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Nondestructive Classification between Normal and Artificially Aged Corn (*Zea mays* L.) Seeds Using Near Infrared Spectroscopy

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ABSTRACT Near infrared (NIR) spectroscopy was used to classify normal and artificially aged nonviable corn (*Zea mays* L., cv. 'Suwon19') seeds. The spectra at 1100-2500 nm were scanned with normal and artificially aged single seeds and analyzed by principle component analysis (PCA). To discriminate normal seeds from artificially aged seeds, a calibration modeling set was developed with a discriminant partial least square 2 (PLS 2) method. The calibration model derived from PLS 2 resulted in 100% classification accuracy of normal and artificially aged (aged) seeds from the raw, the 1st and 2nd derivative spectra. The prediction accuracy of the unknown normal seeds was 88, 100 and 97% from the raw, the 1st and 2nd derivative spectra, and that of the unknown aged seeds was 100% from all the raw, the 1st and 2nd derivative spectra, respectively. The results showed a possibility to separate corn seeds into viable and non-viable using NIR spectroscopy.

Keywords : corn seed, nondestructive classification, near infrared spectroscopy (NIR), calibration, prediction

It would be very important for crop seeds to be maintained good quality for producing healthy and vigorous seedlings. However, all kind of seeds are inevitably deteriorated under unfavorable conditions or during storage. Therefore many studies for detecting viable and nonviable seeds have been performed. Many physiological and biochemical changes which take place during seed deterioration are or have been used to assess seed quality (McDonald 1999).

Previous works in germinability test have usually focused on the biochemical test such as tetrazolium (TZ) or conductivity tests (McDonald 1998). However, these tests have some difficulties, require experience to interpret, need to be bulked and destructive for testing. Also, nonviable seeds of *Cruciferae* (cabbage, broccoli, cauliflower, Chinese cabbage, and radish) could be separated by detecting the leakage of sinapine (Taylor *et al*., 1993; Lee *et al*., 1997) or amino acid (Min 2000; Min 2001). However, the methods need pretreatments and take time; soaking seeds in water and coating with cellulose and separating under UV light etc. Also sinapine was found only in the seeds of *Cruciferae* family.

Today NIR spectroscopy is widely used as a quantitative and qualitative analytical technique to measure moisture, fat, and protein contents of seeds in many crops (Abe *et al*., 1995; Dyer and Feng, 1995; Norris and Hart, 1996). The technique is a nondestructive, fast, and cheap way compared to other analytical techniques, and often need no or minimal sample preparation. Also, NIR spectroscopy is applied to detect rice weevils in wheat kernels (Backer *et al*., 1999) and to classify viable and nonviable seeds of Mexican weeping (*Pinus patula*) (Tigabu and Oden, 2003), Scot pine (*Pinus sylvestris*) (Lestander and Oden, 2002), and gourd (*Lagenaria siceraria*) (Min and Kang, 2003a,b). But these NIR techniques have been applied for classifying the visible indications of physical differences or microorganism contamination between viable and nonviable seeds.

This study was performed to observe the possibility of classifying non-visible differences between the normal and artificial aged corn seeds by NIR spectroscopy on a nondestructive way and without any sample pretreatments.

MATERIALS AND METHODS

Seeds of corn cultivar 'Suwon 19' were used in the present study. The seeds of 'Suwon 19' were obtained from the Gyeongsangbuk-do Agricultural Research and Extension Services in Korea. The germination rate of normal seeds was 98%, while that of artificially aged (aged) seeds was 0%.

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To make nonviable seeds, the normal seeds were aged at 95% relative humidity in a 45±0.1℃ incubator for 7 days. Before incubation, the intact seeds were sprayed by 2% sodium propionate and the seeds divided two groups which one belong to normal and the other subjected to aged treatment. The relative humidity was maintained in a sealed plastic box containing a glycerol-water solution with specific gravity of 1.03 according to the method of Forney & Bandl (1992). The aged seeds and normal seeds were dried at 20℃ in an air circulating incubator until the original weight.

The spectra of 200 normal and 200 aged seeds were analyzed on the single seed bases by NIR spectroscopy (Foss NIRSystem 5000 spectrometer, USA) for calibration set. NIR reflectance spectra, expressed in the form of log (1/R), were collected from the single seeds. Individual seed was placed in a seed holder that allowed the radiation reflected from one side of the entire plat surface of a corn seed (Fig. 1). The seed holder was made by 3 mm thick black alumina plate which had an oval hole in the center of the plate for corn seed fitted in the NIR sample mount. Each observation was the mean value of 32 successive scans with 2 nm increments between 1100-2500 nm. The spectra collected separately from normal and aged seed groups were used to make a calibration and prediction model.

A principle component analysis (PCA) was performed to get an overview of the data as a basis for outlier detection. The NIR spectra were then analyzed for calibration and predicted by a discriminant partial least squares 2 (PLS 2) method using the software package WinISI III (version 1.60, Infrasoft International, USA). The prediction efficiency of the fitted model was evaluated using 100 normal and 100 aged seeds leftover.

The spectra from the normal and aged seeds are shown in Fig. 2. The spectra reflected from all normal and aged seeds are shown in Fig. 2-A, the average spectra of normal and aged seeds in Fig. 2-B, and the 1^{st} and 2^{nd} derivatives of the average spectra of normal and aged seeds in Fig. 2-C and 2-D, respectively.

The principle of the discriminant PLS 2 model was to set the digitized spectra data as two classes; 1 for normal and 2 for aged seeds. If the 2 corresponds to the sample being in the aged seeds group, the 1 to the sample not being in the group (Martens and Naes, 1989; Woo *et al*., 1999). The overall concept of the PLS 2 is that a predicted value of 2.0 is regarded as a perfect match of aged seed (hit), while 1.0 is not in the group (miss) and 1.5 is uncertain, which means that it can go either way. This data analysis was performed at the confidence level of 95% in t-test.

RESULTS AND DISCUSSION

There were no specific different band patterns in the NIR raw spectra between normal and aged seeds except band position, but some differences showed in 1^{st} and 2^{nd} derivatives in peak positions between normal and aged seeds, that might be from the differences of hundreds of physical and chemical components in seeds involved on the spectra bands (Fig. 2). Therefore, the multivariate classification models were developed from the spectra reflected from normal and aged seeds in a NIR by use of the PCA and PLS 2 programs (Martens and Naes, 1989). There was clear-cut separation in the scores of individual seeds between normal and aged seeds in the three-dimensional PCA space as shown in Fig. 3. It means that the normal and aged seed groups had quite different characteristics although the patterns of NIR spectra were quite similar.

In order to find the optimal PLS 2 model, the 1^{st} and 2^{nd} derivative algorithms were utilized. Table 1 shows the calibration results for each model. According to the results, the spectra of normal and aged corn seeds were perfectly classified in the raw, 1^{st}, and 2^{nd} derivative modeling sets. There were no miss and uncertain seeds out of 200 of the normal and aged seeds both in the raw, 1^{st} and 2^{nd} derivative spectra respectively. Therefore raw, 1^{st} and 2^{nd} derivative in the normal and aged seeds all were considered the best calibration model because they had no error. These means there were

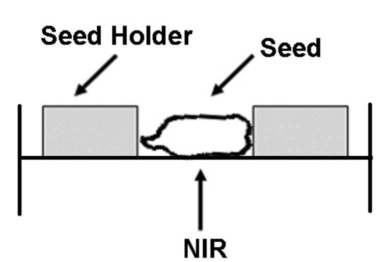

Fig. 1. A schematic diagram to measure NIR spectra of single corn seed. The seed holder is modified for fitting corn seed.

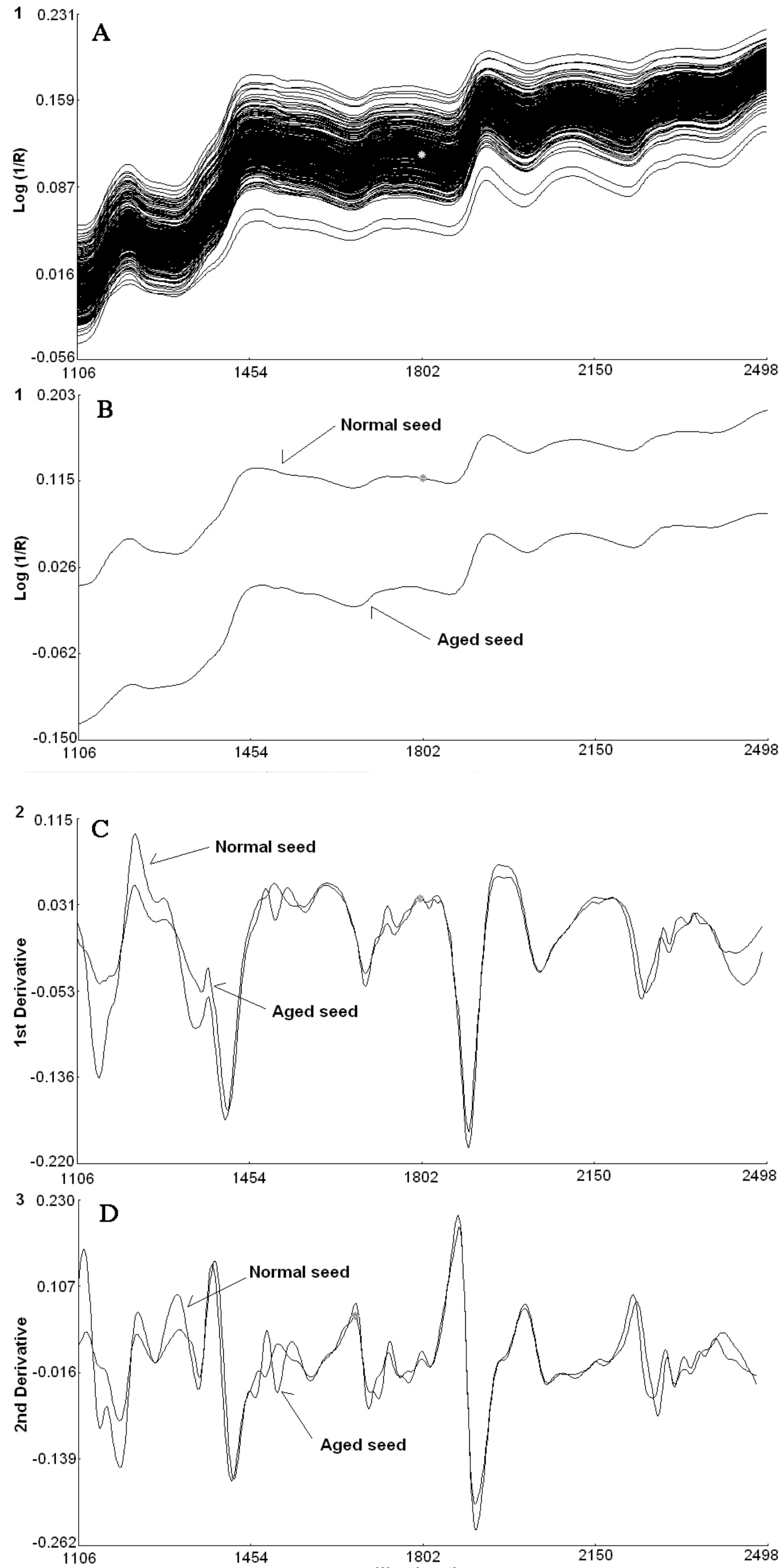

Fig. 2. NIR spectra of corn seeds (A: original, B: mean spectra of the original, C: the 1st derivative of the mean spectra, D: the 2nd derivative of the mean spectra). The patterns of the spectra from the 1st and 2nd derivatives were seen some differences in each peaks between normal and aged seeds, which might be from various physical or chemical differences.

100% of calibration accuracy from raw, 1st and 2nd derivative spectra in the normal and aged seeds respectively. The prediction results of the unknown samples of the corn seeds, which were not used for the calibration set, are shown in Table 2. There were uncertain of 12 and 3 seeds out of 100 of the normal seeds in the raw and 2nd derivative spectra respectively. All the 12 and 3 of uncertain seeds of raw and 2nd derivative spectra belong to the miss sides. Therefore the prediction accuracy of the normal seeds was 88, 100 and 97% in the raw, 1st and 2nd derivative spectra respectively, that means the calibration model from 1st derivative in the normal seeds was the best because they had no miss and 100% of prediction accuracy. In the prediction of aged seeds, there were no miss and uncertain seeds out of 100 in all spectra of the raw, 1st and 2nd derivatives. Therefore the prediction accuracy of the aged seeds was 100% from the raw, 1st and 2nd derivative spectra all, that means the aged seeds could be perfectly classified using the raw, 1st and 2nd calibration spectra.

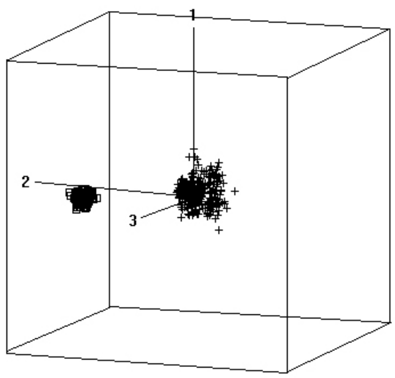

Fig. 3. Principle component score plot for corn seeds (+: normal seeds, □: aged seeds). The classification was very clear between the two groups of the normal and aged seeds, indicating that the spectra between the two groups had quite different factors.

The derivatives of spectra have known two beneficial effects: 1) the improvement of resolution of overlapping peaks and removal of linear baselines and 2) more effective in modeling and testing than the original spectra (Burns and Ciurczak, 1992). However the raw spectra have sometimes good prediction as shown in the aged seed prediction as

Table 1. Hit, miss and uncertain of normal and artificially aged corn seeds in the calibration set classified by PLS 2 models from raw, the 1st and 2nd derivative data sets.

Spectrum	Total seed	Hit	Miss	Uncertain	Calibration accuracy (%)
Normal seed	200				
Raw		200	0	0	100
1st derivative		200	0	0	100
2nd derivative		200	0	0	100
Artificially aged seed	200				
Raw		200	0	0	100
1st derivative		200	0	0	100
2nd derivative		200	0	0	100

Table 2. Hit, miss and uncertain of normal and artificially aged corn seeds in the prediction sets classified by PLS 2 models from raw, the 1st and 2nd derivative data sets.

Spectrum	Total seed	Hit	Miss	Uncertain	Prediction accuracy (%)
Normal seed	100				
Raw		88	0	12	88
1st derivative		100	0	0	100
2nd derivative		97	0	3	97
Artificially aged seed	100				
Raw		100	0	0	100
1st derivative		100	0	0	100
2nd derivative		100	0	0	100

Table 2.

In this experiment, there were no differences in seed appearance and the original NIR spectra patterns from the seeds between normal and aged seeds. Also, the normal seeds were treated by same disinfectant and moisture content as aged seeds, not for being influenced by the chemical or physical differences, but for being influenced only by the differences of physiological viability between the normal and aged seeds. Basavarajappa *et al*. (1991) reported that corn seeds had changed not only the level of phospholipids, ascorbate and fatty acid, but also the content of carbohydrate, reducing sugar, protein and amino acid during accelerated aging. In this study, the NIR spectra could be responded from the chemical differences, as the report, between normal and aged seeds. Regarding the seed aging and changing of physical and chemical properties of seeds, there are many reports. When seeds are deteriorated, usually they are showing the accumulation toxic products of anaerobic respiration such as alcohol and acetaldehyde, increase in the concentrations of reducing sugars, amino acids, and free fatty acids, loss of membrane integrity, and cytogenetical changes (Basra, 1995; Chang and Sung, 1998). Also Seo and Lee (2004) reported that leakage of total sugar and electrolytes and amylase activity were quite different between normal and artificially aged super sweet corn seeds. The NIR spectra was also used to distinguish between the filled and empty seeds of *Pinus patula* Shiede & Deppe (Tigabu and Oden, 2003) and between sound and insect-infested seeds of *Cordia africana* (Tigabu and Oden, 2002), and microorganism contaminated or not on the cotyledon in gourd seeds (Min and Kang, 2003a,b). But in all these cases, the NIR was used for recognizing the physical differences of seeds, not used for recognizing the physiological differences, such as seed viability.

NIR spectra were very effective to classify normal and aged seeds, however we do not know what a certain spectrum corresponding to what a difference of seeds, as many physical and chemical properties of seeds may affect on the NIR spectra. Also, there are diverse aspects of seed deterioration phenomena depending on the diverse seeds. Thus, on the characterization of seed viability and NIR spectra, further study should be needed in the future. Furthermore, we need to verify the method using the natural aged seeds for actual application of the method in the corn study.

In conclusion, an attempt to classify the normal and aged corn seeds on a single seed base using NIR spectroscopy was successful with very high accuracy in this experiment. The use of NIR spectroscopy for detecting seed viability is a very attractive technique, because it is rapid, can be easily automated, and required no pretreatments.

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