ from anomeric carbon of the cowpea coatless grain.

	T_{CH} (ms)	^1H $T_{1\rho}$ (ms)
Sempre Verde	0.27	17.98
Pitiuba	0.45	29.48
Novato	0.52	12.80
Tvu 233	0.17	[a]
Tvu 382	0.24	167.65

[a] The data was not fitted accordingly.

Tvu 233, the ^1H $T_{1\rho}$ value was not properly obtained. However, the data presented the same tendency observed for the seed Tvu 382 (Fig. 6), which indicated that Tvu 233 also has the longer ^1H $T_{1\rho}$ decay. This feature indicates that the cotyledon of the samples Sempre Verde, Pitiuba, and Novato presented the higher rigidity and ordination while the Tvu 233 and Tvu 382 seeds had systems with higher mobility (soft seeds). Additionally, these two seeds are morphologically similar (see Fig. 4 in the corresponding Data in Brief).

The ssNMR was important to observe the overall composition of the native coatless seeds. In general, the seeds are composed by starch, protein and fatty acids. It was shown that the ordination of the seeds was different: the seeds morphologically similar (Tvu 233 and Tvu 382) are composed by less ordinated and soft matter compared to the others species.

4. Conclusions

The oligosaccharides measurement was highly simplified because it did not require any complex pretreatment of the sample apart from the addition of D_2O and buffer. The analyses appointed the Tvu 382 cowpea variety as the one with the lower raffinose content. This information could be useful for breeding program in order to establish cultivars with reduced oligosaccharides content. Additionally, niacin (vitamin B3) was detected in a range from 0.8 to 1.5 $mg \cdot g^{-1}$ and Setentão genotype was the seed that presented the highest content.

The ^{13}C CP and SP/MAS NMR spectrum shows that the cotyledon of the cowpea comprised a truly rigid portion made of starch as well as a soft portion made of amylose, fatty acids and protein. The hydration experiments show the hydration process affecting the dynamics of the cowpea regarding the C6-resonances at ~63 ppm and C4 resonances at ~83 ppm, and showing that these carbons are easily accessible for water. The VCT experiment suggested the softer structure of Tvu 233 and Tvu 382 seeds.

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