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Considerations Regarding the Use of Hyperspectral Imaging Data in Classifications of Food Products, Exemplified by Analysis of Maize Kernels

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Development of robust analytical procedures is critical when using hyperspectral imaging technology in food technology and agriculture. This study used near-isogenic inbred corn lines to address two basic questions: (1) To what extent is classification accuracy increased by grinding maize kernels? (2) Can the classification accuracy of two near-isogenic inbred lines be increased by using a spectral filter to classify only certain hyperspectral profiles from each image cube? Whole kernels and ground kernels in two particle intervals, 0.250−0.354 mm (size 1) and 0.354−0.841 mm (size 2), were examined. Spectral profiles acquired from ground kernels had higher spectral repeatability than data collected from whole kernels. The classification error of discriminant functions from whole kernels was >3 times lower than that of size 1 ground particles. Applying a spectral filter to input data had negligible effect on classifications of hyperspectral profiles from whole kernels and size 2 ground particles, but for size 1 ground particles a considerable increase in accuracy was observed. Independent validation confirmed that distinction between wild type and mutant inbred maize lines could be conducted with >80% accuracy after the proposed spectral filter had been applied to hyperspectral profiles of size 1 ground particles. A combination of discriminant analysis and regression analysis could be used to accurately predict mixture ratios of the two inbred lines. The use of spectral filtering to increase the level of spectral repeatability and the use of hyperspectral imaging technology in large-scale commercial operations are discussed.

KEYWORDS: Discriminant analysis; spectral repeatability; spectral filtering; particle size; corn analysis

INTRODUCTION

With growing interest in applications of ground-based remote sensing in food technologies and agriculture, it is important to both highlight the limitations of spectral-based analyses and develop robust analytical methodologies. There are several important challenges associated with spectral-based analyses of food/agricultural products. For instance, to what extent is it possible to reduce shape effects (variation in projection angle) in the classification of reflectance profiles acquired from target objects, with distinct three-dimensional structures, such as folded crop leaves or whole cereal grains [i.e., hard red wheat types (*1*) or cereal grain breeder selection of seeds on the basis of protein levels (*2*)]? Second, it is important to develop analytical methodology that not only shows high performance in a research environment but also can be implemented on larger commercial scales. That is, near-infrared (NIR) technology has been used widely and with high accuracy in studies of individual grain kernels (*3*−*7*). There are several advantages of conducting analyses on a single-kernel level as kernels can be placed in an integrated sphere or a specially designed box (*8*) to homogenize the distribution of light and therefore eliminate distortion of spectral profiles due to projection angles. Delwiche (*9*) placed each kernel crease-side down to reduce the shape effect of target objects. Placing single kernels in a consistent way before spectral data are acquired will obviously reduce possible shape effects, but it also means much higher handling/processing time and therefore restricts the use of this approach on a larger commercial scale.

Throughout this paper, the term "spectral repeatability" is used to describe the level of noise or spectral variance caused by the three-dimensional structure of target objects. As pointed out by Peleg et al. (*10*), low spatial and spectral repeatability is a common problem in airborne remote sensing, but it may also cause problems/challenges in ground-based remote sensing. From **Figure 1** it is seen that when using a spatial resolution

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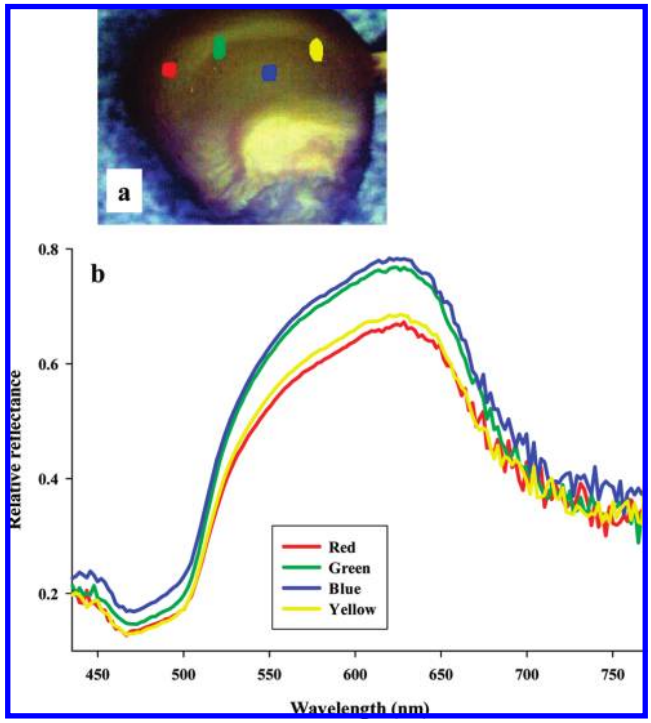

Figure 1. Example of how hyperspectral profiles collected from a single maize kernel are dependent upon projection angles. Each colored dot on the maize kernel (**a**) represents about 300 pixels, and the respective colored profiles are shown (**b**).

that allows acquisition of many pixels from a single kernel, the shape of hyperspectral profiles is highly dependent on where on the kernel the profile was acquired from. In **Figure 1a**, about 112,000 pixels covered the entire maize kernel, and each color dot represents about 300 pixels. The highest variation among average spectral profiles (**Figure 1b**) was seen at 456 nm, in which the highest difference [(maximum − minimum)/(average)] among the four hyperspectral profiles was 31%. Some of that variation is associated with difference in chemical composition within a single kernel, but most of the variability is attributed to the three-dimensional shape of kernels, which determines the projection angle, and it causes a low level of spectral repeatability. In many studies (i.e., refs *3, 4, 7, 9, and 11*), authors average multiple scans of the same object (typically 16−32 scans) to increase the spectral repeatability of input data. Although this method certainly increases the spectral repeatability, a disadvantage appears to be that each object has to be scanned multiple times, which may be a considerable constraint for large-scale operations. Additionally, averaging of pixels makes it more challenging to distinguish between two or more classes if they are mixed in the same sample. For instance, in the classification of hard red winter and hard red spring wheat classes (*1*), classifications based on the average of 16 scans do not provide the end-user with information about the possibility of samples being mixed and, if so, in what ratio. This aspect is not only important for classifications on a nominal scale but is also relevant when, for instance, moisture and/or protein contents are estimated on a large scale.

An alternative to averaging multiple scans of the same object would be to apply a user-defined spectral filter before input data are acquired and to classify each hyperspectral profile separately. That is, hyperspectral imaging can be used to acquire hundreds of spectral profiles from the surface of each target object, but instead of including hyperspectral profiles from all pixels, one could use reflectance value intervals in certain bands as cut levels and only include hyperspectral profiles within predetermined intervals. Applying such a spectral filter would (1) likely increase the spectral repeatability of the input data, (2) not require any averaging, and (3) retain important spatial information (where within the same the given hyperspectral profile was collected) associated with each hyperspectral profile.

This analysis is based on analysis of maize kernels from inbred wild type lines and from near-isogenic (NIL) mutant lines (*12, 13*). We used this maize material to address two basic questions: (1) To what extent is classification accuracy increased by grinding maize kernels? (2) Can the classification accuracy of two near-isogenic inbred lines be increased by using a spectral filter to classify only certain hyperspectral profiles from each image cube? The reasoning behind using inbred maize lines for testing the proposed analysis was that (1) the round shape of maize kernels allows for acquisition of hyperspectral profiles at many projection angles, (2) spectral analysis is widely used in research on the quality of cereals, and (3) paired neo-isogenic inbred lines (wild type versus mutant) comprise subtle differences and would therefore be a challenging model system for testing an improved classification methodology. The analytical approach, based on discriminant analysis, is relevant to most food quality control procedures based on spectral analysis, including analyses of fruits and vegetables (*14*−*16*) and meat quality (*17*), in which dichotomous (i.e., reject vs accept) classification is used.

MATERIALS AND METHODS

Target Objects. Maize kernels used in this study were obtained from a well-established, true inbred line, FR2128B and its near-isogenic mutant line *lox3-4*. Maize plants of both inbred lines were cultivated under field conditions and backcrossed to the inbred line FR2128B for 4−5 generations (*12, 13*). "Wild type" refers to the original FR2128B line, and "mutant" refers to a line in which a 9-lipoxygenase gene was disrupted by an insertion of a *Mutator* transposable element in the coding sequence of the *ZmLOX3* gene (*12*). Genetically, the homozygous mutant individuals are near-isogenic to the recurrent parent, FR2128B line (they share 97.5% of the genome) and are suitable for assessing the effect of the LOX gene knockouts on the plant physiology. No visible defects in weight, size, or other morphological traits of the mutant kernels were detected compared to the wild types (*13*). Kernels of both inbred lines were used either as whole kernels or ground with a cereal grinder (Back to Basics, West Bend, WI) and subsequently sieved into two particle size ranges: 0.250−0.354 mm (size 1) and 0.354−0.841 mm (size 2). Particle mean sizes within each range were unknown. For initial classification, 10 images were collected from each combination of inbred line and level of processing (whole kernels and sizes 1 and 2). In addition, five images were collected from each combination of inbred line and level of processing for validation of classification procedures. Whole maize kernels and/or processed kernels consisted of 5 g samples placed in 5 cm diameter plastic Petri dishes.

Spectral Image Collection. We used a hyperspectral spectral camera (PIKA II, Resonon Inc., Bozeman, MT), which collects 160 bands in the range from 435 to 769 nm. The objective lens had a 35 mm focal length (maximum aperture of F1.4) and was optimized for the visible and NIR spectra. The main specifications of the spectral camera are as follows: interface, Firewire (IEEE 1394b); output, digital (12 bit); 160 bands (spectral) by 640 pixels (spatial); angular field of view, 7 degrees; and spectral resolution, <3 nm. Spectral image cubes were collected under controlled light conditions with a ring light mounted 25 cm above target objects, whereas the hyperspectral camera was mounted 45 cm above target objects. Ambient climate conditions were between 19−22 °C and between 30−40% relative humidity. A piece of white Teflon was used for white calibration, and "relative reflectance" refers to proportional reflectance compared to that obtained from Teflon. Consequently, relative reflectance values ranged from 0 to 1. Each hyperspectral image cube consisted of 250 frames (160,000 pixels) and the spectral with a pixel size of about 0.12 mm^2.

Data Analysis. ENVI 4.0 for Windows (Research Systems Inc., Boulder, CO) was used to collect a random sample of 500 pixels from each hyperspectral image cube, which was exported into PC-SAS 9.0 (SAS Institute, Cary, NC) for statistical analysis. For each maize material, the complete input data file consisted of 10000 hyperspectral profiles (5000 from mutant type and 5000 from wild type). The first part of the analysis concerned generation of a spectral filter with upper and lower reflectance cut levels in the single spectral band that contributed the most to the separation of wild type and mutant. The input file for each maize material was analyzed in a stepwise discriminant analysis (PROC STEPDISC in SAS) to determine the spectral band that contributed the most to the classification of inbred lines. Subsequently, PROC DISCRIM in SAS with the cross-validation option was used to determine the classification error rate (percentage of misclassified hyperspectral profiles). As part of the accuracy assessment, we used the spectral band that contributed the most to each discriminant function and generated frequency distribution curves of correctly or incorrectly classified hyperspectral profiles. In other words, it was determined whether relative reflectance values in a single spectral band could be used as a spectral filter to reduce the error rate. In the second part of the analysis, a new discriminant analysis was conducted on the basis of a reduced input file in which hyperspectral profiles with reflectance values outside a certain range in a single spectral band had been excluded.

Validation. Two kinds of validation were conducted for size 1 ground particles only, as the classification accuracy for whole kernels and size 2 ground particles was considered to be too low. First, 500 hyperspectral profiles were collected randomly from 10 image cubes of both wild type and mutant type maize. Discriminant functions generated on the basis of the reduced input data set of size 1 ground particles were validated with each of the 20 times 500 hyperspectral profiles. One-way ANOVA was used to compare mean classification accuracies for wild type and mutant inbred lines. In a second validation of hyperspectral profiles from size 1 ground particles, material of wild type and mutant type maize was mixed in known ratios (1:9, 1:4, 1:1, 4:1, and 9:1) and analyzed on the basis of the reduced input data set of size 1 ground particles. We collected 10 hyperspectral images for each of the five ratios, and random samples of 1000 hyperspectral profiles from each image were analyzed using the discriminant function generated on the basis of the reduced input data set of size 1 ground particles. The following regression model was used to analyze the relationship between actual (x) and predicted percentage [$F(x)$] in mixtures of size 1 ground materials:

$$F(x) = ax + bx^2 + c \quad (1)$$

a, b, and c are fitted coefficients.

RESULTS

Input Data. There was a clear difference in hyperspectral profiles from whole kernels compared to processed kernels, and sieving the ground material into two particle size categories also had considerable effect on average hyperspectral profiles (**Figure 2**). With very little genetic difference between inbred lines, it was not surprising that average spectral profiles from whole kernels and from ground kernels were very similar in pairwise comparisons of the two inbred lines. However, across the examined spectrum, relative reflectance values were consistently higher from wild types compared to mutants, and as much as 14% difference in average reflectance curves was observed from 470 to 490 nm in size 1 ground particles. For comparison, the highest differences in average reflectance curves from whole kernels and size 2 ground particles were 7 and 10%, respectively.

In the initial stepwise discrimination analyses of wild types and mutants (N = 10000), coefficients of determinations (R^2 values) varied considerably among maize materials (0.141, 225, and 0.529), and there was a positive relationship between size of objects and classification error rate (**Table 1**). It was noticeable that size 1 ground particles were classified with about

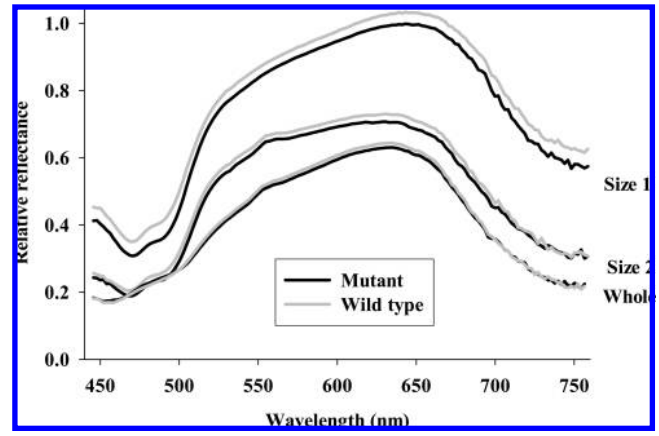

Figure 2. Average hyperspectral profiles (N = 5000) from wild type and mutant inbred lines of whole kernels and sizes 1 (0.250−0.354 mm) and 2 (0.841−0.354 mm) of ground maize.

Table 1. Discriminant Analyses of Whole and Ground Maize Kernels

	full analysis (N = 10000)			reduced analysis			
target object	R^2 value	bands	error (%)	N	R^2 value	bands	error (%)
whole kernels	0.141	52	34.5	4904	0.185	54	33.0
size 2 (0.841−0.354 mm)	0.225	90	28.9	5347	0.225	73	29.6
size 1 (0.250−0.354 mm)	0.529	112	14.6	3302	0.686	73	9.9

3.5 times higher accuracy than whole kernels (**Table 1**). In addition, there was a negative relationship between number of spectral bands that contributed significantly to discriminant functions and classification error rate.

Development of the Spectral Filter. The initial stepwise discrimination analyses showed that the spectral bands that contributed the most to each classification of wild type versus mutant were at 481 nm (whole kernels), 509 nm (size 2), and 505 nm (size 1). We generated frequency distributions curves of relative reflectance values in these spectral bands for pixels that were correctly and incorrectly classified (**Figure 3**). It was seen that the rounded three-dimensional structure of whole kernels meant that reflectance values at 481 nm varied considerably with >1000 pixels in each reflectance interval between 0.05 and 0.40. As seen from **Table 1**, the overall classification error rate was about 35%, and it varied between 30 and 36% for the different reflectance intervals (**Figure 3a**). In the classification of whole kernels, the highest number of correctly classified pixels was found in the reflectance interval between 0.20 and 0.30 in which 1458 of the pixels (67%) in that reflectance interval were correctly classified. Thus, a second discriminant analysis was conducted in which only hyperspectral profiles with relative reflectance values between 0.15 and 0.40 at 481 nm were included, which meant that about 49% of the original hyperspectral profiles were included (N = 4904). On the basis of the reduced input file, the overall error rate was reduced to 32%. The error rate of included hyperspectral profiles was only slightly lower than for those that were excluded, so the spectral filter had only negligible effect on the classification error rate.

In size 2 ground particles, hyperspectral profiles had relative reflectance values at 509 mm between 0.20 and 0.70, and about 80% of the 10000 hyperspectral profiles had relative reflectance values in between 0.30 and 0.50 (**Figure 3b**). Thus, grinding maize kernels considerably reduced the overall variance of

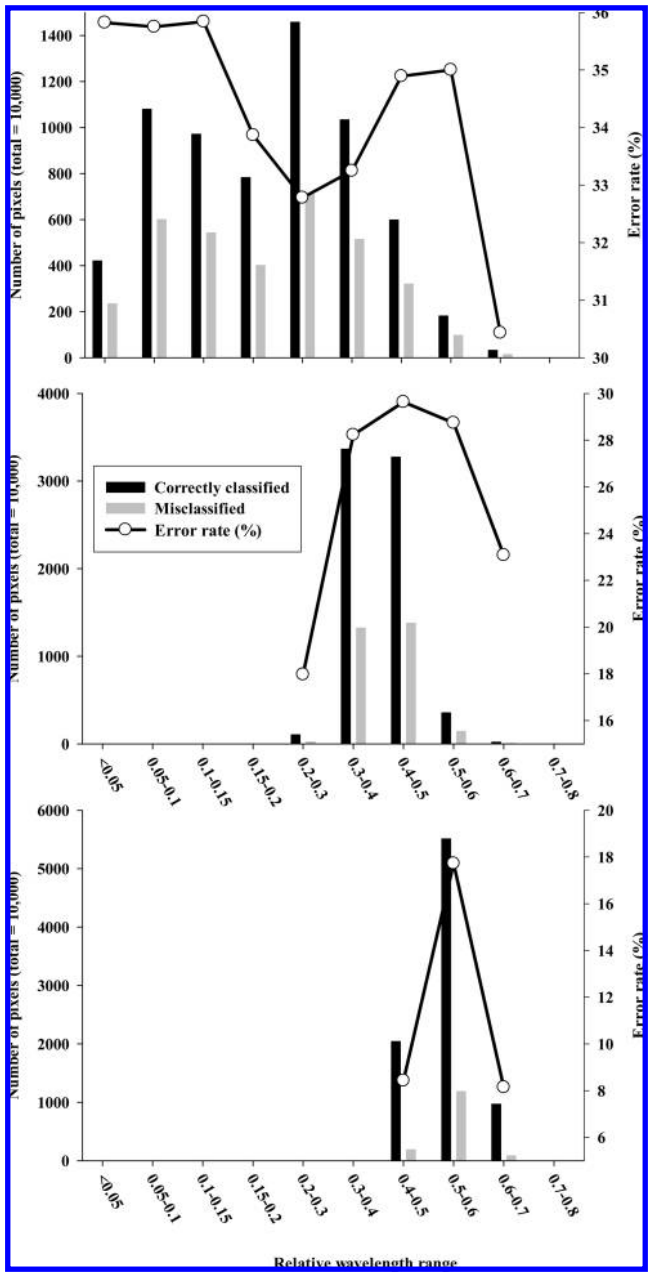

Figure 3. Reflectance values in a single band were examined for whole kernels (**a**, top) (481 nm) and size 2 (**b**, middle) (509 nm), and size 1 (**c**, bottom) (505 nm) ground maize. In each band, we examined the range of reflectance values, and bars denote the numbers of correctly (black) and incorrectly (gray) classified profiles. The classification error denotes the percentage of correctly classified profiles of the total number of profiles.

hyperspectral profiles, and the error rate within the different reflectance intervals was between 18 and 30%. Highest inaccuracy of the classification of wild type and mutants occurred among hyperspectral profiles with relative reflectance values at 509 mm between 0.40 and 0.50. Consequently, a second discriminant analysis was conducted in which these hyperspectral profiles were excluded ($N = 5347$). Using the reduced input file caused a slight increase in error rate from 29 to 30%, so the proposed filtering of input data did not increase the classification accuracy. On the basis of **Figure 3b**, one approach to increase the classification accuracy would be to exclude all hyperspectral profiles with relative reflectance values at 509 nm between 0.30 and 0.60, as the classification accuracy of hyperspectral profiles outside this range was very high (>76%).

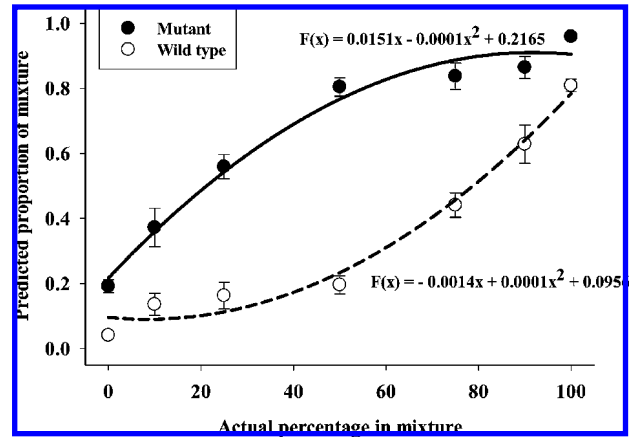

Figure 4. Size 1 (0.250−0.354 mm) ground maize from mutant and wild type was mixed in seven different ratios and used to validate the discriminant functions for both mutant and wild type. For each mixture ratio, we conducted 10 replications. Regression analysis of wild type (eq 1): adjusted R^2 value = 0.97, F value = 96.9, P value < 0.001, $a = -0.0014$, $b = 0.0001$, $c = 0.096$. Regression analysis of mutant (eq 1): adjusted R^2 value = 0.97, F value = 96.9, P value < 0.001, $a = -0.016$, $b = -0.0001$, $c = 0.221$.

However, only very few pixels (660 of 10000 pixels) were found within those relative reflection ranges, and it was considered to be inappropriate to exclude about 95% of hyperspectral profiles in the input data file.

In size 1 ground particles, all relative reflectance values at 505 nm fell within three reflectance intervals 0.40−0.70. The overall classification error rate of all 10000 pixels was 15% (**Table 1**), but it was about 10% higher for hyperspectral profiles with relative reflectance values at 505 nm between 0.50 and 0.60 than outside this reflectance interval (**Figure 3c**). Consequently, a second discriminant analysis was conducted in which hyperspectral profiles with relative reflectance values at 505 nm between 0.50 and 0.60 were excluded ($N = 3302$). Using the reduced input file reduced the error rate from 15 to 10%, which suggested that the combination of grinding maize kernels, analyzing only particles ranging from 0.250 to 0.354 mm in diameter, and applying the proposed spectral filter reduced an already low classification error.

Validation. The first part of the validation showed that, on the basis of mean comparison, there was a highly significant difference in classification accuracy of size 1 ground material of the two inbred lines (F_{10} value = 48.9, $P < 0.01$), as input data from images of wild type maize were classified with 80.8% (±1.9 SE) accuracy and those from mutant maize were classified with about 95.9% (±0.7 SE) accuracy. It is important to mention that application of the spectral filter reduced input data by 50−70% and that mainly hyperspectral profiles from wild type material were excluded by the spectral filter. In the second part of the validation, it was shown that the proportion of mutant maize in mixed samples was consistently overestimated, but with about 20% points when 0−50% of the sample consisted of mutant material with size 1 particle size (**Figure 4**). Regression analyses of both inbred line provided highly significant curve fits (adjusted R^2 values > 0.96, P values < 0.001). Low (<25%) concentration of wild type maize was predicted fairly accurately, but concentrations >25% were generally underestimated. Despite the considerable level of inaccuracy, it is important to remember that the classification accuracy of wild type material only with size 1 particle was about 80%, so the main conclusions on the basis of **Figure 4** were that (1) mixing of the two inbred lines did not seem to

affect the classification accuracy and (2) it would be possible to obtain quite accurate predictions of actual mixture concentrations by using simple regression analyses (eq 1) as calibration curve for predictions based on discriminant analysis.

DISCUSSION

We analyzed whole and ground maize kernels in small Petri dishes, but the same approach could have been used to analyze large volumes of grain, as the only difference would be to collect larger image cubes. In addition, the same analytical approach could be applied to the classification of most other food products. Hyperspectral images were collected with 15 frames per second with a pixel size of about 0.12 mm^2, but with a more powerful computer, the same setup could collect the hyperspectral images 5−10 times faster. In other words, the experimental setup used here is directly applicable to most commercial operations in which hyperspectral imagery may be used in quality control. However, it is important to mention that the analysis was conducted using a number of software packages and that this process would have to be programmed and integrated into one program for it to be used in a commercial setting. We showed that (1) spectral repeatability was negatively associated with size of target objects (whole kernels compared to ground kernels) as grinding and sifting reduced reflectance value ranges within individual bands; (2) R^2 values of the discriminant function from whole kernels were about 3 times lower than that of size 1 ground particles, so also the error rate of stepwise discriminant analyses was negatively associated with size of target objects; (3) applying a spectral filter to input data had negligible effect on classifications of hyperspectral profiles from whole kernels and size 2 ground particles, but for size 1 ground particles a considerable increase in accuracy was observed, (4) independent validation confirmed that distinction between wild type and mutant inbred maize lines could be conducted with >80% accuracy after the proposed spectral filter had been applied to hyperspectral profiles of size 1 ground particles; and (5) a combination of discriminant analysis and regression analysis could be used to accurately predict mixture ratios of the two inbred lines.

Classification Accuracy and Spectral Repeatability. Obviously, the advantages of using spectroscopy in quality control are first and foremost dependent upon the accuracy level of classification procedures. Published studies on classification of grain characteristics (*1*−*4, 9, 11*), fruit and vegetable qualities (*14*−*16*), and meat characteristics (*17*) generally show classification accuracy levels of 70−95%. Many authors acknowledge concerns about spectral repeatability (i.e., ref *18*), but there are only few suggestions as to how to overcome this problem. Although Peleg et al. (*10*) exclusively discussed spatial and spectral repeatability problems associated with airborne imaging, it seems reasonable to broaden the concern even further, as a low level of repeatability may also be found among spectral profiles obtained from points at close proximity (millimeters apart) on a fruit or grain kernel. Recently, Nansen et al. (*19*) showed that variogram analysis may be used in the analysis of hyperspectral data and that emphasis on the spatial structure of hyperspectral image data sets may reduce the sensitivity to both variation in geographic scale and to inherent variation among different image cubes.

Other Concerns. Apart from accuracy, there are other and more practical concerns that become very important when the objective is to develop analytical procedures to be used on a large commercial scale. "Down time", meaning the time machinery and/or workers have to wait on quality control results before proceeding, is enormously important, so it is critical that analyses are completed in a timely fashion and preferably "in-house" to avoid logistical problems associated with mailing of samples to special laboratories or institutes. But clearly, the most important factor, apart from accuracy, is the ability to process large amounts of samples, because thorough quality control is typically implemented to find the famous "needle in the haystack". Most commercial operations run with very low defect levels (in this context, defects may be considered food products with any unwanted characteristics), and the distribution of such defects may be very aggregated, so many samples have to be analyzed to estimate the overall quality. Pasikatan and Dowell (*2*) used a simple band ratio (920/1660 nm) and a high volume color sorter (40 kg/h) to automatically separate individual kernels into high (>12.5%) or low (11.5%) protein content. Pasikatan and Dowell (*2*) showed that accurate classification of an experimental mix of 50:50 high- and low-protein wheat would require more than five resortings of a given wheat sample, and the authors found that several factors other than protein content influenced the wheat classification.

It was within this context that we decided to explore the option of enhancing the accuracy of a fairly standard classification approach (discriminant analysis) by developing a simple spectral filter to increase spectral repeatability of the input spectra and thereby obtain more accurate separation of classes. Generally speaking, the problem of low spectral repeatability of input profiles is similar to working with a photograph out of focus: you can improve it to some extent, but the best thing would have been to focus the camera properly in the first place! The approach described in this study is based on one important assumption—that it is not necessary to classify all pixels. That is, it is inherently assumed that relative reflectance values within each spectral band are distributed in a somewhat consistent fashion, and the second part of the validation was included to account for that. We showed that there was a nonlinear relationship between predictions from discriminant analysis and actual mixture ratios. Despite the nonlinearity of this relationship, fairly simple regression analysis could be used to correct/calibrate the predictions.

Whole or Ground Maize Material. Many studies, including Pasikatan and Dowell (*2*) and Delwiche (*9*), examined whole kernels and the main justifications are that processing (i.e., grinding and sifting) is (1) labor intensive, which may be an important constraint in large-scale operations, and (2) destructive, which is a critical limitation, for instance, in the selection of cereal breeding material. In this study, the classification accuracy of whole kernels was about 68%, whereas it was >80% of size 1 ground material. It is certainly possible that application of a more selective spectral filter could have improved the classification accuracy, but that would have reduced the number of hyperspectral profiles below what was considered to be acceptable in the analyses, but with larger image cubes that would certainly be an option. However, we found a clear trend with classification accuracy negatively correlated with target object size.

Concluding Remarks. Although classification accuracies of 81 and 96% for wild type and mutant, respectively, may not be acceptable in some quality control procedures, it is important to remember that we used maize material in which there was only <3% genetic difference between the two classes. The level of classification error would likely have been considerably lower if more distinctly different materials had been compared. In addition, it may be possible to consider the proposed procedure a first screening step to be followed by more rigorous analyses

(i.e., NIR spectroscopy) in a subsequent step. Application of the spectral filter reduced input data by 50−70%, which means that at least twice as much data can be classified with the same amount of computer power (half of the input data was discarded in the preprocessing stage), so spectral filtering increased the ability to analyze large samples. From the analyses of sample mixtures, we showed that there was a clear relationship between classification accuracy and mixture and that simple curve fits could be used to calibrate predicted mixtures from discriminant analysis.

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LITERATURE CITED

(1) Delwiche, S. R.; Chen, Y.; Hruschka, W. R. Differentiation of hard red wheat by near-infrared analysis of bulk samples. *Cereal Chem.* **1995**, *72*, 243–247.

(2) Pasikatan, M. C.; Dowell, F. E. High-speed NIR segregation of high- and low-protein single wheat seeds. *Cereal Chem.* **2003**, *81*, 145–150.

(3) Fontaine, J.; Schirmer, B.; Horr, J. Near-infrared reflectance spectroscopy (NIRS) enables the fast and accurate prediction of essential amino acid contents. 2. Results for wheat, barley, corn, triticale, wheat bran/middlings, rice bran, and sorghum. *J. Agric. Food Chem.* **2002**, *50*, 3902–3911.

(4) Fontaine, J.; Horr, J.; Schirmer, B. Amino acid contents in raw materials can be precisely analyzed in a global network of near-infrared spectrometers: collaborative trials prove the positive effects of instrument standardization and repeatability files. *J. Agric. Food Chem.* **2004**, *52*, 701–708.

(5) Delwiche, S. R.; Hruschka, W. R. Protein content of bulk wheat from near-infrared reflectance of individual kernels. *Cereal Chem.* **1999**, *77*, 86–88.

(6) Wang, D.; Dowell, F. E.; Chung, D. S. Assessment of heat-damaged wheat kernels using near-infrared spectroscopy. *Cereal Chem.* **2001**, *78*, 625–628.

(7) Baye, T.; Becker, H. C. Analyzing seed weight, fatty acid composition, oil, and protein contents in *Vernonia galamensis* germplasm by near-infrared reflectance spectroscopy. *J. Am. Oil Chem. Soc.* **2004**, *81*, 641–645.

(8) Ram, M. S.; Dowell, F. E.; Seitz, L.; Lookhart, G. Development of standard procedures for a simple, rapid test to determine wheat color class. *Cereal Chem.* **2002**, *79*, 230–237.

(9) Delwiche, S. R. Protein content of single kernels of wheat by near-infrared reflectance spectroscopy. *J. Cereal Sci.* **1998**, *27*, 241–254.

(10) Peleg, K.; Anderson, G. L.; Yang, C. Repeatability of hyperspectral imaging systems—quantification and improvement. *Int. J. Rem. Sens.* **2005**, *26*, 115–139.

(11) Kays, S. E.; Barton, F. E. Rapid prediction of gross energy and utilizable energy in cereal food products using near-infrared reflectance spectroscopy. *J. Agric. Food Chem.* **2002**, *50*, 1284–1289.

(12) Gao, X.; Shim, W. B.; Göbel, C.; Kunze, S.; Feussner, I.; Meeley, R.; Balint-Kurti, P.; Kolomiets, M. Disruption of a maize 9-lipoxygenase results in increased resistance to fungal pathogens and reduced levels of contamination with the mycotoxin fumonisin. *Mol. Plant Microb. Interact.* **2007**, *20*, 922–933.

(13) Gao, X.; Starr, J.; Göbel, C.; Engelberth, J.; Feussner, I.; Tumlinson, J.; Kolomiets, M. Maize 9-lipoxygenase ZmLOX3 controls development, root-specific expression of defense genes and resistance to root-knot nematodes. *Mol. Plant Microb. Interact.* **2008**, *21*, 98–109.

(14) Mehl, P. M.; Chen, Y.; Kim, M. S.; Chan, D. E. Development of hyperspectral imaging technique for the detection of apple surface defects and contaminations. *J. Food Eng.* **2004**, *61*, 67–81.

(15) Lefcout, A. M.; Kim, M. S. Technique for normalizing intensity histograms of images when the approximate size of the target is known: detection of feces on apples using fluorescence imaging. *Comp. Elec. Agric.* **2006**, *50*, 135–147.

(16) Lu, R.; Peng, Y. Hyperspectral scattering for assessing peach fruit firmness. *Biosyst. Eng.* **2006**, *93*, 161–171.

(17) Kong, S. G.; Chen, Y.; Kim, I.; Kim, M. S. Analysis of hyperspectral fluorescence images for poultry skin tumor inspection. *Appl. Opt.* **2004**, *43*, 824–832.

(18) Baghzouz, M.; Devitt, D. A.; Morris, R. L. Evaluating temporal variability in the spectral reflectance response of annual ryegrass to changes in nitrogen applications and leaching fractions. *Int. J. Rem. Sens.* **2006**, *27*, 4137–4157.

(19) Nansen, C.; Macedo, T.; Swanson, R.; Weaver, D. K. Use of spatial structure analysis of hyperspectral data cubes for detection of insect-induced stress in wheat plants. *Int. J. Rem. Sens.* In press.

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