Measuring Grain Protein Concentration with In-line Near Infrared Reflectance Spectroscopy

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ABSTRACT

The advent of near infrared (NIR) on-combine sensors gives growers the opportunity to measure the grain protein concentration of wheat (*Triticum aestivum* L.) during harvest. A study consisting of three sequential experiments (laboratory bench, combine test stand, and field) was conducted to evaluate the performance of an in-line, NIR reflectance spectrometer, referred to as the ProSpectra Grain Analyzer, possessing a factory calibration model. In the laboratory bench experiment, the instrument was mounted to a circulating impeller apparatus designed to simulate a moving stream of grain. The ProSpectra performed well on a validation set of 231 grain samples of soft white winter wheat and explained a high level of protein variability ($R^2 = 0.91$, SEP = 3.1 g kg$^{-1}$) with a slope near unity. In the second experiment, the sensor was installed on a combine test stand constructed from the cross and exit augers, and clean grain elevator of a combine, to create the grain flow conditions found on a combine. Predicted protein was highly correlated ($R^2 = 0.93$, SEP = 4.5 g kg$^{-1}$) with reference protein of nine large (14-kg) wheat samples. During the third experiment, the instrument was placed on the exit auger of a Case IH 1470 combine for the harvest of a 17-ha winter wheat field. Prospectra protein predictions correlated well with reference protein measurements ($R^2 = 0.94$, SEP = 3.1 g kg$^{-1}$). This study demonstrated the feasibility of using in-line NIR reflectance spectroscopy to rapidly (0.5 Hz measurement rate) and accurately (SEP < 5.0 g kg$^{-1}$) measure wheat protein in a moving grain stream.

Near infrared spectroscopy (Williams and Norris, 1987) is an industry-wide technique used today in the analysis of wheat and other cereal grains for protein content. In recent years, consideration has been given to measuring the protein concentration of wheat as it is harvested by a combine (Engel et al., 1997). Interest in this concept is focused on two potential applications. First, optical sensors could be used during harvesting or handling to segregate grain based on protein concentration, thus enabling growers to better capture price premiums in value-added markets that pay premiums for quality (Stafford, 1999; Thylén et al., 2002; McNeill et al., 2005). Second, protein measurements, when integrated with GPS technology, would enable the development of protein maps of farm fields. Grain protein maps have been proposed as surrogates for soil N testing and for use in making variable-rate N fertilizer recommendations (Engel et al., 1999; Long et al., 2000; Taylor et al., 2005).

Recent attempts to measure wheat grain protein concentration on a combine have used online, NIR transmittance-type sensors such as the CropScan 2000G (NIR Technology, Inc., Bankstown, NSW, Australia) and the AccuHarvest On-Combine Grain Analyzer (Zeltex, Hagerstown, MD). An online sensor uses a recirculating transport system to automatically remove a sample from the grain stream, bring it to the analyzer, and return it to the grain stream (Bakeev, 2003). On-combine measurement accuracies of grain protein content of hard red spring wheat have been reported to be 6.6 g kg$^{-1}$ for the CropScan sensor (Long et al., 2005) and 4.9 g kg$^{-1}$ for the AccuHarvest sensor (Long and Rosenthal, 2005). Both instruments are capable of measuring protein at a rate up to 0.13 Hz. Similarly, an NIR reflectance-type sensor, mounted within a mechanical bypass on the combine’s clean grain elevator, was reported to have a prediction accuracy of 5.7 g kg$^{-1}$ and measurement rate of 1 Hz (Maertens et al., 2004).

In contrast to online sensors, in-line sensors operate by measuring protein content directly in the combine grain stream and may be advantaged by a lack of a need for a mechanical sample transport system. A previous test of a prototype, in-line NIR reflectance spectrometer manufactured by Textron Systems (Wilmington, MA), referred to as the ProSpectra Grain Analyzer (von Rosenberg et al., 2000), proved unsuccessful because of hardware problems that were thought to be caused by malfunctioning of the instrument’s internal reference for compensation of variations in source light intensity (Meier, 2004). Recently, the original concept designer: DSquared Development (La Grande, OR), corrected this problem by implementing better temperature and reference-reflection compensation to ensure good signal to noise ratio. In this study, an improved version of the ProSpectra instrument was evaluated for measuring the grain protein concentration of wheat during harvest with a combine.

Abbreviations: NIR, near infrared; PLS, partial least squares; SEP, standard error of prediction.
MATERIALS AND METHODS

Instrument Description

The ProSpectra instrument (Fig. 1) consists of three components: (i) a fiber optical sensor probe, designed for in-line measurement, (ii) an electronic spectrometer unit, which is removed from the immediate vicinity of sensing, and (iii) a computer processing unit with the chemometric and instrument control software DeLight (DSquared Development, LaGrande, OR). Both the sensor probe and spectrometer are designed to withstand heat, dust, vibration, and other rigors of field conditions at harvest. The spectrometer is a closed system that contains a power supply, solid state electronics, and an internal temperature stabilizing unit.

The ProSpectra instrument measures diffuse reflectance spectra at 0.5 nm intervals over a wavelength range from 600 to 1100 nm. The instrument relies on a 1,024 element light sensitive detector array, or Charged Coupled Device, using a proprietary optical system that is thermally stabilized to operate over the range of −30° to +50°C. A tungsten light emitting bulb, reference shutter, and sapphire lens are integrated into the sensor probe. A fiber optic pickup cable transmits the reflected spectra between the probe and detection sensors in the spectrometer unit. This information is used to define the relationship between spectral reflectance (R') and apparent absorbance (A'), or $A' = \log \left(\frac{1}{R'}\right)$ (Murray and Williams, 1987), where $R' = \frac{R_{\text{sample}}}{R_{\text{ref}}}$. Constituent information is calculated with an embedded processor from an average of 100 scans over 2 s, which corresponds to a measurement rate of 0.5 Hz. The sensor’s narrow aperture (2.54 mm) conforms to the size of single grain kernels. Grain must flow past the aperture so that the readings taken within each 2-s scan interval adequately represent the reflectance properties of the grain kernels, which vary with distribution of protein within the endosperm and orientation of kernels to NIR light. During operation, a 100% reflectance reference scan ($R_{\text{ref}}$) is taken every 15 min as needed to recalibrate the sensor.

As an aside, DeLight provides partial least squares (PLS) regression for preparing mathematical calibrations used by the ProSpectra instrument to predict grain protein concentration in unknown samples. Regression by PLS has become the standard tool for modeling linear relations between multivariate measurements, especially where a large number of predictors is necessary (de Jong, 1993). Calibration models can either be factory supplied or created by the user or other third party.

Laboratory Bench Evaluation

Soft white winter wheat grain samples (800 g) were obtained from dryland fields in northeastern Oregon for validation of the factory-supplied calibration model. The samples ($n = 231$) were manually collected from the exit auger of a combine during harvest and ranged from 85 to 140 g kg$^{-1}$ in protein concentration. Samples were cleaned before analysis of grain protein. Subsamples (40 g) were ground in a Udy Mill (UDY Corp., 201 Rome Court, Fort Collins, CO 80524) before N determination by an automated dry combustion or Dumas procedure (Leco Corp., St. Joseph, MI). Reference protein concentrations were calculated by multiplying Leco N by 5.7 and corrected to a 120 mg g$^{-1}$ moisture basis.

During the laboratory bench evaluation phase of this study, the ProSpectra sensor was connected with a 1000-mL cylindrical chamber-circulating impeller apparatus (Fig. 2). Grain was placed in the cylinder and the center mounted impeller spun grain past the probe in a manner that simulated the grain flow and packing conditions found within an auger. The spinner assembly was a closed system, to prevent ambient light from entering the cylinder. The control software DeLight was used to operate the spectrometer and record the spectra diffusely reflected from the kernels as they flowed past the sensor head. The multiple coefficient of determination ($R^2$) and the standard error of prediction (SEP), or standard deviation of the differences between NIR and reference values (Williams, 1987), were used to validate the accuracy of the instrument.
Combine Test Stand Evaluation

A stationary combine test stand was fabricated to evaluate the performance of the Prospectra analyzer under the dynamic flow conditions that are found on a combine. The cross auger, clean grain elevator, and the exit auger from a Gleaner combine harvester were mounted to a standard (1.2 by 1.0 m) plywood pallet bin (Fig. 3). An electric motor, and chain and gear drive were used to turn the elevator and augers at speeds that simulated the grain flow conditions experienced on a combine. The entire apparatus produced a grain stream equivalent to 4 m$^3$ h$^{-1}$. A rectangular opening cut in the housing of the exit auger provided a means for mounting the sensor head directly over the axis of the auger, thereby exposing the sapphire sensing lens of the fiber optic probe to the grain stream. The grain stream moved through the exit auger in a spiral clockwise pattern when viewed from the front, or grain exit end (Fig. 4). It was essential to position the sensor head so the grain stream intercepted the sensor head position. A viable location was found at 30° above bottom center on the cylindrical housing. The auger flighting immediately adjacent to the sensor head was removed to prevent interference with the NIR light reflected from the grain.

Reference grain samples for validation were derived from a large-plot N fertility trial with winter wheat that was conducted in 2005 near Condon, OR. Nine 14-kg grain samples from that study consisted of three club, soft white winter, and hard white winter wheat, and embodied a wide range in protein levels (90–140 g kg$^{-1}$). Each sample was cleaned and split into 10 representative subsamples. Reference protein analysis (corrected to 12 g kg$^{-1}$ moisture) of subsamples was performed in the laboratory with a Foss Infratec 1241 NIR whole grain analyzer. The average of the 10 subsample measurements was taken as the protein value for each of the nine reference samples. Instrument precision was then estimated by obtaining multiple ($n = 30$) measurements on the nine 14-kg reference samples of known protein concentration as they flowed through the combine test stand over a period of 60 s. Model performance was reported as the multiple coefficient of determination ($R^2$), the SEP, and average difference between modeled and reference values (bias). To allow the instrument to be evaluated on the test stand over a period of 5 min, two large grain samples (272 kg each) were obtained from a local grain company. The samples included soft white winter wheat with reportedly 110 g kg$^{-1}$ protein and hard red spring wheat with 130 g kg$^{-1}$ protein. Repeatability was assessed by examining the bias and standard error of repeated measurements, and examining the readings of the check samples for trends of consistency over time.

Field Evaluation

The same instrument, used on the combine test stand, was mounted to the housing of the exit auger in the bulk tank of a Case IH 1470 combine, and tested during the harvest of a...
Reference protein analysis (corrected to 120 g kg$^{-1}$ moisture) of subsamples was performed in the laboratory with a CropScan 2000B NIR whole grain analyzer (NIR Technology Australia, Bankstown, NSW) calibrated for soft white winter wheat. The average of the three subsample measurements for each of the 45 reference samples and the average of the corresponding ProSpectra readings within each 10- to 20-m sampling interval were combined into one data set and analyzed for precision using the $R^2$ and SEP.

**RESULTS AND DISCUSSION**

**Bench and Combine Test Stand Evaluation**

Predicted protein values, obtained with the ProSpectra instrument mounted to the cylindrical chamber-circulating impeller apparatus, exhibited good agreement with reference protein values ($R^2 = 0.91$) and a slope near unity (Fig. 5A). The SEP was 3.1 g kg$^{-1}$, which compares with the precision of laboratory NIR whole grain analyzers (SEP = 2.8–3.6 g kg$^{-1}$; Williams, 1987). When evaluated on the combine test stand, the instrument performed well in predicting the protein concentration of the nine 14-kg grain samples (Fig. 5B). A high level of variance in reference protein values was explained ($R^2 = 0.93$) and the standard error of measurements with the ProSpectra sensor was 4.5 g kg$^{-1}$. The precision of the ProSpectra instrument to indicate the same value on repeated input, or repeatability, was assessed by examining the standard error of measurements of the two 272-kg grain samples over 5 min. A plot of continuous readings about the reference mean was without trends over time (Fig. 6) and had a standard error of <3.0 g kg$^{-1}$ thus revealing that the ProSpectra device has an acceptable degree of instrument repeatability. Biases from the two large grain samples were relatively low (<1.5 g kg$^{-1}$) indicating the instrument overpredicted for as many readings as they underpredicted.

**In-Field Evaluation**

The predicted vs. reference protein measurements were highly correlated ($R^2 = 0.94$) for the instrument evaluated on the combine in the field (Fig. 7). Ability to predict protein is further indicated by the lack of bias and a slope near unity. Measurement accuracies of in-line NIR prediction on the combine in the field and on the combine test stand were comparable (SEP = 3.1 g kg$^{-1}$ vs. 4.5 g kg$^{-1}$). Both tests satisfied company specifications or requirements that targeted an accuracy of <5.0 g kg$^{-1}$ protein. Performance of the ProSpectra instrument on the combine test stand and in the field was not as good as found on the cylindrical chamber-circulating impeller apparatus in the laboratory. This result was expected given the larger grain sample size and measurement errors associated with georeferencing reference samples in the field relative to when analyzed with in-line protein sensing on the combine. Accuracy of the ProSpectra instrument exceeded that of other commercially available on-combine grain quality sensors previously mentioned to be between 4.9 and 6.6 g kg$^{-1}$. These results establish validity for in-line NIR reflectance measurement of protein concentration on a flowing grain stream conveyed by an auger.

The prediction model developed in the laboratory was transferable to the field where it was used to map the grain protein concentration of the 17-ha field near Helix (Fig. 8). The map reveals that crop management practices influence the observed grain protein concentrations. Alternate wheat–conventional fallow generally had low levels of protein (<110 g kg$^{-1}$) followed by volunteer wheat following alternate wheat–conventional fallow and alternate wheat–chemical fallow with intermediate levels (110–130 g kg$^{-1}$), and alternate wheat–pea (Pisum sativum L.) with high levels (>130 g kg$^{-1}$). Crop management systems (i.e., wheat–fallow vs. continuous) likely influenced plant available water and thus help explain the spatial patterns in grain protein levels observed within this field. Indeed, farm fields are inherently variable in topography, soils,
fertility, and other production factors, which are known to influence the pattern of site-specific grain yield and grain quality (Reyns et al., 2000; Fiez et al., 1994). This source of variability plays havoc with the goal of providing a consistent supply of high quality grain for today’s sophisticated buyers.

Ideally, the ultimate application for an in-line NIR sensor would be on a combine harvester for monitoring this variability in protein concentration and controlling a mechanism on the grain bin filling auger that segregates the grain into quantities of low or high protein (Stafford, 1999; Thylén et al., 2002). Bramble et al. (2002) examined the protein variance structure within 46 commercial wheat fields in Kansas and found field and plot (plots within a field) sources of variance partialled 70% of the total variance with standard errors of >83 g kg\(^{-1}\). Our study and the work of Bramble et al. (2002) imply that on-combine grain sensing technology would enable growers to consider site-specific variability as a practical limit for managing the protein content of their wheat. Implementation of on-combine grain segregation will require hauling vehicles capable of accommodating the segregated grain and returning to the field rapidly to maintain high harvesting efficiency (McNeill et al., 2005).

The most recent use of site-specific protein information has been in supporting precision agriculture. For example, maps of grain protein and grain yield, derived from a combine equipped with a GPS receiver, yield monitor, and optical NIR sensor, can be arithmetically combined in a geographic information system to compute N factors that are important in determining the variable-rate N requirements for a grain crop (Long et al., 2000; Taylor et al., 2005). Grain protein and grain yield maps have also been proposed for use in estimating the amount of straw yield after harvest (Engel et al., 2003). Straw yield maps might prove useful in refining precision N recommendations and quantifying the amount of feedstock available in farm fields for cellulosic ethanol production.

CONCLUSIONS

A protein prediction model for the Prospectra Grain Analyzer was developed during the calibration phase of this study. When mounted on the exit auger of a combine test stand, the ProSpectra instrument measured the protein concentration of grain flowing in a stream with excellent accuracy with the percent difference between predicted and reference measurements being <5 g kg\(^{-1}\) (SEP). In the field evaluation phase the instrument was mounted to the exit auger of a Case IH 1470 combine. Protein predictions again correlated well with estimates of protein obtained by reference methods. Our study suggests that in-line NIR reflectance can provide rapid and efficient measurements of grain protein concentration with appropriate limits of precision and accuracy. In-line grain protein analysis is a new concept in production agriculture. The instrument tested in this study represents NIR technology that is continuing to evolve, but shows great promise for what is likely to become a very important development in grain marketing and precision nutrient management.

PREVIOUS PAGE

LEGEND
Grain Protein (g kg\(^{-1}\))
- 145
- 140
- 135
- 130
- 125
- 120
- 115
- 110
- 105
- 100
- 95
- 90
- 85
- 80

Fig. 8. Map of grain protein concentration for 17-ha field of soft white winter wheat with smaller field areas of alternate wheat–conventional fallow (A), volunteer wheat following alternate wheat–conventional fallow (B), alternate wheat–chemical fallow (C), and alternate wheat–pea (D).

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REFERENCES


