Accuracy and Feasibility of Measuring Characteristics of Single Kernels Using Near-Infrared Spectroscopy[1]

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ABSTRACT

Single-kernel near-infrared spectroscopy has been used to measure many grain attributes such as protein, oil, internal insects, transgenic traits, and fungal damage. Analysis of single kernels instead of bulk samples has the advantage of detecting attributes that may only be present in a few kernels in a sample. It can also give the distribution of measured attributes. This paper reviews manual and automated, reflectance and transmittance, and low-speed and high-speed single-kernel NIRS analysis and sorting systems.

Near-infrared spectroscopy (NIRS) is commonly used for measuring characteristics of biological materials. Advantages that make this technology a viable alternative to many analytical techniques include little or no sample preparation, measurements in <1s, and spectrometers that cost less than $2000. Williams and Norris (2001) reviewed the physics of NIRS and summarized the use of this technology for measuring constituents such as protein and moisture since its earliest applications about 30 years ago. The technology has progressed to include predictions of functionality parameters such as protein composition, biochemical properties, dough-handling properties, and bread quality (Delwiche and Weaver 1994; Delwiche et al 1998).

Until the mid-1990's, predictions were made only from ground or whole-grain bulk samples. Ground sample predictions have the advantage of lower experimental error than whole-grain analysis partly because of less influence of particle size on NIR absorption, and constituents are more uniformly mixed throughout the sample. Disadvantages of analyzing ground samples include sample presentation errors caused by packing variability, and the sample is destroyed. Whole-grain analysis advantages include reducing analysis time since little sample preparation is needed, and the kernels are preserved for other analyses. However, analysis of bulk samples may not detect grain attributes such as fungal or insect damage that are not present in all, or most, kernels. Also, bulk-sample analysis does not indicate if an attribute, such as protein, follows a normal, binomial, or skewed distribution in a sample. Information about the distribution of attributes within a sample can indicate if a lot has been mixed with extreme ranges of attributes in order to arrive at an average value.

To address some of the limitations of bulk-sample analysis, NIRS was adapted for analysis of single kernels in the mid-1990's. Advantages of single-kernel analysis include that it can detect attributes that may only be present in a few kernels from a bulk sample, and it can provide information about distribution of attributes within a sample. In addition, specific kernels can be preserved for further analysis or used to propagate specific traits in breeding programs. This

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[1] Paper for presentation at the ICC Conference 2002 " Novel Raw Materials, Technologies, and Products – New Challenge for Quality Control," May 2002, Budapest, Hungary.
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paper provides a review of single-kernel NIRS measurement technology, and the accuracy and feasibility of this technology.

Single-Kernel Measurement Technology

Manual Systems -- Transmittance

Many spectrometers can be fitted with fiber optic probes to facilitate collecting spectra from small objects manually placed in the field of view. Much of the early single-kernel NIRS work used transmitted energy and scanning spectrometers with silicon sensors (Delwiche 1993, 1995; Finney and Norris 1978; Lamb and Hurburgh 1991; Orman and Schumann 1992). While the performance of these spectrophotometers can be very good, they can require several minutes to collect one spectrum. Also, many of these spectrometers contained silicon (Si) sensors and were thus limited to the 400-1100 nm range (Table 1). Most of this early work measured only major constituents such as protein, oil, moisture, and hardness.

Manual Systems -- Reflectance

In the mid-1990's, lead-sulfide (PbS, 1100-2500 nm) and indium-galium-arsinide (InGaAs, 1100-1700 nm) sensors became more commonly used in spectrometers, although they had been available for many years. Whereas Si sensors can only measure absorbance in the third overtone region, PbS and InGaAs sensors can measure absorptions in the first and second overtone regions. Stronger absorptions in these regions allow measurement of additional attributes of biological materials.

In addition to using other sensors, researchers began collecting diffuse reflectance spectra instead of transmittance spectra because it may be easier to develop an automated single-kernel NIR system that uses reflected energy, and less energy is required to collect diffuse reflectance spectra versus transmittance spectra. Examples of attributes that could now be predicted with these sensors that measure absorbance at longer wavelengths include major constituents such as protein content (Delwiche 1998; Delwiche and Hruschka 2000) and attributes such as wheat class (Delwiche and Massie 1996); color class (Wang 1999a, 1999b, 1999c; Dowell 1997; Ram et al 2002); internal insects (Baker et al 1999; Ridgway and Chambers 1996; Cheewapramong and Wehling 2001); scab, deoxynivalenol, and ergosterol in wheat (Dowell et al 1998a, 1999); kernel vitreousness (Dowell 2000); heat damage (Wang 2001); transgenic corn (Avidin) (Kramer et al 2000); aflatoxin and fumonisin in corn (Pearson et al 2001; Dowell et al 2002a); and pecky rice (Wang et al 2002) (Table 1).

Automated Systems

In the late 1990's, several companies started marketing automated single-kernel NIR systems. Perten Instrument (Springfield, Ill.) introduced an automated system (SKCS 4170) for small grains that consisted of a kernel singulator coupled with a Si-InGaAs diode-array spectrometer (400-1700 nm). This system was developed through a cooperative research and development agreement with the Engineering Research Unit of the USDA ARS Grain Marketing and Production Research Center, Manhattan, Kan. The system can collect spectra at a rate of 1 kernel/2s and uses reflected energy. It has been used to measure moisture, grain hardness, color class, protein, internal insects, and bunted kernels in wheat (Dowell et al 1997, 1998; Dowell 1998; Maghirang and Dowell 2002; Maghirang et al 2002) (Table 1). Brimrose (Baltimore, Md.) markets a system (Seedmeister) for large grains such as corn. This system consists of a kernel singulator and an acousto-optic tunable filter, and has a wavelength range of 900-1600 nm. This system can process and sort 1 kernel/s and uses transmitted energy. The cost of each of the Perten and Brimrose systems is about $100,000, and both systems are used in several research facilities.

The Engineering Research Unit of the USDA ARS Grain Marketing and Production Research Center, Manhattan, Kan., has recently developed a simplified single-kernel NIR sensing and sorting system that utilizes a low-cost InGaAs sensor. Perten Instruments is currently developing a commercial version of this lower-cost prototype. InGaAs diode-array sensors can be purchased for <$5000, and Si diode-array sensors can be purchased for <$2000. This decrease in cost, while maintaining accuracy and speed similar to higher cost systems, may make it possible to assemble an automated single-kernel NIR system at a fraction of the cost of the original commercial systems.

High-Speed Sorting

High-speed sorters have commonly been used to remove visible defects in commodities, and recently InGaAs sensors have been incorporated into these systems. Inspection and sorting with these instruments can occur at a rate as high as 10,000 kernels/s, or about 1100 kg/hr (40 bu/hr). Dowell et al (2002b) used a ScanMaster II SM100IE (Satake USA Inc, Houston, Texas) to remove bunted kernels from uninfected seed with 100% accuracy (Table 1). They have also recently used this technology to remove red wheat from white wheat stock (Pasikatan and Dowell 2002), and to sort into high and low protein groups. While these systems cannot quantify levels of attributes in single kernels and are limited to one or two wavelengths, they can rapidly sort samples into two groups that have distinctly different traits. Breeders are now using this technology to purify seed stock, and it may have additional applications in the grain industry.

Single-Kernel Measurement Accuracy and Feasibility

Literature shows that many attributes of single kernels can be measured by reflectance and transmittance spectroscopy with standard errors that are usually suitable for screening purposes (Table 1). While the standard error of single-kernel analysis may be twice that of bulk-sample analysis, the benefit of knowing the distribution of attributes within a sample outweighs this loss in accuracy for many applications. The increase in sensitivity and reductions in cost as this technology advances will help increase its acceptance in all industry segments.

While many attributes can be measured with this technology, it is limited to those that are present in sufficient quantities to significantly affect NIR absorption. Thus, it may not be possible to detect attributes that comprise <0.1% of the kernel mass. It is useful for detecting the presence of internal insects and predicting the level of protein or oil, but it may not be useful for detecting minute traces of some attributes such as those that may be associated with some transgenic traits and are present at the ppm or ppb level. While literature shows that levels of transgenic traits, fumonisin, and aflatoxin in corn (Kramer et al 2000; Pearson et al 2001; Dowell et al 2002a) and vomitoxin in wheat (Dowell et al 1999b) can be predicted, the standard errors of these predictions are very high. The ability of NIRS to predict levels of transgenic traits or toxins is likely due to the high correlation of these attributes to changes in other intrinsic characteristics such as protein levels, vitrousness, changes in the protein-starch matrix, etc.

In summary, single-kernel NIRS provides the grain industry with a useful tool for measuring the distribution of attributes within samples, and provides a means to measure attributes such as internal insects and fungal damage that may be present in a small percentage of kernels. Current cost reductions and increases in sensor sensitivities, combined with sorting capabilities, should help this technology gain widespread use throughout all industry segments.

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Table 1. Compilation of research conducted showing the feasibility and accuracy of using NIR single-kernel analysis for measuring selected grain characteristics.

Commodity	Parameter	Wavelength Range (nm)	References	Statistics		
				R^2	SECV	%CC
Wheat	Moisture content	400-1700	Dowell et al., 1997	0.97	0.43	-
	Protein content	850-1050	Delwiche, 1995	0.85-0.93	SEP: 0.42-0.94	-
		400-1100	Abe et al., 1996	-	SEP: 0.77	-
		400-1700	Dowell et al., 1997	0.94	0.75	-
		1100-2498	Delwiche, 1998	-	0.46-0.72	-
		1100-2498	Delwiche and Hruschka, 2000	0.95 - 0.99	0.16-0.63	-
	Wheat class	551-2476	Delwiche and Massie, 1996	-	-	63-98
	Color class	400-2000	Wang et al., 1999b	-	-	98
		400-2000	Wang et al., 1999c	-	-	95 – 98
		400-1700	Dowell, 1997	-	-	98
		400-1700	Dowell, 1998	-	-	99
		490-640	Shadow and Carrasco, 2000	-	-	100
		400-1700	Ram et al., 2002	-	-	100
	Dominant R alleles	400-2000	Wang et al., 1999a	-	-	78 - 100
	Internal insects	400-2500	Ridgway and Chambers, 1996	0.57-0.83	-	-
		400-1700	Dowell et al., 1998	-	-	99
		400-1100	Ridgway et al., 1999	-	-	90-96
		1100-1900	Cheewapramong and Wehling, 2001	-	-	90-97
		400-1700	Maghirang et al., 2002	-	-	93
	Parasitized insect	400-1700	Baker et al., 1999	-	-	75–100
		700-1700	Dowell et al., 2000	-	-	62-94
	Bunted kernels	400-1700	Dowell et al., 1997	-	-	93
		675	Dowell et al., 2000b	-	-	100
	Scab	400-1700	Dowell et al., 1999	-	-	100
	DON, Ergosterol	400-1700	Dowell et al., 1999	0.64	40 ppm DON	100
	Vitreousness	400-1700	Dowell, 2000	-	-	75 - 100
	Heat damage	400-1700	Wang et al., 2001	-	-	94 - 100
	Hardness	740-1139	Delwiche, 1993	0.68	SEP: 16.8	-
		400-1700	Maghirang and Dowell, 2002	0.91	7.7	100
Corn	Moisture content	700-1100	Finney Jr. and Norris, 1978	-	Std error: ~2%	-
	Avidin	400-1700	Kramer et al., 2000	0.29	526 ppm	77 – 92
	Aflatoxin, Fumonisin	400-1700	Pearson et al., 2001; Dowell et al., 2002	-	-	86 – 100
	Oil	850-1050	Orman and Schuman, 1992	0.56	1.3	-
Soybeans	Moisture content	800-1100	Lamb and Hurburgh Jr., 1991	-	SEP: 0.65-0.69	-
	Protein content	400-1100	Abe et al., 1996	-	SEP: 0.67	-
Rice	Pecky rice	400-1700	Wang et al., 2002	-	-	99 – 100

R^2 = coefficient of determination; SECV = standard error of cross validation; SEP = standard error of prediction; %CC = percentage of correct classification.