Original Article
Journal of Biosystems Engineering

J. of Biosystems Eng. 38(4):318-326. (2013. 12)
http://dx.doi.org/10.5307/JBE.2013.38.4.318
eISSN : 2234-1862
pISSN : 1738-1266

Determination of Germination Quality of Cucumber (*Cucumis Sativus*) Seed by LED-Induced Hyperspectral Reflectance Imaging

Changyeun Mo[1], Jongguk Lim[1]*, Kangjin Lee[1], Sukwon Kang[1], Moon S. Kim[2], Giyoung Kim[1], Byoung-Kwan Cho[3]*

[1]*National Academy of Agricultural Science, Rural Development Administration, 249 Seodun-dong, Gwonseon-gu, Suwon, Gyeonggi-do 441-100, South Korea*
[2]*Environmental Microbiology and Food Safety Laboratory, Agricultural Research Service, US Department of Agriculture, PowderMill Rd. Bldg.303, BARC-East, Beltsville, MD20705, USA*
[3]*Department of Biosystems Machinery Engineering, College of Agricultural and Life Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, South Korea*

Received: November 8th, 2013; Revised: November 25th, 2013; Accepted: November 26th, 2013

Abstract

Purpose: We developed a viability evaluation method for cucumber (*Cucumis sativus*) seed using hyperspectral reflectance imaging. **Methods:** Reflectance spectra of cucumber seeds in the 400 to 1000 nm range were collected from hyperspectral reflectance images obtained using blue, green, and red LED illumination. A partial least squares–discriminant analysis (PLS-DA) was developed to predict viable and non-viable seeds. Various ranges of spectra induced by four types of LEDs (Blue, Green, Red, and RGB) were investigated to develop the classification models. **Results:** PLS-DA models for spectra in the 600 to 700 nm range showed 98.5% discrimination accuracy for both viable and non-viable seeds. Using images based on the PLS-DA model, the discrimination accuracy for viable and non-viable seeds was 100% and 99%, respectively **Conclusions:** Hyperspectral reflectance images made using LED light can be used to select high quality cucumber seeds.

Keywords: Cucumber seed, Germination prediction, Hyperspectral imaging, PLS-DA, LED

Introduction

The size of seed market in the world has reached $7.8 billion in 2011; as a result, plant variety protection has expanded to secure food supply and to ensure competitiveness of agricultural products on the international market, and competition for superior crop varieties has accelerated. Seeds are one of the most fundamental elements of agriculture. Good quality seeds improve agricultural products and reduce the unnecessary loss caused by defective seeds in all production process, including sowing, growing, and harvesting. Cucumbers (*Cucumis sativus*), which contain most of the essential nutrients, are an important vegetable crop. These vegetables are used as ingredients in food, cosmetics, and health food. Selection of healthy seeds with high germination rates is important for increasing the yield of high-quality cucumbers.

Physical properties such as weight, specific gravity, and color are used to select superior seeds of high quality. Germination, biochemical, and physical tests are also used to evaluate seed quality (International Seed Testing Association, ISTA, 2008). Germination tests require 8-14 days for evaluation. Tetrazolium or electrical conductivity tests can be used to rapidly evaluate seed viability, but require specialized training and experience. Therefore, near infrared reflectance spectroscopy, or Raman spectroscopy, has been used to measure components of seeds and to

***Corresponding author:** Jongguk Lim
Tel: +82-31-290-1966; **Fax:** +82-31-290-1900
E-mail: limjg@korea.kr
Byoung-Kwan Cho
Tel: +82-42-821-6715; **Fax:** +82-42-823-6246
E-mail: chobk@cnu.ac.kr

estimate seed viability (Min & Kang, 2003; Shetty et al., 2011; Shin et al., 2012; Reitzenstein et al., 2007; Silva et al., 2008). Spectroscopy is used to determine the concentration of specific compounds in a sample by analysis of average spectrum information obtained from the whole sample. This involves diluting the information spatially. However, in hyperspectral reflectance spectroscopy, image information and spectral information about the target object is collected simultaneously for each pixel simultaneously. Hyperspectral reflectance spectroscopy has gained attention as a non-destructive estimation technique. Research and applications in the field of food and agriculture that use hyperspectral reflectance spectroscopy have increased in recent years (Kim et al 2001; Lee et al. 2008; Cho et al. 2011). There has also been research into multispectral and hyperspectral imaging techniques that use LED illumination instead of Tungsten-halogen illumination to get high output of blue, green, red wavelengths (Lawrence et al, 2005; Qin et al, 2011).

We developed a partial least square regression-discrimination (PLS-DA) model based on hyperspectral reflectance spectroscopy to select cucumber seeds with high germination rates. In addition, optimal conditions for LED illumination were investigated and used to develop an algorithm for seed discrimination using hyperspectral imaging.

Materials and Methods

Materials

Cucumber seeds that were not treated with any coating were purchased from a local seller (Jeil Seed Inc., Suwon, Korea) in 2012. In order to identify the germination characteristics of aging, 100 seeds were kept in a chamber, at a constant relative humidity of 96% and temperature of 45°C for 18 days to artificially accelerate aging. Seeds were then dried at 22°C for 24 h so that they would have identical moisture content. All of the seeds, 100 normal seeds and 100 aged seeds, were vacuum packed and stored at 4°C. The seeds equilibrated to room temperature prior to the hyperspectral imaging and germination.

The hyperspectral imaging system

The hyperspectral imaging system was composed of a hyperspectral image measurement unit, light source, sample transfer unit, and data acquisition unit (Figure 1). The hyperspectral image measurement unit had three parts: an electron multiplying charge-coupled device camera (EMCCD, MegaLuca R, ANDOR Technology, South Windsor, CT, USA) with 1004 × 1002 pixels, an imaging spectrograph (VNIR, Headwall Photonics, Fitchburg, MA, USA) that produced a spectrum for each wavelength between 400 and 1000 nm, and a C-mount object lens (F1.9 - 24 mm,

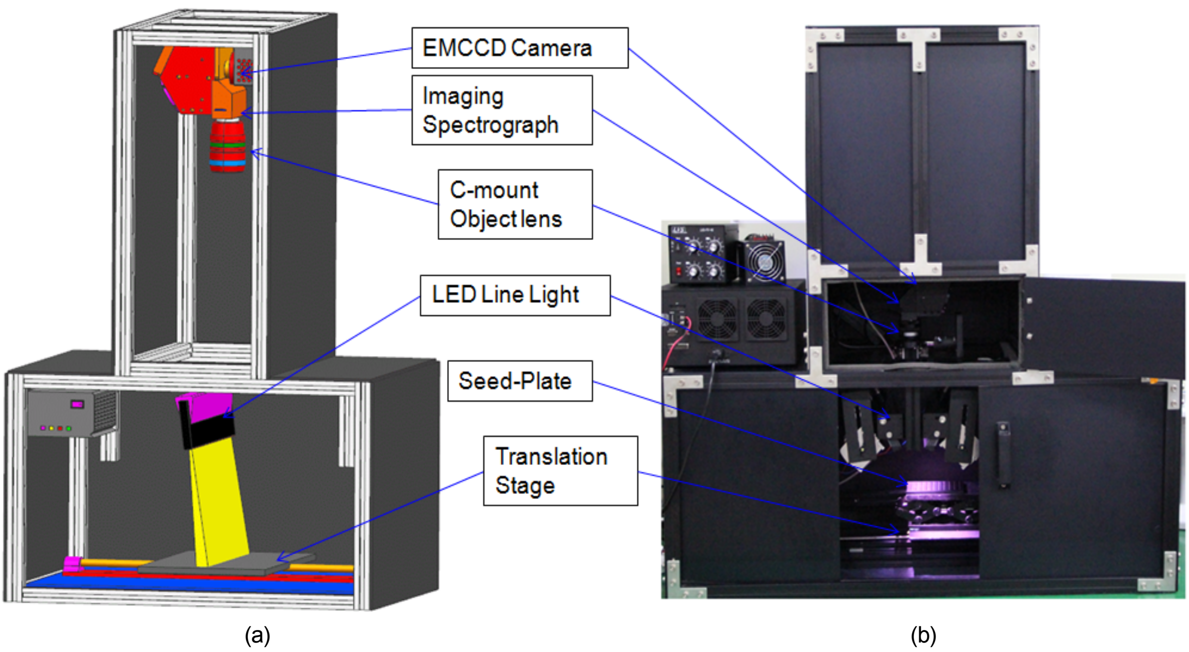


Figure 1. (a) Schematic diagram and (b) photo of the hyperspectral reflectance imaging system.

Schneider Optics, Hauppauge, NY, USA) with a 25-mm focal length. The hyperspectral image measurement unit had a resolution of 8 × 8 μm and was cooled to - 20°C using a thermoelectric cooling method. In addition, it captured 14-bit images at a rate of 12.5 MHz. Three types of light emitting diodes (LEDs, HPLS-RGB, LVS co. Ltd, Incheon, Korea), including a blue LED (462 nm peak wavelength), green LED (525 nm peak wavelength), and a red LED (625 nm peak wavelength). The output of each LED light source was controlled individually between 0 and 100%. Circular optical fiber cable was connected to the light source, and line-shape optical fiber cable was fixed perpendicular to the direction of transfer, and at a 15-degree incline. Light was emitted from the optical fibers in the shape of the thin line. The light reflected from the sample passed through the camera lens and a 25 μm × 18 mm (width × length) aperture slit. The reflected light then was separated by wavelength by the imaging spectrograph. The spectra image amplified that by the EMCCD camera was stored. The sample transfer unit was composed of a moving table, step motor (XN10-0180-m02-21, Velmex, New York, USA), and step motor drive. Samples were fixed to the table, which moved in the traverse direction, was controlled by the step interval and the number of steps.

Acquiring hyperspectral image spectra

The sample seeds were arranged in a 10 × 10 grid and fixed on the translation stage. Hyperspectral images of the top and bottom of all 200 samples were captured three times, for a total of 1200 images. The images were acquired by scanning the line with three LED light sources. The exposure time, traverse distance, total number of moving steps, and distance from the hyperspectral imaging camera to the sample were set to 2 ms, 0.1 mm, 800, and 315 mm, respectively. Hyperspectral images, each with 125 wavebands in the range of 400-1000 nm were composed of 640 × 800 pixels. After obtaining hyperspectral images of seeds, the seeds were vacuum packed and kept in a chamber at constant temperature of 4°C and relative humidity of 60%.

In order to check the stability of the light source, reflectance spectra of a white Teflon plate that had reflectivity of 99%, were measured hourly under the following conditions: 100% blue LED, 100% green LED, and 15% red LED. As shown in Figure 2, the reflectance spectra of blue LED, green LED, and red LED were in

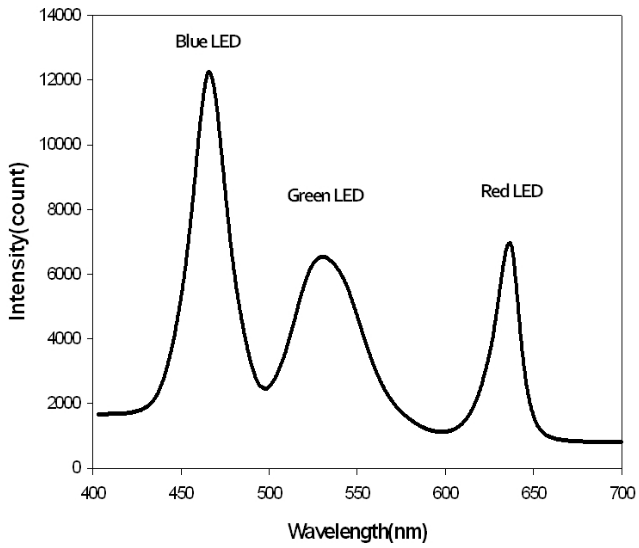


Figure 2. Spectrum of reference panel under blue, green, and red LED illumination.

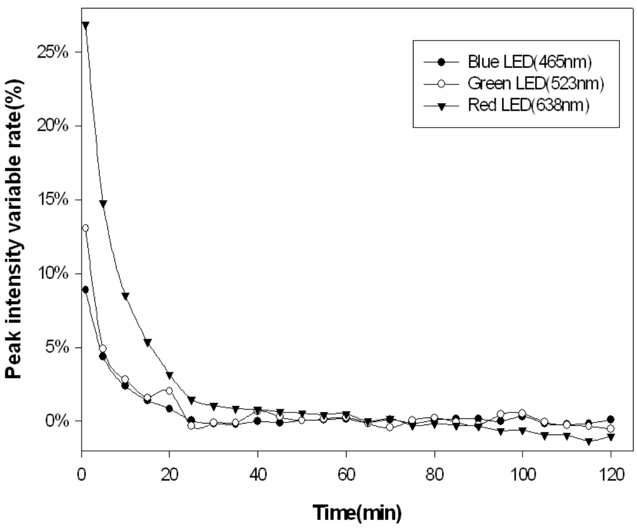


Figure 3. Peak intensity variance of blue LED peak (465 nm), green LED peak (523 nm), and red LED peak (638 nm).

the 400-500 nm, 500-600 nm region, and 600-700 nm region, respectively. Figure 3 shows the peak variance of intensity rate of each light source. The blue LED stabilized the fastest, and it took about 20 minutes for all three light sources to stabilize. The variation of the peak after stabilizing was less than ± 1.5%. Hyperspectral images were collected after the light sources had been allowed to stabilize for 20 minutes.

Germination test

After obtaining hyperspectral reflectance images, a germination test was performed following the guidelines prescribed by the International Seed Testing Association (2008) to discriminate the viable from non-viable seeds. Twenty seeds were arranged at regular intervals (5 × 4) on wet paper. The wet paper was rolled, put into a plastic container, and placed on the middle shelf of chamber with relative humidity of 65% and a constant temperature of 25°C. After eight days, seeds with an emerged radicle that was at least 1 mm in length were considered to have germinated.

Pretreatment of hyperspectral image data

The hyperspectral images of seeds were transformed into hyperspectral reflectance images using equation (1) in order to remove the noise from the device and the influence of uneven light source intensities. Hyperspectral images of a dark reference were obtained without any light source in order to determine device noise. Hyperspectral images of the white reference, a Teflon plate that exhibited reflectivity of more than 99%, were obtained to calibrate the intensity of the light source at each vertical pixel.

$$I_{reflectance}(i)(\%) = \frac{I_{raw}(i) - I_{dark}}{I_{white} - I_{dark}} \times 100 \quad (1)$$

where

$I_{reflectance}$ is the corrected reflectance image at i_{th} wavelength,

I_{raw} is the raw hyperspectral image at i_{th} wavelength,

I_{dark} is the hyperspectral image of dark reference at i_{th} wavelength,

I_{white} is the hyperspectral image of white reference at i_{th} wavelength

The sample holder was removed from the transformed hyperspectral reflectance images, and the seed portion of each hyperspectral reflectance spectrum was extracted. Because there were 323-798 spectra for each seed, the spectra were averaged for further analysis.

Prediction of germination using hyperspectral reflectance spectra

A model for predicting viability was developed using PLS-DA and the classification rate (%) of this model was evaluated. PLS-DA models for three different kinds of LED (blue, green, and red) and a combination of the three LEDs, RGB LED, were developed and compared to determine the optimal wavelength range for determination of seed viability. The 1200 spectra we obtained were divided into two groups in order to develop the PLS-DA model. We used 800 randomly-selected spectra to develop the model and 400 spectra to test it. The PLS-DA was used to separate seeds into two groups, based on the spectra set (Alexandrakis et al., 2008). The model categorizes spectra as either '0' or '2': '0' referred to non-viable seeds, '2' referred to viable seeds.

We used a cross-validation method for the PLS-DA models and the accuracy of each model was represented by its determination coefficient of calibration (Rc^2), standard error of calibration (SEC), determination coefficient of validation (Rv^2), standard error of prediction (SEP), and optimal factor (F).

Predicting germination using hyperspectral reflectance images

As shown in equation (2), PLS images were composed of estimated values which were obtained by applying regression coefficients, i.e. weights for each wavelength of the PLS-DA model, to the spectra of each pixel.

$$PLS\ Image = \Sigma_{i=1}^{n} I_i R_i + C \quad (2)$$

where

I_i is the i_{th} image of n spectral images,

R_i is the regression coefficient from the PLS-DA model,

C is the constant of the PLS-DA model

MATLAB (version 7.0.4, The Mathworks, Natick, MA, USA) was used to extract and analyze the hyperspectral image data, and Unscrambler v 9.2 (CAMO Co., Oslo, Norway) was used to develop and evaluate PLS-DA models.

Results and Discussion

Germination test

To identify characteristics of aged cucumber seeds, a germination test was performed using 20 healthy seeds and 20 artificially aged seeds. The germination rate was greater than 85% for seeds aged fewer than 10 days, but began to decrease with duration of aging treatment thereafter. Viability was lost by the 18th day of aging treatment. Therefore, we used 100 normal seeds and 100 seeds aged for 18 days to develop our model for predicting viability from hyperspectral images. The overall germination rate for healthy and aged seeds was 50.5%.

Spectral characteristics of cucumber seeds

Figure 4 shows the average spectra of pixels extracted from images of 10 viable and 10 non-viable seeds. Reflectance spectra of the seeds are shown for the following wavelength ranges: 400-500 nm (blue LED), 500-600 nm (green LED), and 600-700 nm (red LED). The intensities of the spectra for viable seeds were greater than those for non-viable seeds in all three wavelength ranges. The absorption wavelength of chlorophyll, 675 nm, was included in the wavelength range of the red LED (640-700 nm). Viable seeds had larger spectrum peaks than non-viable seeds because low chlorophyll fluorescence increased the intensity of the spectrum peak in viable seeds (Merzlyak N.M. et al, 2003). In other words, low chlorophyll fluorescence was associated with increased germination rate (Jalink et al, 1998). Thus, germination quality could be predicted using the spectral reflectance from three kinds of LED illumination.

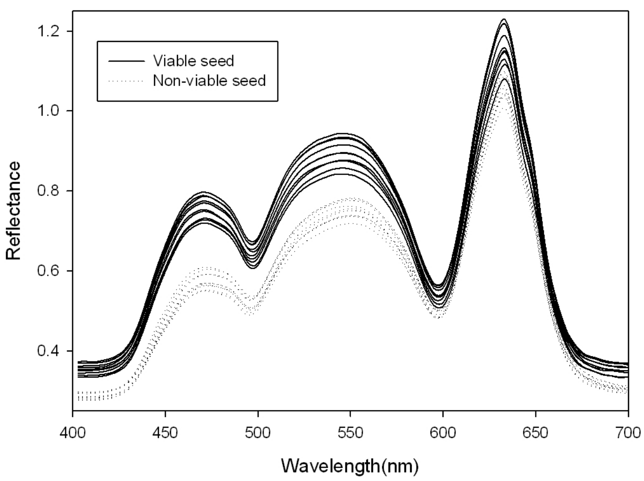


Figure 4. Average spectra extracted from images of viable and non-viable cucumber seeds illuminated by Blue, Green, and Red LEDs.

Prediction model for germination quality using hyperspectral reflectance spectra

A PLS-DA model was developed to discriminate viable seeds from non-viable seeds using hyperspectral reflectance spectra (Table 1). Estimated values that were greater than the classification value were considered to be viable seeds. Otherwise, they were considered to be non-viable seeds. The classification value that resulted in minimum error was selected for use in the model.

Prediction model for germination quality using blue LED reflectance spectra

The Rc^2 and SEC for the PLS-DA model developed using reflectance spectra for blue LED illumination were 0.8918 and 0.329, respectively. Cross-validation showed that R_V^2 and SEP for this model were 0.8907 and 0.3312, respectively. Figure 5 shows the X-variance and Y-variance explained by the calibration validation models by number of factors. The optimal number of factors for the discrimination model was four. Using a CV of 1.04, the model had a discrimination accuracy of 96.5% for viable seeds and 97.5% for non-viable seeds (Figure 6(a)).

Prediction model for germination quality using green LED reflectance spectra

The R_c^2 was 0.9118 and the SEC was 0.2911 for the PLS-DA model developed using reflectance spectra for green LED illumination. Cross-validation resulted in an R_V^2 of 0.9111 and SEP of 0.2986. The optimal number

Table 1. Comparison of PLS-DA Models and classifications of cucumber seed viability based on reflectance spectra from blue, green, red, and RGB LED illumination

Light source	Wavelength range(nm)	Calibration		Validation		F	Prediction		
							CCR		CV
		Rc^2	SEC	Rv^2	SEP		VS	NVS	
Blue LED	400-500	0.8918	0.329	0.8907	0.3312	4	96.5%	97.5%	1.04
Green LED	500-600	0.9118	0.2971	0.9111	0.2986	3	96.0%	96.5%	1.06
Red LED	600-700	0.9180	0.2865	0.9165	0.2895	6	98.5%	98.5%	1.18
RGB LED	400-700	0.9106	0.2993	0.9098	0.3008	3	96.0%	97.0%	1.02

Abbreviations: F, number of factors; CCR, correct classification rate; VS, viable seed; NVS, non-viable seed; CV, classification value; RGB LED, mixed blue, green, and red LEDs.

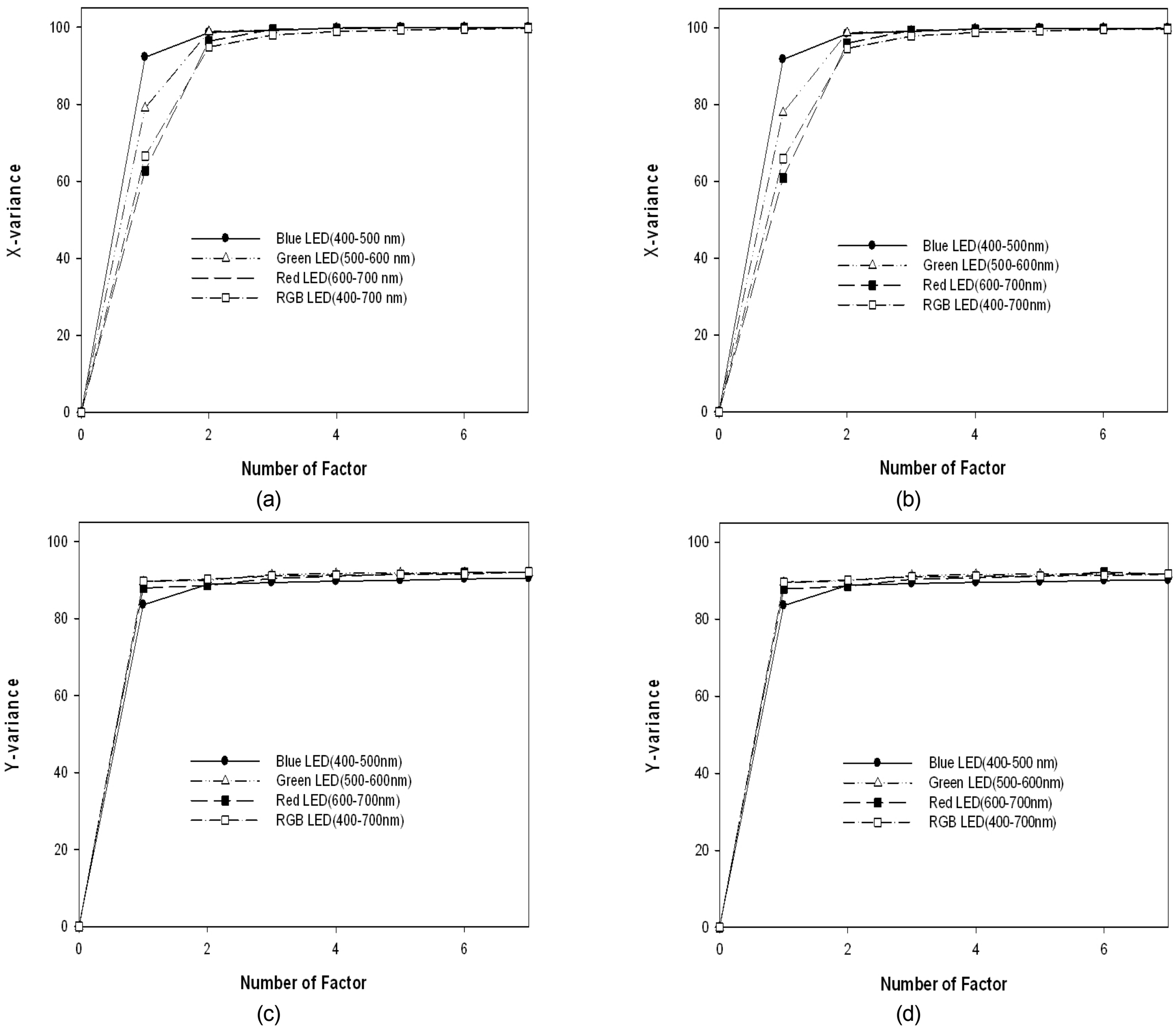


Figure 5. X-variance explained by (a) the calibration model and (b) the validation model and Y-variance explained by (c) the calibration model and (d) the validation model by number of factors.

of factors for the model was three (Figure 5). Figure 6(b) shows the results of prediction using a classification value of 1.06: the discrimination accuracy for viable seeds and non-viable seeds was 96% and 96.5%, respectively.

Prediction model for germination quality using red LED reflectance spectra

For the model developed using reflectance spectra for red LED illumination, the R_c^2 and SEC were 0.918 and 0.2865, respectively. R_V^2 and SEP of the cross-validation model were 0.9165 and 0.2895, respectively. The percentage of the X-variance and Y-variance explained by the models increased with the number factor more slowly than for models constructed using other LED illuminations. The optimal number of factors was six (Figure 5). The CV of this model was 1.18, and the discrimination accuracy of both viable seeds and non-viable seeds was 98.5 (Figure. 6(c)).

Prediction model for germination quality using RGB LED reflectance spectra

The PLS-DA model developed using reflectance spectra for RGB LED illumination had an optimal number of three factors. The calibration model had an R_c^2 0.9106 and an SEC of 0.2993. The R_V^2 and SEP of the validation model were 0.9098 and 0.3008, respectively. Figure 6(d) shows the predictions of this model using a CV of 1.04. The discrimination accuracy of the model for viable and non-viable seeds was 96.0% and 97.0%, respectively.

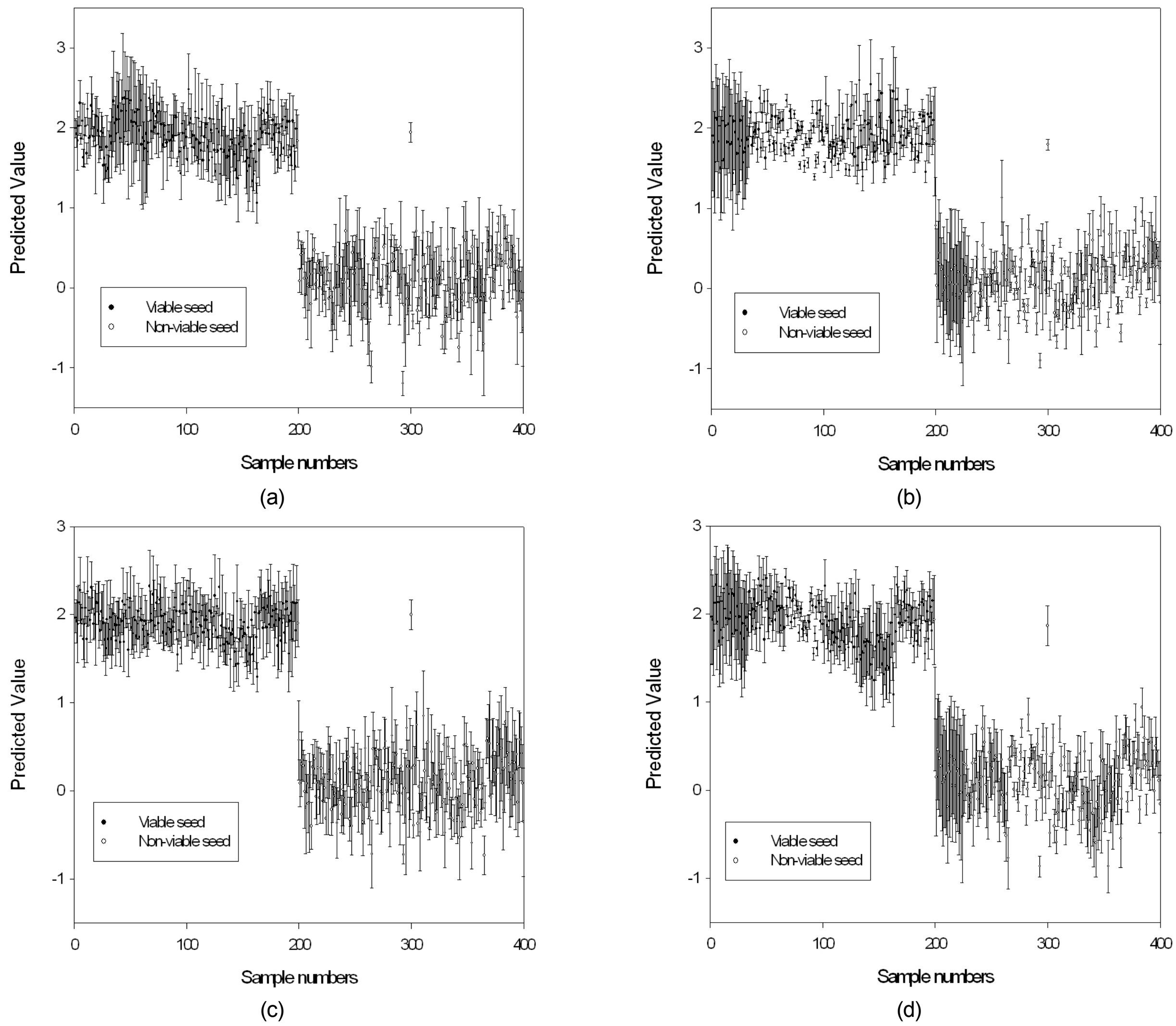
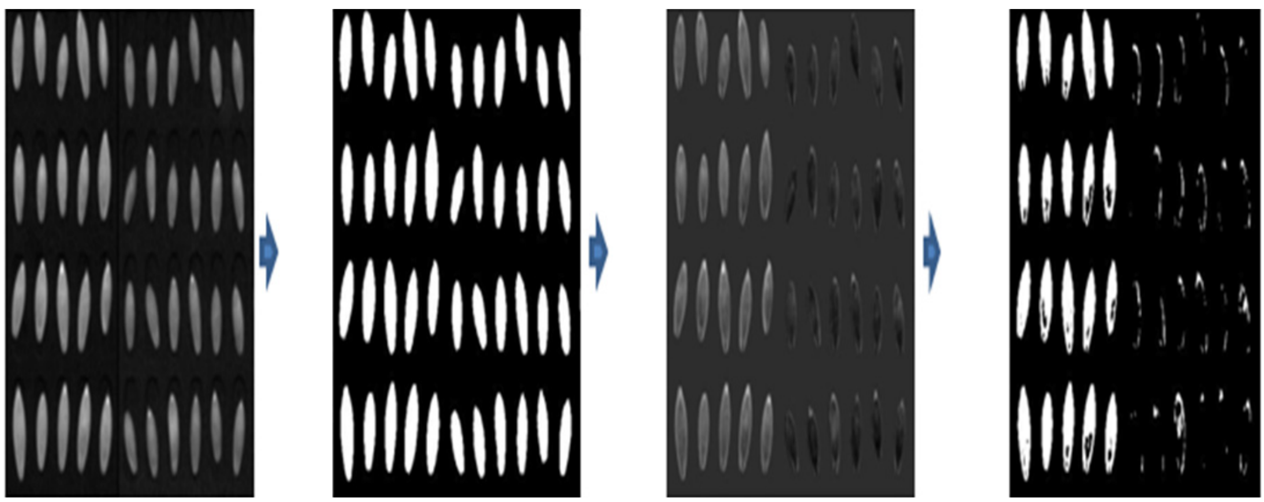


Figure 6. PLS-DA prediction results for discrimination between viable and non-viable cucumber seeds using reflectance spectra for (a) blue LED (400~500 nm), (b) green LED (500~600 nm), (c) red LED (600~700 nm), (d) RGB LED (400~700 nm).



(a) 407 nm image (b) masking image (c) PLS-Image (d) PLS-binary Image

Figure 7. PLS Imaging processing for classification of viable and non-viable cucumber seeds.

Among the developed models, the PLS-DA model using red LED illumination reflectance spectra showed the highest discrimination accuracy since the model includes information about chlorophyll (Jalink et al., 1998).

Determining germination quality using hyperspectral reflectance images

Hyperspectral data processing was performed to determine germination quality using the hyperspectral image data of RGB LED illumination, which showed the highest discrimination accuracy. As shown in Figure 7, images 407 nm reflectance from seeds were converted into binary images; values greater than the threshold value were converted to '1' and those less than the threshold value were converted to '0'. The backgrounds were then erased, and a masking image was made of only the seeds. PLS images were created by applying regression coefficients from the PLSR model to the hyperspectral images and then applying the masking image. PLS images were converted into binary images using the CV of the PLS-DA for red LED reflectance spectra in Table 1 (1.18), as the threshold value of PLS image. Binary PLS images represented the vigorous parts of seeds.

A total of 212,272 spectra from 400 seed images were used to verify the PLS-DA model for red LED illumination spectra (Figure 8). Figure 7(d) shows binary PLS images of seeds, and shows the vigorous part of each seed (image value '1'). There were 356-773 pixel spectra per seed. If more than 43% of the pixels for a given seed had an image value of 1, the seed was considered to be viable. If less than 43% of the pixels had an image value of 1, the seed was considered to be non-viable. The discrimination accuracy for 198 viable seeds and 202 non-viable seeds was 100% and 99.0%, respectively (Table 2). Discriminating viable seeds using the ratio of vigorous pixels was more accurate than discriminating averaged spectrum of seed using PLS-DA models. The hyperspectral imaging technique is more appropriate for determination of seed viability than conventional spectroscopy using averaged spectrum.

Table 2. Discrimination between viable and non-viable cucumber seeds using PLS-images and a PLS-DA model

	Number of seeds	Prediction		
		Viable seeds	Non-viable seeds	Accuracy
Viable seeds	198	198	0	100%
Non-viable seeds	202	2	200	99.0%

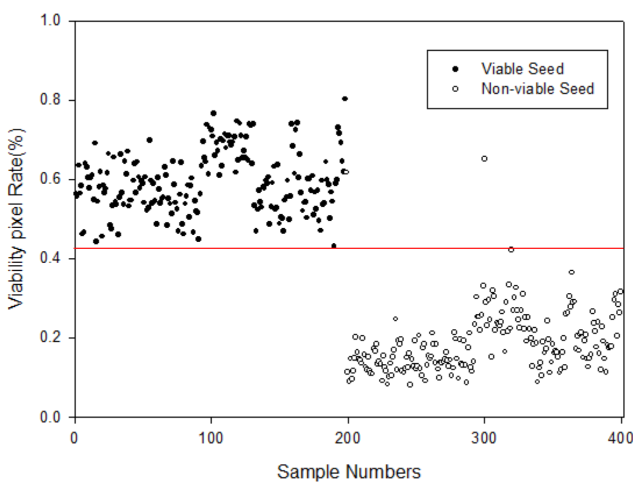


Figure 8. PLS image prediction results for discrimination between viable and non-viable cucumber seeds using spectral images of seeds illuminated by an RGB LED (400~700 nm) to which a PLS-DA was applied.

Conclusions

The purpose of this study was to develop a new technique to discriminate between viable and non-viable cucumber seeds. A PLS-DA model was developed using hyperspectral image of cucumber seeds induced by LED illumination. In addition, an algorithm for hyperspectral image processing was developed using optimal PLS-DA model.

The PLS-DA model developed using hyperspectral reflectance image spectrum of red LED illumination (wavelength region of 600-700 nm) showed 98.5% discrimination accuracy for both viable and non-viable seeds. Of the LEDs tested, illumination with a red LED resulted in the best discrimination between viable and non-viable seeds. Hyperspectral reflectance images were successfully used identify aged, non-viable seeds.

Processing hyperspectral images using the PLS-DA model resulted in discrimination accuracies of 100% and 99% for viable seeds and non-viable seeds, respectively. Hyperspectral reflectance imaging has good potential for identifying superior seeds in massive quantities of seeds.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgement

This study was carried out with the support of "Research Program for Agricultural Science & Technology Development (Project No. PJ009399)", National Academy of Agricultural Science, Rural Development Administration, Republic of Korea.

References

Alexandrakis, D., G. Downey and A. G. M. Scannell. 2008. Detection and identification of bacteriain an isolated system with near-infrared spectroscopy and multivariate analysis. Journal of Agricultural and Food Chemistry 56:3431-3437.

Cho, B. K., I. S. Beak, N. G. Lee, C. H. MO. 2011. Study on bruise detection of 'Fuji' apple using hyperspectral reflectance imagery. Journal of Biosystems Engineering. 36: 484-490.

ISTA. 2008. International rules for seed testing. edition 2008. International Seed Testing Association.

Jalink, H., R. V. Scoor, A. Frandas, J. G. Pijlen and J. B. Raoul. 1998. Chlorophyll fluorescence of Brassica oleracea seeds as a non-destructive marker for seed maturity and seed performance. Journal of Seed Science Research 8:437-443.

Kim, M. S., Y. R. Chen and P. M. Mehl. 2001. Hyperspectral reflectance and fluorescence imaging system for food quality and safety. Transactions of the American Society of Agricultural Engineers 44(3):721-729.

Lawrence, K. C., B. Park, W. R. Windham and G. W. Heitschmidt. 2005. LED lighting for use in multispectral and hyperspectral imaging. ASAE Paper No. 053073. ASABE Annual International Meeting.

Lee, K. J., S. W. Kang, S. R. Delwiche, M. S. Kim and S. H. Noh. 2008. Correlation analysis of hyperspectral imagery for multispectral wavelength selection for detection of defects on apples. Sensing and Instrumentation for Food Quality and Safety 2:90-96.

Merzlyak, M. N., A. E. Solovchenko and A. A. Gitelson. 2003. Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. Postharvest Biology and Technology 27:197-211.

Min, T. G. and W. S. Kang. 2003. Nondestructive separation of viable and non-viable gourd (Lagenaria siceraria) seeds using single seed near infrared reflectance spectroscopy. Korean Society for Horticultural Science 44:545-548.

Reitzenstein, S., P. Rösch, M. A. Strehle, D. Berg, M. Baranska, H. Schulz, E. Rudloff and J. Popp. 2007. Nondestructive analysis of single rapeseeds by means of Raman spectroscopy. Journal of Raman Spectroscopy 38(3):301-308.

Qin, J., K. Chao, M. S. Kim,, S. Kang and W. Jun. 2011. Detection of organic residues on poultry processing equipment surfaces by LED-induced fluorescence imaging. Applied Engineering in Agriculture. 27(1):153-161.

Shetty, N., T. G. Min, M. H. Olesen and B. Boelt. 2011. Optimal sample size for predicting viability of cabbage and radish seeds based on near infrared spectra of single seeds. Journal of Near Infrared Spectroscopy 19:451-461.

Shin, K., H. Chung and C. W. Kwak. 2012. Transmission Raman measurement directly through packed corn kernels to improve sample representation and accuracy of compositional analysis. Analyst 137:3690-3696.

Silva, C. E., P. Vandenabeele, H. G. Edwards and L. F. de Oliveira. 2008. NIR-FT-Raman spectroscopic analytical characterization of the fruits, seeds, and phytotherapeutic oils from rosehips. Analytical and Bioanalytical Chemistry 392(7-8):1489-1496.