Research Article
doi: 10.1590/01047760202330013414
vol(30), 2024

CERNE

Use of near infrared spectroscopy for estimating moisture content in Brazilian cherry (*Eugenia uniflora* L.) seeds submitted to dehydration

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SILVICULTURE

ABSTRACT

Background: The determination of seed moisture content is of utmost importance for the evaluation of its quality. Near-infrared (NIR) spectroscopy has been successfully applied to estimate properties of biological materials. However, studies using this technique on forest seeds are still scarce. Thus, the aim of this study was to explore the potential of NIR spectroscopy for estimating the moisture content of *E. uniflora* seeds. NIR spectra were obtained using optical fiber from 100 individual seeds that were dehydrated in silica gel for 2, 7, 14, and 21 days. Subsequently, the seeds were subjected to the moisture test by the oven method, and had moisture contents of 52.4%, 41.7%, 33.1%, 21.8%, and 18.1%, respectively.

Results: Principal component analysis (PCA) of the spectral signatures explained 100% of the data variability. The moisture content was associated with the spectra by partial least squares regression (PLS-R) and the predictive model presented a coefficient of determination in cross-validation (R^2cv) of 0.88 and the root mean square error of cross-validation (RMSEcv) was 5.43%.

Conclusion: The statistics associated with the models indicate that NIR spectroscopy has potential for estimating the moisture content of *E. uniflora* seeds.

Keywords: Seed desiccation; seed physiology; seed technology.

HIGHLIGHTS

The use of Near-infrared (NIR) spectroscopy coupled with multivariate analysis proved to be efficient for predicting the moisture content of *E. uniflora* seeds, with a coefficient of determination of 0.88 and a root mean square error of 5.43%, indicating that the model is robust and generated reliable estimates.

The moisture content determination of *E. uniflora* seeds through NIR has the advantages of being non-destructive and much faster when compared to the conventional oven method.

ALVES, M. C. S.; SILVA, T. A.; TONETTI, O. A. O.; JOSÉ, A. C.; HEIN, P. R. G.; FARIA, J. M. Use of near infrared spectroscopy for estimating moisture content in Brazilian cherry (*Eugenia uniflora* L.) seeds submitted to dehydration clones. CERNE, v. 30, 2024, e-103414, doi: 10.1590/01047760202330013414

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Received: April 9/2024 Accepted: August 8/2024

CERNE
ISSN 0104-7760

INTRODUCTION

The moisture content is one of the parameters that is most related to seed quality. From it, it is possible to determine the harvest time, how it will be handled after harvest and for how long it remains viable without losing its physiological quality (Garcia and Coelho, 2021).

There are several methods for determining the moisture content of seeds, with the most used and recommended by the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA) being the method using an air circulation oven (Besharati et al., 2021; Hay et al., 2023). This method is also cited by the Rules for Seed Analysis (Brazil, 2009) and follows a destructive procedure, which often compromises the study, since, in the forestry sector, seed lots are almost always represented by reduced quantities. In addition, the method requires time in an oven at 105°C for 24 hours. Therefore, it is important that accurate and fast methods are available to determine the moisture content of seeds.

The use of rapid and non-destructive analytical techniques, such as near-infrared (NIR) spectroscopy, has been widely studied for the determination of different characteristics and evaluation of the quality of agricultural products (Mendez et al., 2019; Aguiar et al., 2022), wood (Medeiros et al. 2023) and paper (Lima et al. 2022). This technique is based on electromagnetic radiation in the near-infrared region, which extends from a wavelength of 750 to 2500 nm (or a wavenumber of 12500 cm^{-1} to 4000 cm^{-1}), and interacts with organic molecules, especially in -CH, -OH, -NH, -SH, and C=O interactions (Cao et al., 2020).

Water is a component that is intensely detected by electromagnetic radiation in the NIR, so samples with high water content (> 80%) are strongly dominated by the water band, consequently, the most intense absorption appears in the wavelength range of 1940 nm (Larios et al., 2020). When the samples undergo a dehydration process, changes occur in the spectra and a sharp decrease in the absorption of infrared intensity in the wavelength range of water (Sakare et al., 2020). The determination of moisture content from the NIR spectrum does not reflect only water due to the specificity of the wavelength range of 1940 nm, but also the loss of volatiles during the drying process (Genisheva et al., 2018).

Near-infrared spectroscopy presents a number of advantages over traditional analytical methods (Zhou et al., 2020). In addition to being a non-destructive technique, it requires minimal or no sample preparation, no reagent is used, so no residue is produced; it is a multianalytical technique, that is, several constituents of the same sample can be obtained simultaneously, and its accuracy can be high (Larios et al., 2020; Ribeiro et al., 2020;). Regarding the disadvantages of the technique, it is observed the dependence on calibration procedures, these obtained from traditional analytical methods. Another disadvantage is the complexity in choosing the models that best represent the data (Paz et al., 2019).

Numerous studies have applied NIR technology to seeds of annual plants and agricultural crops, such as peanuts (Raigar et al., 2024), soybeans (Silva et al., 2024), and coffee (Macedo et al., 2021). Agelet and Hurburgh (2014) reviewed critical aspects of NIRS for single seed analysis, including reference methods, sample morphology, and spectrometer suitability. In the context of moisture estimation in grains, Zhang and Guo (2020) evaluated maize seed moisture using visible/near-infrared (Vis/NIR) technology. Their findings indicated that PLSR models based on NIR spectra outperformed Vis/NIR models in predicting maize seed moisture content.

To our knowledge, no studies have explored the application of NIR technology for estimating moisture content in *Eugenia uniflora* seeds. Therefore, this study aims to develop near-infrared spectroscopy models capable of assessing the moisture content of dehydrated *E. uniflora* seeds.

MATERIAL AND METHODS

Seed collection and processing

Ripe *E. uniflora* fruits were collected in november 2017 from mother trees located in Lavras, MG (21º14´ S and 45º00´ W), and seed processing was carried out by macerating the fruits in a sieve under running water.

Seed dehydration

Moisture content was determined individually for freshly collected seeds and for seeds that were dehydrated in silica gel for 2, 7, 14, and 21 days to achieve target moisture levels of 40%, 30%, 20%, and 10%, for a total of 100 *E. uniflora* seeds.

Dehydration was performed in a laboratory at a temperature of 20 ºC in a hygrostat box made of rigid, dark plastic with a ventilation system. The seeds were placed on a screen to prevent direct contact with the desiccant material and weighed periodically.

The target weights corresponding to the different target moisture levels were calculated according to Hong and Ellis (1996). Every time a target weight was reached, a sample was removed for the acquisition of spectra and subsequent moisture determination in an oven.

Near-infrared (NIR) spectral acquisition

The spectral signatures were obtained using a Bruker FT-NIR spectrometer (model MPA, Bruker Optik GmbH, Ettlingen, Germany) in a climate-controlled room (20 °C and 60% relative humidity). The spectra were measured directly on the seeds before the moisture test for each treatment. The acquisition of the spectra was performed in the range of 12500 to 3500 cm^{-1} (800 to 2857 nm) with spectral resolution of 8 cm^{-1} and 16 scans per reading in diffuse reflectance mode. The multivariate analyses were performed using two databases: 1) from 12500 to 4000 cm^{-1} and 2) from 9000 to 4000 cm^{-1}. The spectrometer is connected to a computer that stores the data of the collected spectra using the OPUS software, version 7.0. Twenty (20) seeds from each treatment were individually analyzed using a fiber optic probe.

Moisture Test as a Reference Method

After reading the spectra, the moisture content was determined for the freshly collected seeds and for those that had been dehydrated. In each treatment, 20 seeds were individually cut in half, placed in an oven at 105 °C for 24 hours under forced air circulation, in accordance with the Rules for Seed Analysis – RAS (Brasil, 2009). The moisture content was expressed as a percentage based on the wet weight.

Multivariate data analysis

Multivariate data analysis was performed using the Chemoface® software version 1.61 (Nunes et al., 2012). Principal component analysis (PCA) was performed on raw data to obtain an overview of the data and to detect outliers.

Partial Least Squares Regression (PLS-R) was used to relate the NIR spectral signatures to the data obtained by the reference method performed in the laboratory for the moisture test of seeds. The number of latent variables was defined based on maximizing the coefficient of determination and minimizing the validation error standard. The calibration of the models was validated using the leave-one-out (full) cross-validation method. Mathematical treatments and spectral band selection were applied to improve the models. Normalization (Norm), Standard normal variation (SNV), Multiplicative scatter correction (MSC) and first and second derivatives were computed for spectra using the Savitzky–Golay algorithm with 13 smoothing points and either 2 or 3 polynomial orders directly within the Chemoface software (https://www.ufla.br/chemoface/), aiming to diminish noise and enhance signal quality.

RESULTS

Seed dehydration and moisture determination

The mean and standard deviation of the moisture content of freshly collected seeds obtained in the laboratory were 52.4% and 4.1%, respectively, while the moisture content predicted by the NIR model was 52.4 ± 2.6%. Seeds subjected to dehydration in silica gel for 2 days reached a mean and standard deviation of 41.7% ± 6.9%. After 7 days of dehydration, the seeds reached a moisture content of 33.1% ± 11.6%. After 14 days, the seeds had a moisture content of 21.8% ± 11.6% and, after 21 days of exposure to silica gel, the seeds had a mean and standard deviation of 18.1% ± 9.2% (Figures 1 and 2).

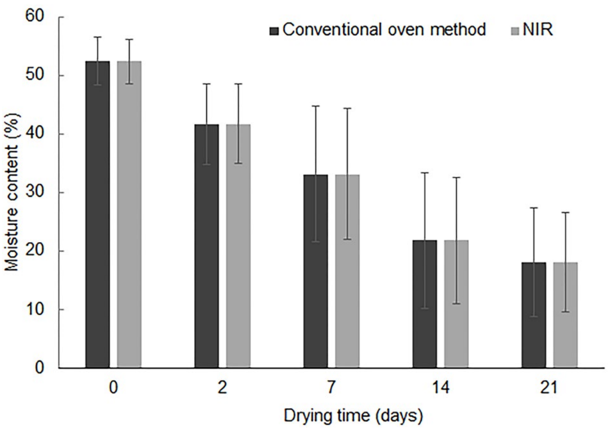

Figure 1: Mean and standard deviation values of the moisture content obtained through the conventional oven method and those predicted by NIR spectra in *E. uniflora* seeds.

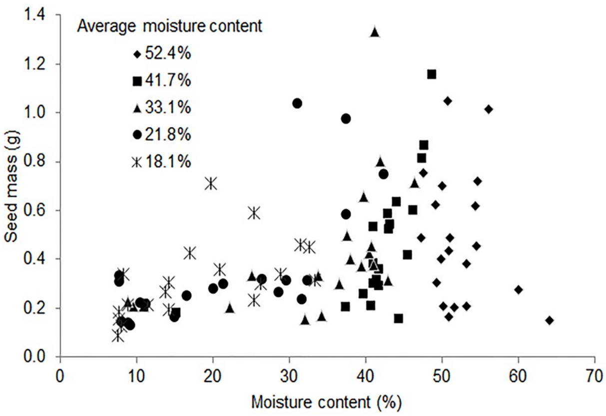

Figure 2: Moisture content (% wet basis) individual of *E. uniflora* seeds obtained through dehydration in silica gel.

NIR spectral signature

The acquisition of the spectra of *E. uniflora* seeds was performed in the range of 12500 to 3500 cm^{-1} (800 to 2857 nm). However, to improve the quality of the information contained in the average spectra based on absorbance, the range extending from 12500 to 9000 cm^{-1} (800 to 1111 nm) was excluded due to the presence of much noise due to the variation of moisture in the material (Figure 3A-B).

Principal component analysis (PCA) was performed for a preliminary evaluation of the behavior of the spectra and possible separation of the moisture content of *E. uniflora* seeds that were or were not subjected to dehydration. The principal components PC1 and PC2, based on the raw data, explained 100% of the entire variance of the spectral data, with PC1 explaining 97.3% of the data variability. There was a clear separation of seeds that were exposed to dehydration from those that were not dehydrated (freshly collected seeds) (Figure 4).

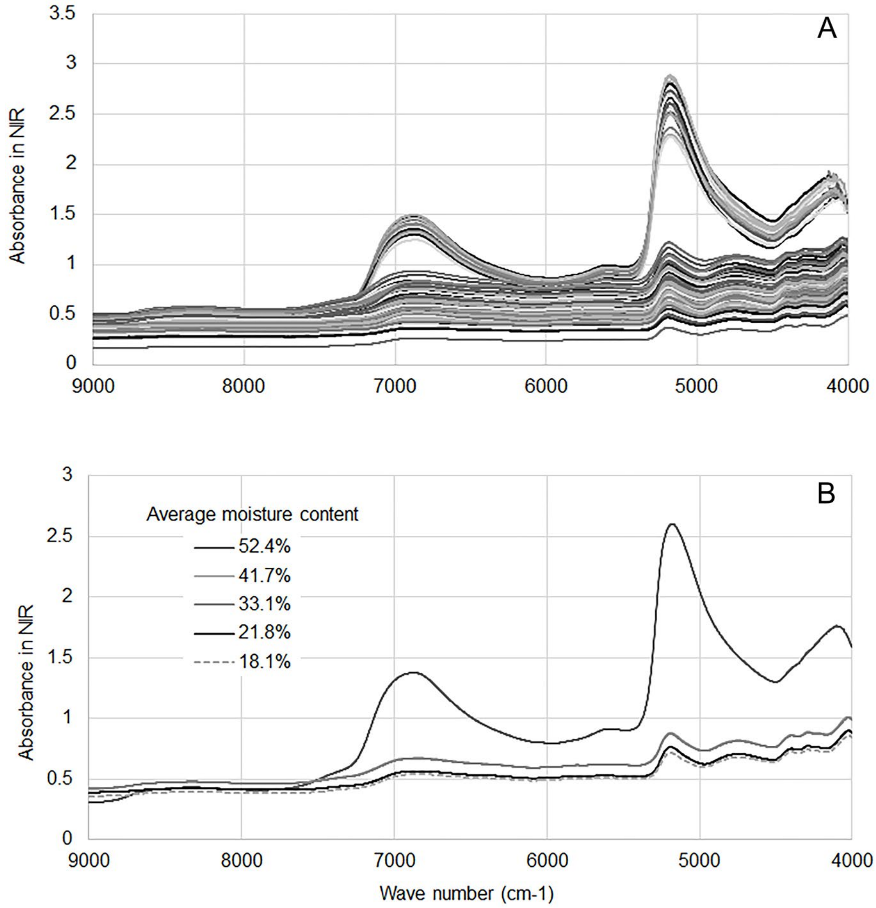

Figure 3: NIR spectra of *E. uniflora* seeds submitted or not to dehydration. (A) Crude spectra of all seeds; (B) Average of the spectra by average moisture.

Estimation of moisture content from NIR spectroscopy

Various mathematical treatments were applied to build the best model to predict the moisture content of *E. uniflora* seeds. PLS-R models were developed using the entire NIR spectra (from 12500 to 4000 cm^{-1}) and a selected NIR range (from 9000 to 4000 cm^{-1}). The best model from PLS-R was the one where the wavenumber range of 12500 to 9000 cm^{-1} (800 to 1111 nm) was excluded due to noise, and the mathematical treatment of multiplicative scatter correction (MSC) was applied. With this model, a cross-validation coefficient of determination (R^2cv) of 0.88 and a cross-validation root mean square error (RMSEcv) of 5.430 were obtained (Table 1). These data indicate that the models are robust and generated reliable estimates.

Figure 5 shows a plot of the moisture content obtained by the oven method versus the predicted value through NIR spectra for the best model (model 11 from Table 1). A strong association is observed between the measured values and those predicted by the model. Estimates from Model 11 are based on the NIR range of 9,000 to 4,000 cm^{-1} following the application of Multiplicative Scatter Correction. This model predicts moisture content based on NIR signatures with a root mean square error of 5.43%.

DISCUSSION

Dehydration, moisture testing, and spectral properties

E. uniflora seeds show uneven drying (Figure 2). The moisture content of freshly collected seeds was more homogeneous than that of seeds that were subjected to dehydration in silica gel (Figure 1). NIR predictions of moisture content compared to values obtained by the oven method were close, especially for seeds with higher moisture contents. As the seeds dried, these measured and predicted values fluctuated.

It is important to note that NIR spectra readings were made with the presence of the seed coat. Therefore, it is inferred that as the seeds dry, the seed coat becomes stiffer and this causes the reading to penetrate only very superficially into the seed cotyledons. The existing literature does not clearly report whether this pattern occurs in other species. The penetration depth in organic materials such as seeds should be investigated further to determine if it is consistent across other species where NIR is used to estimate moisture content. For instance, Erdogdu et al. (2015) evaluated the penetration depths of different spice commodities (such as black pepper seeds, paprika powder, and oregano leaves) under infrared radiation. They reported that the penetration depth values for black pepper seeds ranged from 0.26 to 0.36 cm in terms of infrared absorption through a glass petri dish.

NIR radiation penetration in seed cotyledons is a topic of interest due to its potential effects on seed germination, growth, and biochemical processes. Understanding how NIR radiation interacts with cotyledons can provide insights into plant development and stress responses.

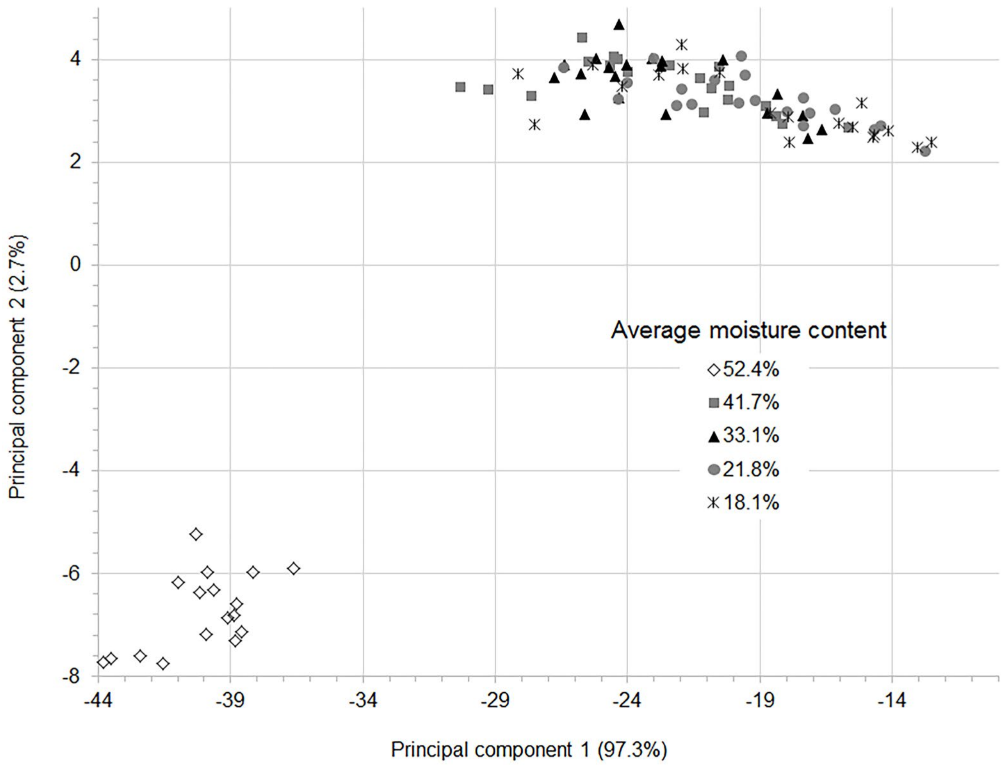

Figure 4: Two-dimensional dispersion plot for PC1 and PC2 of the principal component analysis (PCA) of NIR spectra from seeds of *E. uniflora* at different moisture levels.

Table 1: Statistics associated to the Partial Least Squares Regression in calibration and cross-validation for estimating seed moisture of *E. uniflora* by NIR spectra.

Model	Database	Treat.	R²c	RMSEc	R²cv	RMSEcv	LV	RPD
1	12.5-4.0	-	0.80	6.868	0.74	7.951	7	1.96
2	12.5-4.0	Norm.	0.90	4.857	0.85	6.043	7	2.58
3	12.5-4.0	1d	0.81	6.801	0.73	8.176	7	1.90
4	12.5-4.0	2d	0.68	8.826	0.61	9.669	4	1.61
5	12.5-4.0	MSC	0.92	4.294	0.87	5.499	7	2.83
6	12.5-4.0	SNV	0.92	4.437	0.87	5.669	7	2.75
7	9.0-4.0	-	0.84	6.245	0.78	7.348	7	2.12
8	9.0-4.0	Norm.	0.88	5.353	0.84	6.310	6	2.47
9	9.0-4.0	1d	0.84	6.106	0.75	7.772	7	2.00
10	9.0-4.0	2d	0.76	7.619	0.67	8.906	4	1.75
11	9.0-4.0	MSC	0.90	4.783	0.88	5.430	5	2.87
12	9.0-4.0	SNV	0.90	4.877	0.87	5.531	5	2.81

Legend: Treat. – treatment; R²c - coefficient of determination for calibration; RMSEc - root mean square error for calibration; R²cv - coefficient of determination for cross-validation; RMSEcv - root mean square error for cross-validation; LV - latent variables; RPD - relative performance difference; All - all samples; 1d - first derivative; 2d - second derivative; Norm. - normalization; MSC - multiplicative scatter correction; SNV - standard normal variate; database: 12.5-4.0 - wavenumber range from 12.500 to 4.000 cm^{-1}; 9.0-4.0 - wavenumber range from 9.000 to 4.000 cm^{-1}.

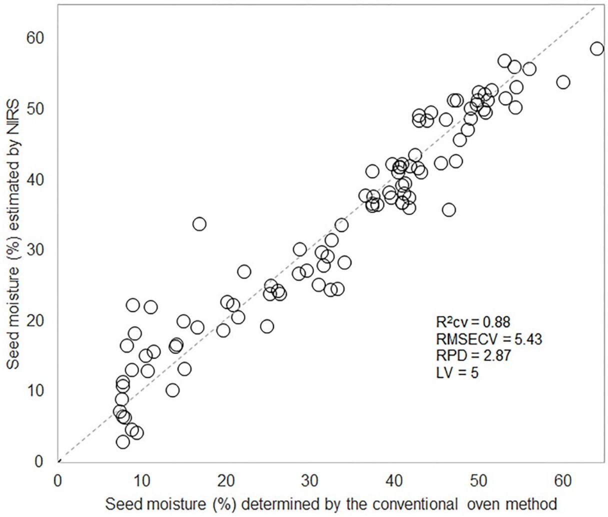

Figure 5: Regression graph with values obtained through the oven method and predicted by the NIR, for moisture content of *E. uniflora* seeds in percentage.

Inspection of the spectra indicated that several wavelength regions reflect chemical information related to moisture content. NIR spectra of *E. uniflora* seeds showed variation in absorbance along the wavelength with visible differences in absorbance between freshly collected seeds and those subjected to dehydration. The largest absorption band observed for both treatments was around 5168 cm^{-1} (1935 nm) (Figure 3), a region of the spectrum associated with variation in water content of biological materials (Workman and Weyer, 2012).

In this study, dehydration caused a sharp decrease in absorbance in the spectrum at the wavelength corresponding to water. According to Büning-Pfaue (2003), there is a decrease in absorption in the wavelength range corresponding to water in samples undergoing dehydration. Thus, due to the specificity of the 1940 nm wavelength range for water molecules, the drying process can be monitored due to the decrease in NIR absorbance caused by drying (Reh et al., 2006; Strabeli et al., 2023).

Based on the PCA scores, it was not possible to detect outliers. The spectral variation explained (PC1 + PC2) based on the data was 100%. A clear separation was observed between the seed samples that were dehydrated on different days and those that were not dehydrated (Figure 4). Adnan et al. (2017) used near-infrared spectroscopy to predict moisture in coffee beans. The authors applied PCA for outlier inspection and separation of *Coffea arabica* and *Coffea canephora* species and reported a spectral variation in the raw data of 99% for PC1 and PC2.

Moisture content estimation from NIR

Various mathematical treatments were applied to the spectra of *E. uniflora* seeds subjected to dehydration or not, in order to reduce noise and obtain a satisfactory R^2 capable of generating models that better estimate the moisture content of the seeds. In this study, the model characterized as the best was the one that generated the highest R^2, resulted in the smallest number of latent variables, and the smallest mean squared error for cross-validation. Thus, after excluding the range of 12500 to 9000 cm^{-1} and applying the MSC mathematical treatment, the model 11 with R^2cv of 0.88 and RMSECV of 5.43 and was generated using only 5 latent variables (Table 1).

Adnan et al. (2017) used these selection criteria to choose the best model to predict the moisture content of *Coffea arabica* and *Coffea canephora* grains, and obtained an R^2cv of 0.96. For these authors, the choice of a good model cannot be based only on R^2cv, RMSEcv, or RPD, which reflect predictive power. It is important to consider the number of latent variables, as a model using fewer latent variables is less prone to overfitting.

The PLS-R model based on the spectral data of *E. uniflora* seeds generated good accuracy for both calibration and validation (Figure 5). The data measured by NIR are close to the values estimated in the method for determining the moisture content based on the Rules for Seed Analysis. It is suggested that this technique can be applied to seeds of other species, since it has the potential to replace traditional methods when there is a need for a rapid and non-destructive prediction of samples, especially when it is desired to estimate the moisture content of a larger quantity of seeds and/or grains for the control of the production process.

This study reported promising results, but encountered technical and operational limitations. Firstly, uncertainties remain regarding the depth to which NIR radiation penetrates seeds and how moisture content and seed coat thickness affect its interaction with seed molecules. Additionally, each treatment analyzed only 20 seeds due to material constraints. Furthermore, the study was restricted to analyzing data at four specific time points (2, 7, 14, and 21 days). Despite these challenges, the study successfully demonstrated the tool's capability to rapidly and reliably estimate humidity. These findings underscore the importance of expanding future research to encompass a broader range of treatments, moisture levels, and dehydration durations across seeds from various plant species.

CONCLUSION

The use of near-infrared (NIR) spectroscopy coupled with multivariate analysis proved to be efficient for predicting the moisture content of *E. uniflora* seeds. The exploration of NIR spectra, followed by principal component analysis (PCA), indicates that spectral measurements can be used to discriminate between freshly collected *E. uniflora* seeds and those subjected to dehydration. From the partial least squares regression (PLS-R) analysis with spectral band selection and application of the SNV treatment to the data, a predictive model was obtained that presented a coefficient of determination (R^2cv) of 0.88 and the root mean square error (RMSEcv) of 5.43%. These data indicate that the model is robust and generated reliable estimates.

AUTHORSHIP CONTRIBUTION

Project Idea: JMRF; PRGH
Funding: PRGH; JMRF
Database: MCSA; PRGH; JMRF
Processing: MCSA; PRGH; JMRF
Analysis: MCSA; PRGH; JMRF; OAOT; ACJ
Writing: MCSA; PRGH; JMRF
Review: MCSA; PRGH; JMRF; TAS; OAOT; ACJ

ACKNOWLEDGMENTS

J.M.R.F is a CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil) research fellow (Process PQ 317013/2021-1). A.C.J is a CNPq research fellow (Process PQ 317242/2021-0). J.M.R.F thanks Fapemig (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) for partially funding the research (Processes PPM 00145/17 and APQ 00742/23). Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the master's scholarship for M.C.S. Alves.

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