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Comparative nondestructive measurement of corn seed viability using Fourier transform near-infrared (FT-NIR) and Raman spectroscopy

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ABSTRACT

The commercialization of agriculture has driven the need to ascertain the quality of agricultural inputs, especially seeds in order to optimize output and increase economic returns. Seed viability is a critical consideration for ensuring a reasonably high harvest. More often than not, farmers experience losses after a significant percentage of seeds fail to germinate after planting. The loss of seed viability may be due to a number of reasons such as overheating during drying, physical damage during post-harvest processing, and ageing during storage. It is therefore critical for seed companies to sufficiently inspect their products and uphold them to acceptable seed quality standards in order to gain credibility and ensure business sustainability. In this study, the Fourier transform near-infrared (FT-NIR) and Raman spectroscopy techniques were used for evaluating seed viability to investigate their comparative advantages with regard to the corn viability test and classification. The techniques were applied to white, yellow, and purple corns with 300 samples in each category. The 300 sample corn seeds were divided into two groups of 150 seeds each; one group was heat-treated using microwaving, and the other was used as the control. Sample spectra from treated and untreated corn seeds were collected using an FT-NIR spectrometer in the wave range of 1000–2500 nm, and then Raman spectrometer in the wave range of 170–3200 cm^{-1}. The collected spectra were divided into training and testing sets, corresponding to 70% and 30% of the total, respectively for calibration and validation of the techniques. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to assess the spectral data from FT-NIR and Raman spectroscopy. The analysis results indicated that FT-NIR spectroscopy correctly classified viable and nonviable seeds for all the three categories of corns with a high accuracy of 100% and a predictive ability of more than 95%. Moreover, Raman spectroscopy demonstrated reasonably high classification accuracy with PLS-DA, but a significant number of seeds were overlapping when using PCA. In addition, an analysis of variance (ANOVA) indicated that the difference between treated and untreated corn seeds was not statistically significant ($P < 0.05$). The study demonstrated that FT-NIR spectroscopy is superior to Raman spectroscopy in evaluating corn seed viability.

1. Introduction

Seed viability and vigorous growth of crops are of utmost interest to every farmer. The viability of seeds may be inhibited due to several reasons such as overheating during drying, physical damage during post-harvest processing, and ageing during storage. Farmers may acquire seeds for planting from seed companies, research institutes, or by self-breeding. In the recent past, most farmers have been increasingly lured to buy seeds from seed companies, which offer attractive prices and assurances of high productivity. More often than not, the prime interest of seed companies is to increase their sales and sustain their businesses as much as possible, whereas that of farmer is value for money, and that every seed bought should germinate. Therefore, to ensure business optimization, seed companies have to secure farmers' confidence and credibility by supplying sufficiently viable seeds for client satisfaction. This requires that corn seed supplier companies invest in corn viability test and classification technologies capable of efficiently handling bulk volumes of seeds.

It is difficult or even impossible to accurately and precisely separate large volumes of nonviable seeds from normal ones by human means, using the naked eye or low-capability instruments [1]. A variety of product inspection techniques ranging from low-capability gadgets to sophisticated high-tech equipment have

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evolved over time to aid the detection and classification of undesired agricultural products [2].

Some of the conventional methods, including human sorting, immunoassay tests, and polymerase chain reactions, [3] have several shortcomings such as subjectivity, invasiveness, low specificity, long time consumption and low accuracy [4]. On the other hand, modern spectroscopic techniques, such as Fourier transform-infrared (FT-IR), Fourier transform near-infrared (FT-NIR), nuclear magnetic resonance (NMR), ultraviolet–visible (UV–vis), Raman, and hyperspectral imaging, have exhibited great potential for detection, qualification, and quantification of target physical and chemical attributes of agricultural products [5–7].

In principle, the vibrational spectroscopic techniques exploit the differences in biochemical composition, such as starch, protein, and moisture contents, that exist between aged and normal seeds. Aged corns often have reduced amounts of protein and starch, which are easily detectible in the near-infrared (NIR) range of the electromagnetic spectrum. The major chemical difference between aged and normal corn seeds is the decreased H^+ATPase, an enzyme of protein nature, which occurs during ageing. The enzyme activates the seed membrane cells to divide and expand, whereas corn ageing causes loss of activity of the enzyme, and consequently loss of germination [8,9].

The advantages of spectroscopic techniques over conventional methods in detecting seed viability include high accuracy and precision, non-invasiveness, and rapidity. Therefore, it is necessary to investigate the feasibility of using spectroscopic techniques to identify and classify viable and nonviable corn kernels.

The aims of this study were to investigate the possibility of using FT-NIR and Raman spectroscopy in classifying viable and nonviable corns, and evaluate their comparative performance advantages.

2. Materials and methods

2.1. Seed selection and aging treatment

Three categories of commercial hybrid corn kernels (*Zea mays* L.), namely, white, yellow, and purple, were used in the experiment. For each category, 300 kernels were considered with 150 seeds used as the aged sample and the other 150 seeds used as the control. Artificial ageing was induced in the corn samples using microwave heat treatment at 1000 W input power and 40 s exposure time, which were earlier optimized for this experiment. The heat treated kernels are assumed inactive and nonviable with zero germination potential. For each category of corn seeds, the treated and untreated samples could not be differentiated by the naked eye. The samples were kept in polyethylene bottles for at least 24 h before spectral measurement.

2.2. Spectral data collection

Two spectral measurement techniques, namely, FT-NIR and Raman spectroscopy, were used to collect spectral signatures from the treated and untreated corn samples.

2.2.1. Fourier transform near-infrared spectroscopy

Spectral data from 300 kernels of corn for each of the three categories (white, yellow, and purple) were acquired using an FT-NIR spectrometer (Antaris II FT-NIR analyzer, Thermo Scientific Co., USA). The reflectance spectra were acquired on a single-seed basis, one after the other. The kernel placed on the sample holder was irradiated by the NIR halogen light, which was then reflected from the seed to the detector [10]. The treated and untreated corn seeds were tested individually at the wavelength range of 4000–10000 cm^{-1} (1000–2500 nm) at 8 cm^{-1} intervals. For each corn kernel measured, 32 successive scans were done, and the mean spectra were obtained. The background scan was always conducted with a golden slit before acquiring the spectrum from each kernel.

2.2.2. Raman spectroscopy

The three categories of corn kernels were scanned using a portable i-Raman spectrometer (BWTEK Inc., USA) to obtain the spectral data from each kernel. The Raman spectrometer had a charge-coupled device (CCD) detector of 14×900 μm pixel size and a 785 nm near-infrared laser. The standard 785 nm excitation laser was selected to reduce the background and sample fluorescence.

The treated and untreated kernels were individually measured for each of the three categories. The measurement was carried out in the Raman shift range from 170 to 3200 cm^{-1} at the spectral resolution of 4 cm^{-1}. For every sample, an integration time of 8000 ms and four scans were used to generate high quality of Raman spectra, and the averaged spectrum file was saved for analysis.

2.3. Chemometric analysis

The spectral data from the treated and untreated corn in each category were analyzed using the software MATLAB (Version 7.10.0, The Mathworks, Natik, MA, USA) for the two spectroscopic measurement techniques, i.e., FT-NIR and Raman spectroscopy. In this study, a simple and efficient polynomial curve fitting method was employed to remove the fluorescence effect and to correct the baseline of the obtained Raman spectra. Polynomial fitting involves determining the proper order polynomial for obtaining a baseline through iterative calculation.

Two multivariate data analysis techniques were applied, namely principal component analysis (PCA), and partial least squares–discriminant analysis (PLS-DA) to develop classification models for the viable and nonviable corn kernels. PCA was used for dimensionality reduction and visualization of data distribution with regard to distinction between nonviable and viable corn samples. Dimensionality reduction is done by transforming the many variables into fewer meaningful components, known as principal components (PCs), which can be related to the variables. In the new coordinate system of PCs, the PC loadings, which are correlation coefficients (weights), are used to estimate how much each of the original variables contribute to each of the PCs.

For PLS-DA, the spectral data from FT-NIR and corrected Raman spectra were preprocessed through baseline correction and normalization (mean, max, and range) across their respective wavelength ranges to remove any possible spectral noise due to systematic and/or environmental errors. The normalized spectra were further preprocessed using several methods, such as multiplicative scattering correction (MSC), standard normal variate (SNV), and Savitzky–Golay filtering functions, to produce the first and second signal-smoothing derivatives of the spectra, which in turn reduce peak overlap. According to Chen et al. [11], MSC is essential for leveling the spectra by resolving spectral differences in the data, whereas SNV is a correction method necessary for removing slope variation from the spectra generated by scatter and variation of pixel size [12]. In this experiment, the spectral data from each seed were acquired with the illumination directed on the germ side. The side of illumination reception on the seed is a critical consideration as the germ side often exhibits higher reflectivity compared with the back side of the seed [13]. The average spectra of viable and nonviable seeds of both FT-NIR and Raman spectroscopy are depicted in Fig. 1(a) and (b), respectively.

In each technique, 300 sample spectra (150 treated and 150 untreated) were randomly divided into two sets, namely training, and test sets. The training set (70% = 210 samples) was used to construct the chemometric calibration models for classification using multivariate statistical analysis, whereas the test set (30% = 90 samples) was used to validate the constructed model based on