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Special Supplement: Pulses

Classification of artificially aged lentil seeds using Fourier transform near-infrared spectroscopy[1]

Marcelo Augusto Rocha Limão[2], Daniel Teixeira Pinheiro[3], André Dantas de Medeiros[2], João Paulo Oliveira Ribeiro[4], Denise Cunha Fernandes dos Santos Dias[2], Laércio Junio da Silva[2]

ABSTRACT

Seed deterioration is among the main causes of loss in physiological potential. This study aimed to investigate the potential of Fourier transform near-infrared (FT-NIR) spectroscopy to classify lentil seeds subjected to artificial aging at 41 ºC and 100 % of relative humidity for 24, 48, 72, 96 and 120 hours, with unaged seeds used as control. After obtaining the spectra, the seeds were submitted to germination tests, and the percentages of normal, vigorous and weak seedlings, as well as dead seeds, were recorded. From the spectral data, models were obtained using the Partial Least Squares - Discriminant Analysis (PLS-DA) method, separating 70 % of the data for training and 30 % for validation. Pre-processing using the second derivative of Savitzky-Golay (SG) and the combination of the second derivative of SG + multiplicative scatter correction resulted in accuracy and *kappa* values in training of 0.94 and 0.92, respectively. The FT-NIR spectroscopy showed to be a promising tool for classifying artificially aged lentil seeds, considering their physiological potential.

KEYWORDS: *Lens culinaris*, seed physiological potential, artificial intelligence.

RESUMO

Classificação de sementes de lentilha envelhecidas artificialmente usando espectroscopia de infravermelho próximo com transformada de Fourier

A deterioração de sementes está entre as principais causas da perda do potencial fisiológico. Objetivou-se investigar o potencial da espectroscopia de infravermelho próximo com transformada de Fourier (FT-NIR) para classificar sementes de lentilha envelhecidas artificialmente a 41 º C e umidade relativa de100 % por períodos de 24, 48, 72, 96 e 120 h. Como controle, utilizaram-se sementes não envelhecidas. Após a obtenção dos espectros, as sementes foram submetidas ao teste de germinação e contabilizadas a porcentagem de plântulas normais, vigorosas, fracas e sementes mortas. A partir dos dados espectrais foram obtidos modelos utilizando-se o método Partial Least Squares - Discriminant Analysis (PLS-DA), separando-se 70 % dos dados para treinamento e 30 % para validação. O pré-processamento utilizando a segunda derivada de Savitzky-Golay (SG) e a combinação da segunda derivada de SG + multiplicative scatter correction resultaram em valores de acurácia e *kappa* no treinamento de 0.94 e 0.92, respectivamente. A espectroscopia FT-NIR mostrou-se uma ferramenta promissora para a classificação de sementes artificialmente envelhecidas de lentilha, quanto ao potencial fisiológico.

PALAVRAS-CHAVE: *Lens culinaris*, potencial fisiológico de sementes, inteligência artificial.

INTRODUCTION

Pulses are edible dry seeds, typically from leguminous plants such as lentil, chickpea, pea and bean (Faris et al. 2020). They are generally classified as biofortified foods, rich in proteins, fibers, minerals and vitamins, which have drawn increasing attention to their role in health, nutrition and food security (Foschi et al. 2020). Among these, lentil (*Lens culinaris* Medik.) is the fastest-growing legume for direct human consumption and serves as an important source of dietary protein (Khazaei et al. 2019).

In Brazil, lentils are considered an excellent option for irrigated winter agriculture, with yields reaching up to 1,500 kg ha^{-1} (Nascimento & Bagolin 2022). This agronomic potential has driven an expansion in cultivated areas, highlighting the need

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[2] Universidade Federal de Viçosa, Departamento de Agronomia, Viçosa, MG, Brazil.
Email/ORCID: marcelo.limao@ufv.br/0000-0003-1265-1014; andre.d.medeiros@ufv.br/0000-0002-1097-0292; dcdias@ufv.br/0000-0002-0596-2490; laercio.silva@ufv.br/0000-0001-7202-0420.
[3] Centro Universitário do Triângulo, Uberlândia, MG, Brazil. *Email/ORCID*: danielpinheiroagro@gmail.com/0000-0002-8060-8790.
[4] Fundação MS, Maracaju, MS, Brazil. *Email/ORCID*: joaoribeiro@fundacaoms.org.br/0000-0002-1163-3546.
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for high-physiological-potential seeds in the market (Joshi et al. 2017).

Seed physiological potential is the combination of its germination, vigor and longevity. High-quality seeds ensure uniform emergence, better plant establishment, reduced stand gaps, and greater stress and pathogen tolerance. They also help to maximize the genetic potential of a cultivar while supporting production sustainability and a more efficient use of inputs (Marcos-Filho 2020).

The accelerated aging test is used to artificially deteriorate seeds by exposing them to high temperature and relative humidity (Marcos-Filho 2020). These conditions trigger a series of physiological and biochemical changes that lead to quality loss and serve as indicators of initial seed vigor (Peng et al. 2017, Jiang et al. 2018, Andrade et al. 2020, Morais et al. 2021, Souza et al. 2023, Silva et al. 2024, Limão et al. 2025).

Another promising technique is the Fourier transform near-infrared (FT-NIR) spectroscopy (Orrillo et al. 2019, Limão et al. 2025), an easy-to-use method with minimal sample preparation and no reagent consumption, that provides non-invasive, non-destructive, real-time information (Zimmerleiter et al. 2020). However, the accurate assessment of spectral data requires chemometric methods such as the Partial Least Squares - Discriminant Analysis (PLS-DA), Linear Discriminant Analysis (LDA), Support Vector Machines (SVM) and Random Forest, which enable correlating spectral data with the chemical composition and physiological potential of seeds (Silva et al. 2024, Soares et al. 2024).

Various studies have demonstrated the efficiency of the FT-NIR spectroscopy in predicting seed quality in different crops, including chickpea (Ribeiro et al. 2021), wheat (Fan et al. 2020), Japanese cabbage (Ma et al. 2020), *Brachiaria* (Souza et al. 2023) and soybean (Silva et al. 2024, Soares et al. 2024), among others. Furthermore, it can quantify oil, protein and fatty acid content (Fassio et al. 2015, Li et al. 2018, Serson et al. 2020). This technique has also been reported as effective in classifying aged maize (Andrade et al. 2020, Wang et al. 2020) and watermelon seeds (Yasmin et al. 2019). Recently, Limão et al. (2025) demonstrated promising results using FT-NIR spectral models to predict physiological potential classes in lentil seed lots. However, information using seeds with different deterioration levels is also important for understanding the application of this technique to lentil seeds. Thus, this study aimed to investigate the potential of FT-NIR spectroscopy combined with multivariate data analysis to classify artificially aged lentil seeds.

MATERIAL AND METHODS

The study was conducted at the Universidade Federal de Viçosa, in Viçosa, Minas Gerais state, Brazil, in 2022. Lentil (*Lens culinaris* Medik.) seeds of the 'Silvina' cultivar were used, provided by the Embrapa Hortaliças.

Initially, the seed moisture content was determined by the oven-drying method at 105 ºC, for 24 hours (Brasil 2009), with results expressed as percentage.

To obtain seeds with different deterioration levels, artificial aging was performed according to Marcos-Filho (2020). For each aging period, 250 seeds were evenly distributed in a single layer on a metallic screen within a plastic Gerbox-type container, with 40 mL of distilled water added to the bottom. The boxes were sealed to obtain approximately 100 % of relative humidity inside and placed in a BOD chamber at 41 ºC for 24, 48, 72, 96 and 120 hours. Unaged seeds were used as the control treatment.

Two hundred seeds were randomly selected from each artificial aging treatment for spectral data acquisition using an FT-NIR spectrometer (Thermo Scientific Antaris II) and placed individually in a 1-cm² circular metal holder adapted for lentils. For each seed, 3,111 spectral points were collected within the 1,000-2,500 nm wavelength range. Reflectance spectra were expressed as log (1/R), where R represents reflectance.

After spectral acquisition, the seeds from each treatment were germinated according to Brasil (2009). Germination (normal seedlings) was recorded on the fifth and tenth day after planting. The percentages of vigorous seedlings (length > 5.0 cm, with well-developed roots and shoots), weak seedlings (≤ 4.9 cm, with underdeveloped roots or shoots) and dead seeds were also quantified.

The raw spectral data were pre-processed using the standard normal variate (SNV), multiplicative scatter correction (MSC), first Savitzky-Golay (SG) derivative and second SG derivative, as well as their combinations: first SG derivative + MSC; second SG derivative + MSC; first SG derivative + SNV; and second SG derivative + SNV, all using an 11-point wavelength window.

Based on the germination test results after artificial aging, the following physiological potential classes were established: high physiological potential - control and 24-hour treatments (> 81 % of vigorous seedlings); medium physiological potential: 48-hour and 72-hour treatments (60-80 %); low physiological potential: 96-hour and 120-hour treatments (< 59 %). Thus, the number of seeds per class was 400.

The germination test followed a completely randomized design, with six artificial aging periods (control, 24, 48, 72, 96 and 120 hours) and four replicates. The data were tested for normality of errors using the Shapiro-Wilk test and for homogeneity of variances using the Bartlett's test. The means obtained from the germination test across aging periods were submitted to regression analysis. All analyses were conducted using the R software version 4.1.1 (R Core Team 2025).

Spectral data were initially submitted to exploratory analysis using the Principal Component Analysis (PCA), and classification models were then developed using PLS-DA (Barker & Rayens 2003).

The model performance was assessed based on accuracy and the *kappa* coefficient. Additionally, the most relevant wavelength ranges contributing to each physiological potential class were identified from the best-performing model. PLS-DA analyses were conducted using the Caret package in R software (R Core Team 2025).

RESULTS AND DISCUSSION

Unaged control seeds exhibited the highest percentages of germination and vigorous seedlings (98 and 93 %, respectively), followed by seeds artificially aged for 24 hours, with 89 and 81 %, respectively. Consequently, seeds subjected to shorter aging periods (0 and 24 hours) showed lower proportions of weak seedlings and dead seeds (Figure 1).

Figure 1 shows that increased exposure to accelerated aging reduced the seed germination capacity and physiological potential. Conversely, the percentages of weak seedlings and dead seeds increased with longer aging periods (Figure 1).

Seeds submitted to 48 and 72 hours of aging showed similar germination rates (82 and 80 %, respectively) and vigorous seedling percentages (64 %). However, there was a marked decrease in seeds aged for 96 and 120 hours, with germination rates falling to 67 and 32 %, and the percentage of vigorous seedlings to 41 and 6 %, respectively. At 96 hours, 33 % of the seedlings were classified as weak; whereas, at 120 hours, 68 % of the seeds were dead (Figure 1).

Overall, the results confirm that the physiological potential of lentil seeds declines as the duration of artificial aging increases. Vigor loss was proportional to the length of exposure, with the 120-hour period causing the most significant decreases in germination and seedling vigor, along with increases in weak seedlings and dead seeds (Figure 1).

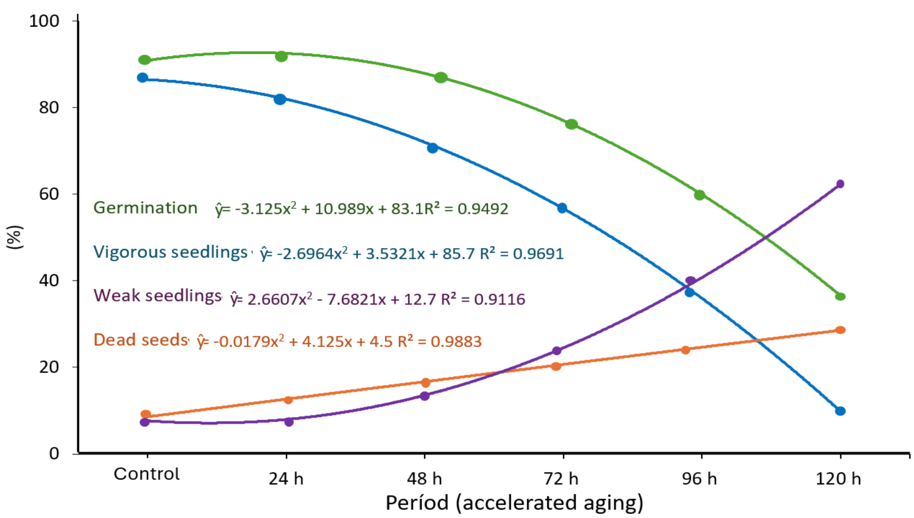

Figure 1. Germination (%) and physiological potential of lentil seeds expressed as percentage of vigorous seedlings, weak seedlings and dead seeds after artificial aging periods.

Seed deterioration is a natural and irreversible process that can be significantly accelerated depending on environmental conditions (Ebone et al. 2019). Artificial aging intensifies several cellular changes responsible for the decline in germination and vigor. These include membrane lipid peroxidation (Veselovsky & Veselova 2012), protein (Min et al. 2017) and nucleic acid breakdown (Fleming et al. 2018), and increased production of reactive-oxygen species (ROS) (Kurek et al. 2019, Zhang et al. 2021, Li et al. 2022, Pinheiro et al. 2023), among others. These changes are primarily associated with the significant increase in seed respiratory rates under high temperature and relative humidity, as reported for lentils and other pulses (Chidananda et al. 2014).

It is important to underscore that the primary storage compounds in lentil seeds are proteins, fibers, carbohydrates and micronutrients (Khazaei et al. 2017). As such, deterioration in this species likely involves changes in these compounds, such as protein degradation (Min et al. 2017) and impaired reserve mobilization to the embryonic axis (Felix et al. 2020), among others. Limão et al. (2025) found that accelerated aging for 48 hours at 41 ºC effectively discriminated the physiological potential of lentil seeds and was associated with low soluble protein content. These combined factors likely contributed to the significant reduction in the seeds' physiological potential (Figure 1).

Figure 2 presents the 1,200 raw spectral data points collected (Figure 2A) and the average spectra (Figure 2B) of the three physiological potential classes defined according to germination and seedling vigour results obtained after ageing treatments. By average spectra (Figure 2B), it is possible to identify differences in absorbance peaks between the classes. In general, the highest absorbance values were obtained for the class with high quality seeds in the entire spectral range (1,000-2,500 nm).

Pre-processing aims to correct the additive and multiplicative effects in the spectra, given that they contain a combination of tones and overtones that increase noise and can obscure results (Wu et al. 2009). Therefore, applying and combining pre-processing methods are essential to efficiently select a model and, consequently, obtain the best classification results (Souza et al. 2023, Soares et al. 2024). Several pre-processing methods and their combinations were tested to identify the most efficient classification model, defined by its ability to predict seed physiological potential classes (Table 1). Using the second SG derivative for pre-processing yielded training accuracy and *kappa* coefficient values of 1.0, indicating a high classification accuracy and precision. For validation, the accuracy and *kappa* values were 0.96 and 0.94, respectively. For pre-processing using the second SG derivative + MSC, accuracy and *kappa* values during calibration were 0.99, with accuracy of 0.94 and *kappa* of 0.92 during validation (Table 1).

For all other pre-processing methods during validation, accuracy values ranged from 0.82 to 0.93, and *kappa* values were below 0.90, varying from 0.73 to 0.89, resulting in intermediate values, when compared to the aforementioned pre-processing methods (Table 1).

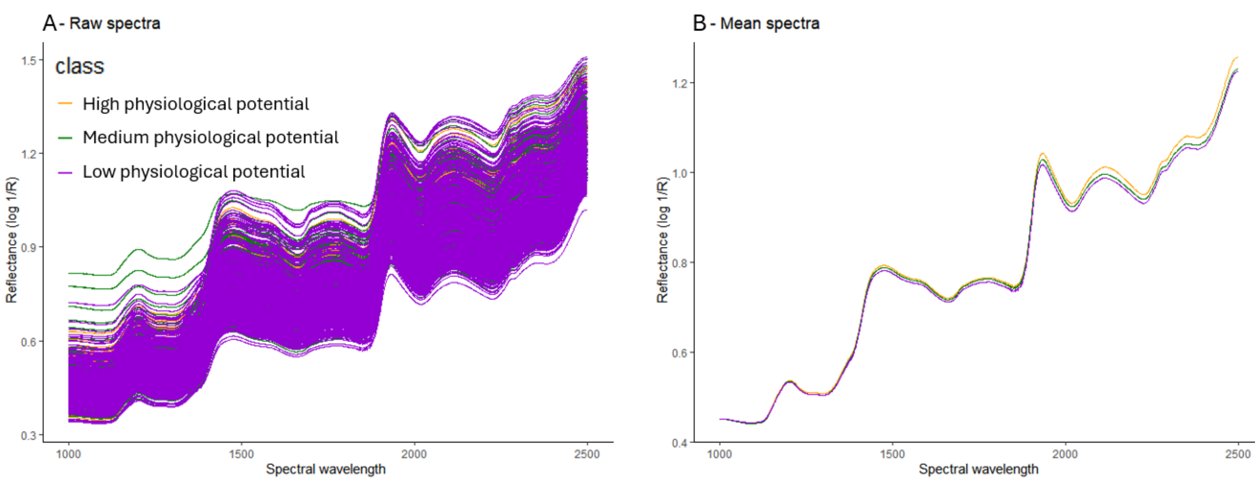

Figure 2. Raw spectral data of artificially aged lentil seeds (A) and spectral means (B).

Table 1. Accuracy and *kappa* coefficient values for training and validation (test) across pre-processing methods used in the classification models.

Pre-processing	Metrics			
	Calibration (n = 840 seeds)		Validation (n = 360 seeds)	
	Accuracy	*Kappa*	Accuracy	*Kappa*
Standard normal variate (SNV)	0.85	0.77	0.83	0.74
Multiplicative scatter correction (MSC)	0.85	0.78	0.82	0.73
First Savitzky-Golay	0.96	0.95	0.93	0.89
Second Savitzky-Golay	1.00	1.00	0.96	0.94
First Savitzky-Golay + MSC	0.96	0.94	0.92	0.88
Second Savitzky-Golay + MSC	0.99	0.99	0.94	0.92
First Savitzky-Golay + SNV	0.88	0.82	0.85	0.77
Second Savitzky-Golay + SNV	0.95	0.92	0.84	0.77

n: number of spectra used for training or testing the classification model.

Limão et al. (2025) evaluated different pre-processing techniques (standard normal variate, multiplicative scatter correction, first and second SG derivatives) and found that the second SG derivative achieved the highest accuracy (~99 %), particularly when combined with PLS-DA models for classifying lentil seed physiological potential using FT-NIR.

Other studies have also evaluated the use of pre-processing techniques to improve the accuracy and *kappa* values of PLS-DA classification models based on FT-NIR spectra in soybean (Soares et al. 2024), wheat (Fan et al. 2020), maize (Andrade et al. 2020) and *Chamaecyparis obtusa* seeds (Mukasa et al. 2019). Ribeiro et al. (2021) observed that PLS-DA models using the first-order derivative effectively differentiated chickpea seed quality after pre-harvest herbicide treatments using FT-NIR spectroscopy. In *Brachiaria* seeds, FT-NIR data combined with PLS-DA models was a sensitive, rapid and non-destructive approach for estimating the physiological potential of *Urochloa decumbens* seed lots, mainly when second derivative pre-processing using the SG filter was used (Souza et al. 2023).

Confusion matrices were created (Figure 3) for the two most effective pre-processing methods for classifying seed physiological potential: second SG derivative and the combination of the second derivative + multiplicative scatter correction. During calibration, both pre-processing methods achieved 100 % of classification accuracy for the high, medium and low-quality classes. However, in the validation phase, the second SG derivative obtained classification accuracies of 95, 97.5 and 96.6 % for high, medium and low physiological potential, respectively. When the second SG derivative was combined with multiplicative scatter correction, the accuracy levels were 92, 95 and 97 % for the same respective classes (Figure 3).

The wavelengths that contributed most to the PLS-DA classification model, using the second SG derivative for high, medium and low-quality classes, were 1,000-1,100 nm, 1,900 nm and 2,300-2,500 nm, respectively (Figure 4).

The most prominent wavelength peaks associated with the different physiological potential classes corresponded to water content (approximately 13 % across all treatments) and organic compounds such as proteins, carbohydrates and lipids, which overlap along the wavelength. The regions of greatest importance for the model were found between 1,000 and 1,100 nm, correlated with the functional groups N-H, C-H and O-H, associated with protein and carbohydrate content. This is consistent with the high protein content of lentil seeds (Ambrose et al. 2016, Mukasa et al. 2019), a trait linked to the physiological potential level (Limão et al. 2025). The peak at 1,900 nm is attributed to the O-H functional group, associated with water and carbohydrates. Peaks between 2,300 and 2,500 nm correspond to the C-H+C-C and C-H+C-H groups, which include polysaccharides, proteins and minor lipid absorbance (Figure 4) (Shenk et al. 2001, Brito et al. 2008, Ambrose et al 2016, Xu et al. 2020). The importance of these spectral bands is directly related to seed deterioration, which results in the degradation of biochemical compounds and, consequently, affects the seed physiological potential (Soares et al. 2024).

Taken together, these findings indicate that FT-NIR spectroscopy is an effective, non-destructive

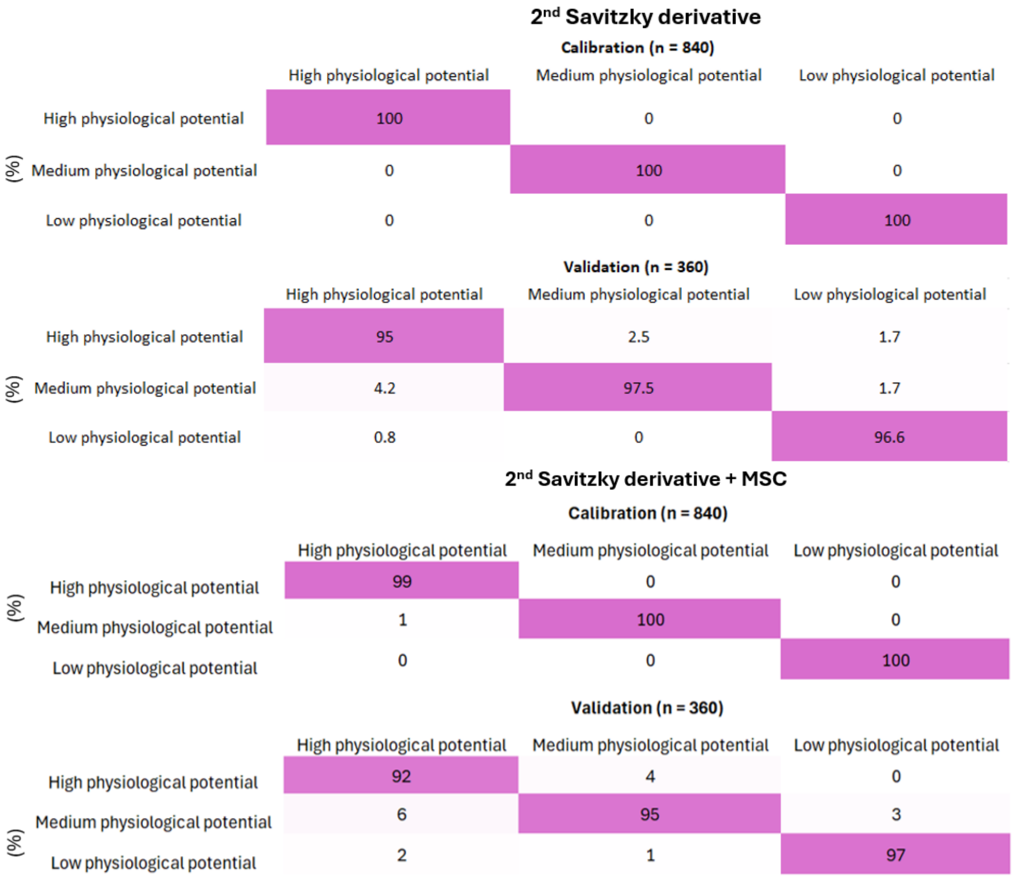

Figure 3. Confusion matrix for lentil seed classification into physiological potential levels via the PLS-DA model, using spectral data pre-processed by the second Savitzky-Golay derivative and a combination of the second Savitzky-Golay + multiplicative scatter correction.

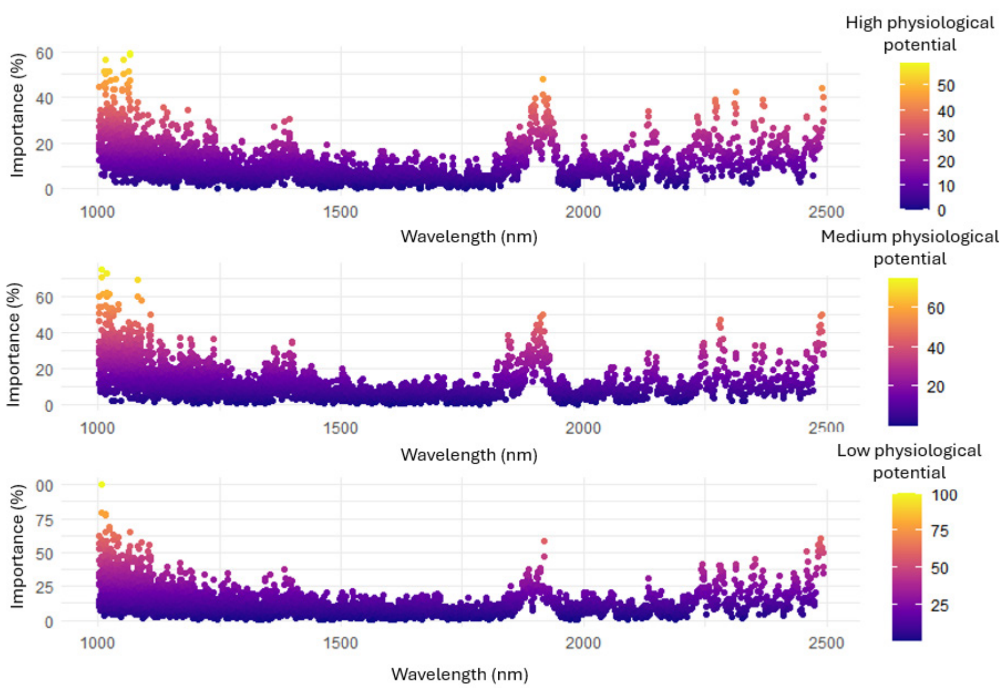

Figure 4. Wavelength variable importance for PLS-DA classification of lentil seed physiological potential levels.

tool for classifying lentil seeds according to their physiological potential, providing fast and accurate results.

CONCLUSIONS

1. Near-Infrared (FT-NIR) spectroscopy is effective in classifying artificially aged lentil seeds according to their physiological potential;
2. The most efficient Partial Least Squares - Discriminant Analysis (PLS-DA) models were obtained by preprocessing the spectral data using the second Savitzky-Golay (SG) derivative and the combination of the second SG derivative + multiplicative scatter correction.

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