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Prediction of dry matter, carbon and ash contents and identification of *Calycophyllum spruceanum* (Benth) organs by Near-Infrared spectrophotometry

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ABSTRACT

Calycophyllum spruceanum (Benth) is a tree of the Amazon region popularly known as 'mulateiro', whose wood has multiple uses, from energy to folk medicine. Thus, it is extremely important to evaluate desirable characteristics, through quick methods that meet the principles of green chemistry. The objective of this study was to evaluate the potential of the near-infrared (NIR) spectroscopy technique for the prediction of dry matter, carbon and ash contents and identification of parts of *C. spruceanum* trees. The Partial Least Squares (PLS) regression model applied within the spectral range of 400–2498 nm proved to be adequate to estimate the dry matter, carbon and ash contents in *C. spruceanum* sapwood and bark samples, with R^2 above 0.90 in both calibration and validation. Hierarchical cluster analysis and principal component analysis techniques when applied to the spectra were able to efficiently separate bark from sapwood. The results show that the NIR technique developed can be used in the determination of dry matter, carbon and ash contents in *C. spruceanum*, in addition to its discriminatory power in the separation of parts of the plant.

1. Introduction

Calycophyllum spruceanum (Benth) is a tree native to the Amazon region, popularly known in Portuguese as 'mulateiro', 'pau-mulato' or 'pau-mulato-de-várzea', which has dense wood with multiple uses [1]. In recent years, energy characteristics of the species have been investigated, and its potential for having fixed carbon and ash contents suitable for this purpose has been verified Andrade [2]. Indicating the need for a better assessment of dry matter content, carbon content and ash for the use of the material to produce medicines (anti-aging, antioxidant, antimicrobial, emollient, wound healing, hemostatic, contraceptive, stimulant, and anti-diabetic properties) and fuel.

However, the analytical methods used in the determination of organic carbon and ash are time consuming and use several reagents harmful to the environment and to the health of the technician responsible for the operation. Moreover, the waste generated requires previous treatments for disposal, which makes the analysis even more expensive. However, in recent decades near-infrared (NIR) spectroscopy has become an important and efficient method in the quantification of physical and chemical attributes of soils and plants [3,4,5], as well as in pattern recognition for soil class separation and plant phenotyping [6,7,8]. This technique has evolved and the equipment has become more accessible, enabling the acquisition of spectral data matrices in several laboratories.

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NIR spectroscopy has been replacing traditional analysis techniques due to its practicality, accuracy and speed in obtaining the results, because the technique does not require any preparation of the samples, besides not using reagents, and it does not destroy or modify the sample, being considered a technique that meets the requirements of Green Chemistry [9,10].

In several articles published in the specialized scientific literature, the efficiency of NIR technique in analysis of forest species has been verified. The technique has been a viable alternative in the evaluations of wood quality [11,12], species identification [7], discrimination of genotypes of the same species [8], prediction of chemical and physical attributes [13,12], among others.

C. spruceanum wood is used in various ways in several areas, for example in tool handles, picture frames, window frames, among others, and its bark is used in teas in folk medicine to assist in the treatment of wounds and mycoses. Thus, the proposal of a method for prediction of chemical and physical characteristics and rapid identification and classification of the part of the *C. spruceanum* tree (plant) or species is of paramount importance for a more appropriate destination of each part of the plant. Additionally, the generated database may be able to meet the forensic context, considering that the differentiation between bark and sapwood is fundamental to identify the origin of the material, since it is sold as a medicinal plant. According to the legislation of environmental crimes (Law 9,605/98), the sale and exhibition for commercial purposes of wood, firewood, coal and other products of plant origin without proper authorization or in disagreement with it is characterized as environmental crime; and according to Brazilian law no. 8,072/90, the falsification, corruption, adulteration or alteration of a product intended for therapeutic or medicinal purposes is considered a heinous crime [14].

In view of the above, the present study aims to evaluate the potential of NIR spectroscopy technique associated with multivariate statistical methods for the prediction of physical (moisture content) and chemical (carbon and ash contents) characteristics and identification of parts of *C. spruceanum* trees.

2. Material and methods

2.1. Sample collection and preparation

A total of 136 samples were collected from adult trees of *Calycophyllum spruceanum* (Benth). The samples were collected in plantations (10°03′18″S; 67°69′04″W) formed by origins from seven natural populations, established in 2006 (Bujari), Manoel Urbano and Tarauacá (2007), Sena Madureira and Feijó (2008), Porto Acre and Rio Branco (2009), municipalities in the states of Acre. Each provenance consisted of 6 lines, with 50 individuals each, spade 3x2m. Tree samples were also collected in primary forests (09°76′78″S; 67°60′07″W) located in Porto Acre.The samples were randomly collected using the destructive method by cutting the trees (felling) using discs with thickness of approximately 15 cm were collected in the trunk at 1.3 m high.

In the laboratory the samples were dried in an air circulation oven (Technal TE-394, Brazil) at a temperature of 65 °C for approximately 72 h to facilitate the milling process. Subsequently, the bark and sapwood were separated, ground in a knife mill with a 2-mm-mesh sieve and packed in plastic flasks.

2.2. Organic carbon, ash and dry matter contents

The analyses of organic carbon were performed by wet oxidation–reduction, according to the method of [15], ash content was determined by the muffle furnace (Jung0812, Brazil) method at a temperature of 550 °C for approximately 4 h, and dry matter was quantified by the principle of gravimetry, which consisted of weighing 5 g of the sample in a porcelain crucible and then drying at a temperature of 105 °C until constant weight, being expressed in % (m/m).

2.3. Near-Infrared spectroscopy

The near-infrared (NIR) spectra were obtained in a NIRS spectrophotometer FOSS DS2500 (FOSS Analytical SA, DK 3400 HillerØed, Denmark), with an average of 64 scans and spectral resolution of 0.5 nm at wavelength from 400 to 2498 nm, using about 2 g of samples in capsules of 3.8 cm internal diameter quartz window for spectral reading in the reflectance mode. The instrument should be preheated at least 30 min before using. Background spectra were obtained using the Spectralon standard (barium sulfate) with 100% reflectance.

2.4. Statistical assessments

2.4.1. Univariate statistical analysis

The variables carbon and ash were transformed through Box-Cox and logarithm, respectively, after their normality was tested (PROC UNIVARIATE, SAS 9.4) [16]. The transformed variables and dry matter were subjected to analysis of variance and their means were compared by Tukey test ($p < 0.05$) using the GLM PROC procedure of SAS 9.4. Linear correlations (Pearson) between the contents of the variables analyzed and their significance ($p < 0.05$) were obtained through the PROC CORR procedure of SAS 9.4.

2.4.2. Multivariate statistical analysis

Multivariate statistical analyses were performed using Uncrambler® X software (version 10.2, CAMO Software Inc., Norway). To construct the calibration model, the original spectra were pre-processed, with the data centered on the mean, by the SNV (Standard Normal Variate) method, and the first derivative using the Savitzky-Golay filter with 9 points on the right and 9 points on the left. Two thirds of the samples were separated for the calibration set and the remaining one third was used in the validation set, using the Kennard-Stone algorithm [17].

After the application of the pre-processing techniques, the predictive models were constructed using the Linear Regression technique by Partial Least Squares - PLS. The quality of the model was evaluated by the parameters: Root Mean Square Error of Calibration (RMSEC), Root Mean Square Error of Prediction (RMSEP), coefficient of determination (R^2), Ratio of Prediction to Deviation (RPD) and number of latent variables.

Identification/authentication and separation of samples of *C. spruceanum* bark and sapwood were performed through the non-supervised techniques of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) using Pirouette 3.11 software.

3. Results and discussion

The contents of carbon, ash and dry matter are presented in Table 1. It was verified that the carbon content in the sapwood, which had mean of 48.54% with variation between 43.74% and 53.51%, was higher than that quantified in the bark (mean of 35.65%, ranging from 29.88% to 42.64%). The difference in carbon content between sapwood and bark was statistically significant.

As for the ash content, there was also a statistically significant difference between sapwood and bark. However, for this attribute, the barks had the highest values, mean of 12.78% and values ranging from 9.17% to 22.55%. In the sapwood, the mean content was 1.45%, with variation from 0.63% to 2.35%.

There was also a statistically significant difference between the dry matter contents in sapwood and bark. The sapwood had higher mean dry matter content (95.85%) compared to the bark (91.47%).

Higher carbon and dry matter content and lower ash content were observed in sapwood, which can be used in direct combustion processes to produce thermal or thermoelectric energy, since they already have superior characteristics in relation to the bark.

Several authors have described the carbon contents in different tree compartments (stem, trunk or stem, branches, leaves, bark and roots)

Table 1
Mean contents, upper limit (UL) and lower limit (LL), standard deviation (SD) and coefficient of variation (CV) of carbon, ash and dry matter contents in % in the sapwood and bark of *C. spruceanum*.

	Constituent					
Variation	Carbon		Ash		Dry matter	
	Sapwood	Bark	Sapwood	Bark	Sapwood	Bark
Mean (%) ± Standard error	48.54 ± 0.25 A	35.65 ± 0.49B	1.45 ± 0.03B	12.78 ± 0.41 A	95.85 ± 2.12 A	91.47 ± 0.93B
UL (%)	53.51	48.85	2.35	22.55	98.4	92.78
LL (%)	43.74	29.88	0.63	9.17	87.02	89.01
CV(%)	2.54	8.06	18.71	20.24	1.80	0.83

$p < 0.0001$.

and these values are similar to those found in the present study. Higuchi and Carvalho Jr. [18] evaluated carbon content in several species of the Terra Firme Dense Humid Rainforest, in the Manaus region, and observed average carbon contents of 48% in the trunk and 39% in the leaves. Balbinot et al. [19] found, for *Pinus taeda* at 5 years of age, average contents in needles, branches, bark, wood and roots of 47.3%, 43%, 40%, 45.7% and 42.8%, respectively. Saidelles et al. [20], studying the carbon contents in *Acacia mearnsii* De Wild., at 4 years of age, found values of 45.8%, 41.39%, 40.87%, 40.68%, 42.13% and 41.92% for leaf, living twig, dead twig, bark, wood and root, respectively. The carbon contents found in the present study are within the variation of contents observed in other forest species.

In a study conducted by IPEF [21], when determining ash contents for some *Eucalyptus* species, the values found in wood and bark were 0.31% and 6.40% for *E. grandis*, 0.41% and 6.14% for *E. saligna*, and 0.41% and 1.57% for *E. microcorys*. It can be observed that the contents found for *C. spruceanum* were higher compared to the species studied in different regions of Brazil.

The analysis of Pearson's correlation (Table 2) between the variables showed that there were significant correlations, both positive and negative, for the attributes studied (dry matter, carbon and ash). Dry matter values were positively correlated with carbon content (0.7451) and negatively correlated with ash content (-0.7963). There was a strong negative correlation between carbon and ash (-0.9521). Thus, there is no need to analyze, by traditional methods, all the attributes evaluated, because they can be estimated only using only a single analysis.

Fig. 1 shows the original NIR spectra of *C. spruceanum* sapwood and bark samples. It was possible to observe an elevation in the baseline and a scattering of the NIR spectra, caused by the non-homogeneity of the samples, that is, by the differences in their packing and orientation. Thus, it was necessary to perform pre-processing of spectral signals in order to remove or smooth spectral noises, elevation in the baseline and the effect of light mirroring because of diffuse reflectance, through the first derivative and SNV mathematical methods so that the identification/authentication model is not biased, or noise model.

It is possible to notice wide and intense bands in the region between 400 and 750 nm (visible region). In regions close to 1,450 and 1,900 nm, it is also possible to observe bands that correspond to the first O–H stretch overtone and the band of combination between stretch and angular deformation of water O–H, respectively [22].

The Partial Least Square (PLS) regression model applied in the spectral ranges of 1000–2498 nm, 400–2498 nm and 1000–2498 nm was adequate to estimate the dry matter in *C. spruceanum* sapwood and bark samples in a global model, but to carbon and ash contents was necessary to create models separately for *C. spruceanum* sapwood and bark samples with different ranges, respectively (Table 3). This was necessary to avoid the two centroid groups formation in a global model.

Table 2
Pearson correlation matrix for Dry Matter, Carbon and Ash.

	Dry Matter	Carbon	Ash
Dry Matter	1	0.774	−0.795
Carbon	0.771	1	−0.954
Ash	−0.795	−0.954	1

$p < 0.0001$.

The three attributes evaluated showed, in the calibration, low root mean square errors (RMSEC) and high coefficients of determination (R^2), values higher than 0.90. In the external validation for the predictive model, as in calibration, there was a low root mean square error. The RPD values (Table 3) for the three evaluated characteristics were high, suggesting that the calibration and validation model used is adequate to estimate the contents of dry matter (Fig. 2), carbon (Fig. 3) and ash (Fig. 4) of *C. spruceanum* sapwood and bark through the acquisition of NIR spectra, according to criteria established by Dunn et al. [23] and Chang et al. [24], who suggest an excellent RPD when the observed value exceeds 2.

The results found in this study are similar to those reported in the scientific literature. Silva et al. [25], in a study on the characterization of the energy properties of tropical wood residues, observed R^2 of 0.93 for fixed carbon in the wood in the calibration and 0.85 in the validation between the traditional method and the NIR method, with RPD of 1.8 characterized as acceptable. Dallagnol et al. [26], who tested the potential for predicting carbon content by NIR for the species *Merostachys skvortzovii*, found correlation coefficients in both calibration ($R^2 = 0.822$) and validation ($R^2 = 0.764$) that are below those observed in the present study. This fact shows that the established model is adequate to replace traditional methods with NIR in the estimation of carbon, ash and dry matter contents in *C. spruceanum*.

Fig. 5, shows the dendrogram resulting from the application of the Hierarchical Cluster Analysis - HCA technique in the NIR spectral data of *C. spruceanum* sapwood and bark. The technique proved to be adequate to separate the bark from the sapwood using spectral treatment with data centered on mean, Euclidean metric distance and Incremental Linkage algorithm. In Fig. 5, it is possible to observe the formation of two large clusters, well defined and with low similarity to each other: sapwood samples in green and bark samples in red.

In order to corroborate the data obtained through HCA in the discrimination of *C. spruceanum* sapwood and bark using NIR spectra, the Principal Component Analysis (PCA) technique was also applied as an unsupervised pattern recognition method. In both techniques, HCA and PCA, the same spectral region was used with the data centered on the mean. Fig. 6 shows the score graph with the representation of PC1 *vs* PC2, in which it is possible to observe the discrimination of 2 well-defined and distant clusters according to the PC1 axis: *C. spruceanum* bark samples in red (positive scores) and *C. spruceanum* sapwood samples in green (negative scores). The discrimination between the two types of *C. spruceanum* samples (sapwood and bark) occurs due to differences in the chemical composition of the two types of samples, as shown in Table 2.

Only two principal components were responsible for describing 99.92% of the total variance of the data, 99.43% attributed to PC1 and 0.49% to PC2. Thus, it can be seen that the PCA is in accordance with the result obtained through HCA. Additionally, in view of the results obtained, the potential for application of both techniques in this type of discrimination is verified.

Great potential has been reported in the application of NIR spectroscopy for phenotyping of plant genetic materials. Panero et al. [27]

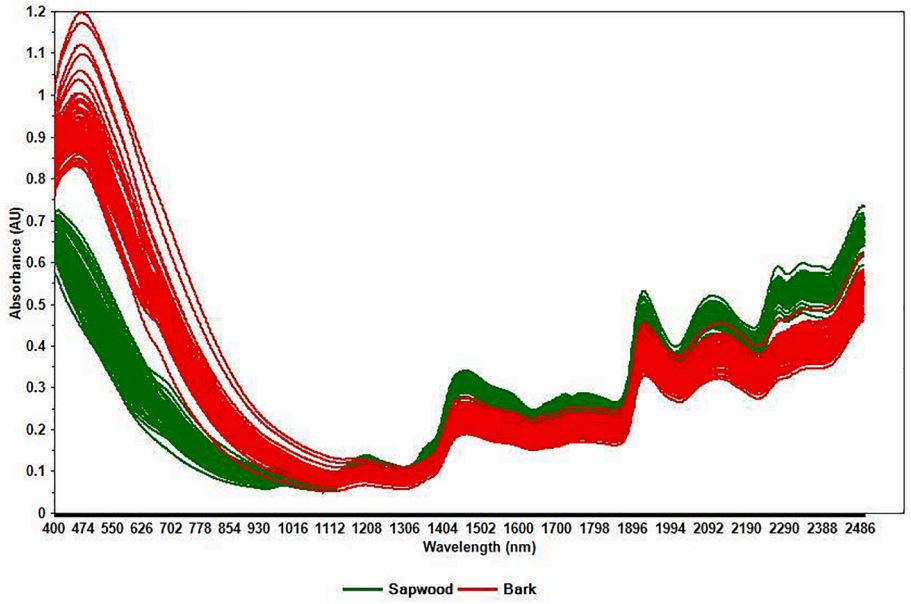

Fig. 1. Original spectra (400–2500 nm) of *C. spruceanum* bark and sapwood samples.

Table 3
Results of statistical analyses of calibration and external validation sets for dry matter, carbon and ash (%) in *C. spruceanum*.

Attribute	Pre-treatment/ Spectral range	N° LV[2]	N Cal[3]	R^2 Cal	RMSEC[5] (%)	N Val[4]	R^2 Val	RMSEP[6] (%)	RPD[7]
Dry matter (%) (sapwood and bark)	SNV – SG-1 (9)[1] 1000–2498 nm	4	87	0.96	0.45	43	0.95	0.49	5
Carbon (%) (sapwood)	SNV – SG-1 (11)[8] 400–2498 nm	9	43	0.92	0.11	27	0.85	0.15	2.6
Carbon (%) (bark)	SNV – SG-1 (11) 400–2498 nm	9	42	0.90	0.48	23	0.87	0.69	2.7
Ash (%) (sapwood)	SNV – SG-1 (11) 400–2498 nm	9	43	0.95	0.04	27	0.84	0.08	2.5
Ash (%) (bark)	SNV – SG-1 (11) 400–2498 nm	9	42	0.96	0.31	23	0.93	0.59	4

[1]SNV – SG-1 (9) – Standard Normal Variate, Savitzky-Golay first derivative with 9 points on the right and 9 points on the left, [2]LV – latent variables, [3]N Cal – number of samples in the calibration set, [4]N Val – number of samples in the validation set, R^2 – coefficient of determination, [5]RMSEC – Root Mean Square Error of Calibration, [6]RMSEP – Root Mean Square Error of Prediction, [7]RPD – Ratio of Prediction to Deviation, [8]SNV – SG-1 (11) – Standard Normal Variate, Savitzky-Golay first derivative with 11 points on the right and 11 points on the left.

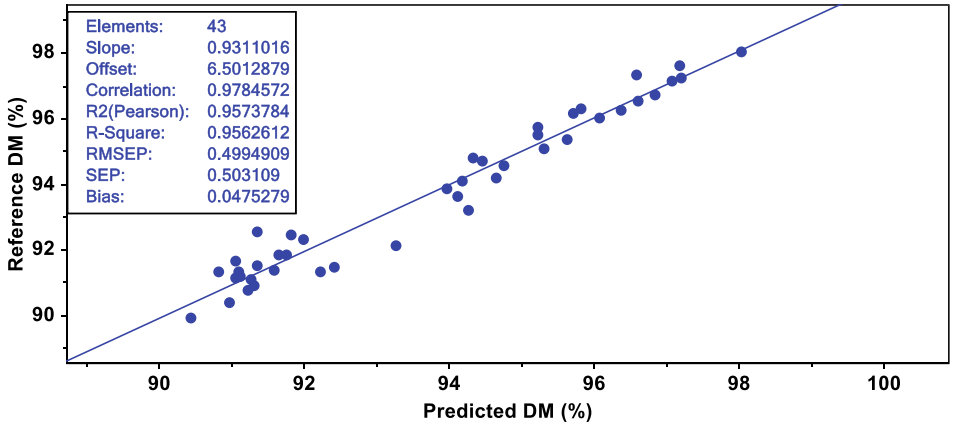

Fig. 2. Reference values *versus* predicted values for dry matter content (%) in C.spruceanum sapwood and bark samples (external validation).

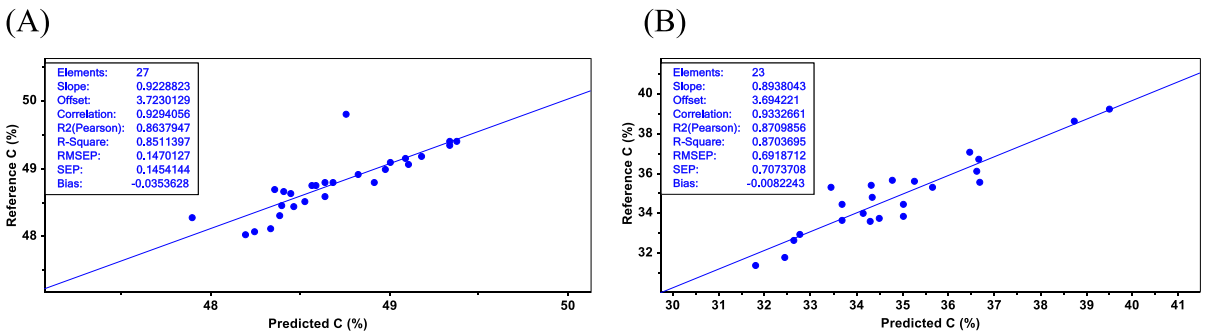

Fig. 3. Reference values *versus* predicted values for carbon content (%) in *C. spruceanum* sapwood (A) and bark (B) samples (external validation).

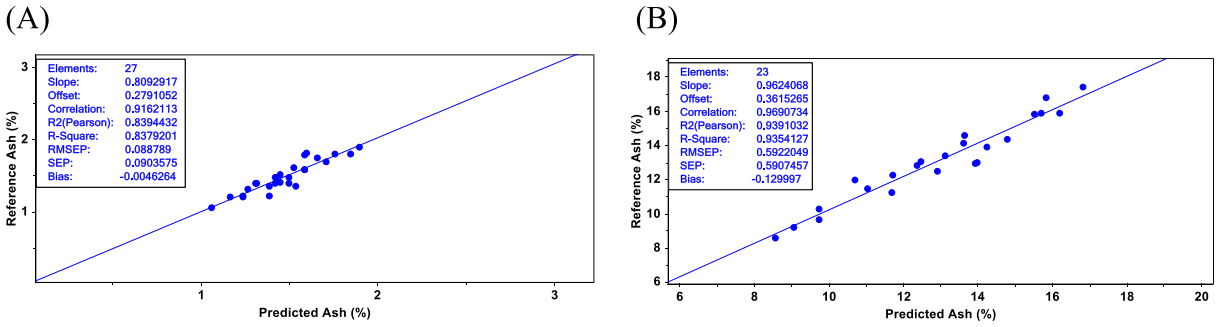

Fig. 4. Reference values *versus* predicted values for ash content (%) in *C. spruceanum* sapwood (A) and bark (B) samples (external validation).

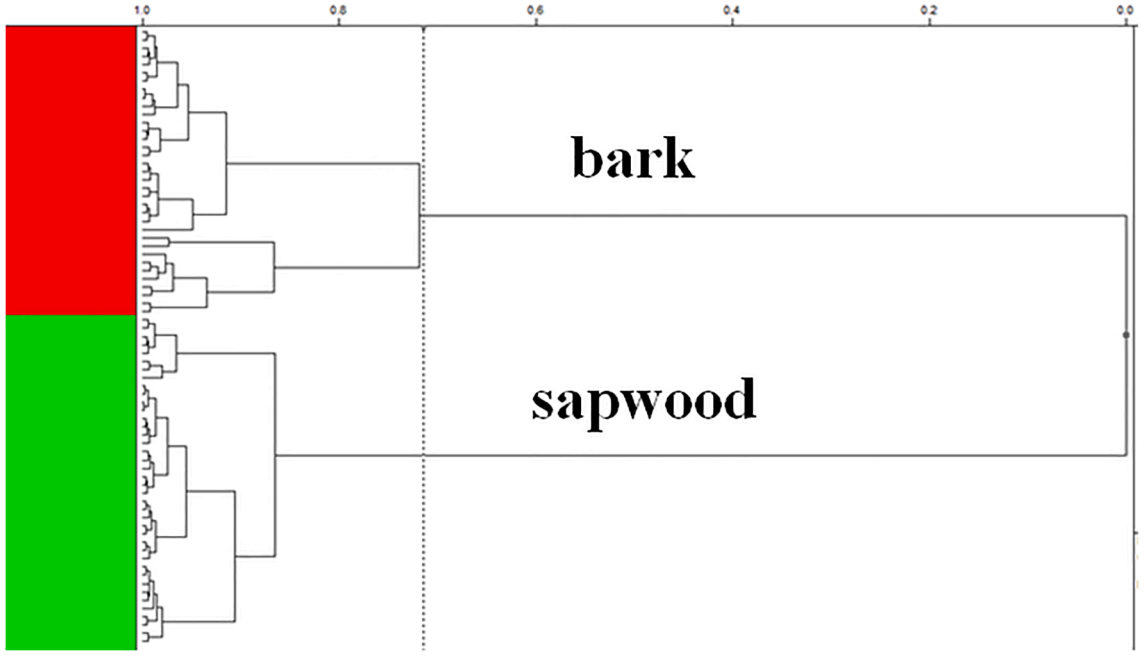

Fig. 5. Discrimination of the samples of sapwood (green) and bark (red) of *C. spruceanum*, obtained by HCA technique with data centered on mean, Euclidean metric distance and Incremental Linkage method.

successfully managed to separate soybean genotypes using the NIR technique associated with multivariate analyses. Similar results were obtained by André et al. [8], who verified that in the spectral range from 400 to 2500 nm this technique is efficient in the discrimination of clones, leaves of different phenological stages and health in rubber tree. These results show the potential for using NIR spectroscopy to identify genetic materials, it and can be applied for various purposes, such as in breeding programs and characterization of materials deposited in germplasms banks.

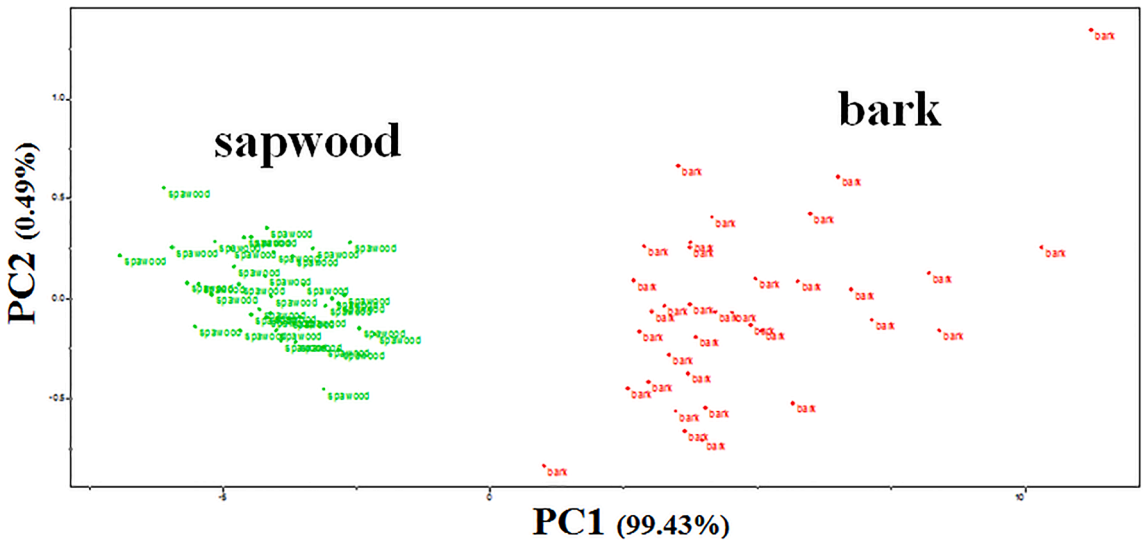

Fig. 6. PC1 *vs* PC2 scores representing the discrimination of the samples of *C. spruceanum* sapwood and bark, after the results obtained using the spectra.

4. Conclusions

There is a strong correlation between the contents of dry matter, carbon, ash of *C. spruceanum*, so it is possible to evaluate only one of these components and estimate the others from it.

The use of near-infrared spectroscopy associated with multivariate calibration methods allowed the development of a fast and nondestructive model for the analysis of dry matter, carbon and ash contents in *C. spruceanum* samples.

With the data of Vis-NIR spectroscopy and multivariate classification techniques (PCA and HCA), it was also possible to verify two clusters of samples, showing that the exploratory analysis make it possible, by graphical visualization, to obtain quick information about the similarity between the origin of the samples (bark and sapwood) of *C. spruceanum*.

CRediT authorship contribution statement

Lucas Dalmolin Ciarnoschi: Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Luis Claudio de Oliveira:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Maria Lucia Ferreira Simeone:** Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Francisco dos Santos Panero:** Writing – original draft, Writing – review & editing. **Pedro dos Santos Panero:** Writing – original draft, Writing – review & editing. **Anselmo Ruiz Rodriguez:** Writing – original draft, Writing – review & editing. **Elenilson G. Alves Filho:** Writing – original draft, Writing – review & editing. **Marcos Gervasio Pereira:** Writing – original draft, Writing – review & editing. **Luciélio Manoel da Silva:** Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

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