A Single Analytical Platform for the Rapid and Simultaneous Measurement of Protein, Oil, and β-Glucan Contents of Oats Using Near-Infrared Reflectance Spectroscopy

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ABSTRACT

Effective near-infrared reflectance spectroscopy (NIRS) predictive calibrations were developed for simultaneous multiple component measurement of constituents (protein, oil, and β-glucan contents) in whole and ground oat groats. The use of whole oat groats as a starting material represents an advancement in the science as it precludes the need for sample grinding. Samples were collected from the 2015 and 2016 crop years from various locations in the United States (South Dakota, North Dakota, Washington, Iowa, and Wisconsin), representing a large geographical region and diverse genetic range ($N = 500$). Predictive calibration equations were developed based on the modified partial least squares (MPLS) regression technique. Reference analyses were done using standard methods approved by AACC International and AOCS (AACCI Method 32-23.01 for β-glucan content, AACCI Method 46-30.01 for crude protein content, AOCS Standard Procedure Am 5-04 for oil content, and AACCI Method 44-15.02 for moisture content). The use of validation sample sets for each constituent, which were independent of samples used in NIRS calibration development, served as additional evidence of accuracy and precision. High coefficient of determination (R^2) and one minus variance ratio (1-VR) and low standard error of calibration (SEC) and standard error of cross-validation (SECV) values provided evidence supporting the accuracy and precision of the calibration models developed for estimation of oat β-glucan, protein, and oil contents. The NIRS calibration for estimation of β-glucan content of ground oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.94, 0.16, 0.22, and 0.87, respectively. Protein calibration for ground oat groats yielded R^2, 1-VR, SEC, and SECV values of 0.94, 0.93, 0.61, and 0.64, respectively. Calibration employing ground oat groats for oil content estimation yielded high R^2 and 1-VR values of 0.93 and 0.92, respectively, and low SEC and SECV values of 0.23 and 0.26, respectively. Whole oat groat NIRS calibrations proved to be as effective as ground groat calibrations. Whole oat groat β-glucan calibrations yielded excellent R^2, SEC, SECV, and 1-VR values of 0.93, 0.18, 0.23, and 0.89, respectively. For protein calibrations of whole oat groats, R^2, SEC, SECV, and 1-VR values were 0.92, 0.70, 0.80, and 0.89, respectively. Oil content calibration developed with whole oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.90, 0.27, 0.30, and 0.88, respectively. This study showed that NIRS is an accurate and effective technology for oat quality measurement in plant breeding programs and food processing.

Knowledge about the composition of agricultural materials used in foods is essential for sound decision-making by geneticists, plant breeders, millers, and bakers. Food processors rely on accurate information on the quality of crops that arrive from the field. Reference methods based on chemical and enzymatic analyses provide the basis for determination of accuracy and precision. However, these methods are hard to implement and require highly trained analysts. Such methods also take time to implement and may be cost-prohibitive, especially if large sample throughput is required. Plant breeding programs, in particular, need cost-effective and fast screening techniques. Near-infrared reflectance spectroscopy (NIRS) provides an analytical platform for rapid and robust measurement of multiple constituents once reference methods have been perfected. NIRS, thus, is a correlative tool that relies on the existence of methods that accurately quantify the target compound, be it protein, carbohydrates, oils, or dietary fiber.

Measurement of individual constituents provides little information about the causes of variability of a constituent. Interrelationships between multiple grain constituents can be gauged through a more comprehensive understanding of seed composition and makeup. Plant breeders, for example, need to interpret variability of nutrients and their relationships to other agronomic traits, such as yield and kernel size, in the context of sources of variations such as genetics, environment, and interactions (20). Cereal chemists may have a better understanding of grain functionality based on a more comprehensive knowledge of variations in nutrient composition. This article reports on the development of calibrations for estimation of protein, oils, and β-glucan in oats (both in whole and ground oat groats).

NIR spectra of samples also serve as a repository of information that could be used to devise analytical methodologies for grain constituents whose importance have not yet been fully realized. Information contained within the spectra may be valuable for future development of correlative methods once sound analytical methods can be developed for those constituents. Routine noninvasive measurement of multiple constituents such as amino acids, fatty acids, dietary fiber moieties, and bioactive ingredients may be possible using a single analytical NIRS platform if they are supported by the existence of sound reference methods.

Oat (*Avena sativa*) is a unique cereal crop with significant benefits for human health and nutrition. This cereal grain has been shown to have multiple health benefits for humans, including mitigating the risks for heart disease (2,13,21,27). β-Glucan, a soluble dietary fiber found in the range of 2 to 6% in oats, is associated with the reduction of postprandial serum glucose levels in humans and animals (6). This effect of β-glucans on blood glucose is significant for the management of diabetes. Oats are also endowed with other beneficial compounds, such as antioxidants, an excellent amino acids profile in their protein, and polyunsaturated fatty acids in their oils. The oat protein content also contributes to their health benefits. Oat protein content is unique among grains because it contains significant levels of

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legumin-like globulins (23) and has a high biological value as a food source (11). Although oats are used primarily in breakfast cereals and breakfast bars, expanding the range of products made with oats would greatly increase the number of consumers who could benefit from its health-promoting attributes. To expand the range of products made with oats, we need to better understand the chemical and nutritional properties of oat constituents and how they affect the quality of final products.

The United States is the world's largest importer of oats. The U.S. oat milling industry currently relies on Canada as the source of most of its oat grain imports (3.1 million tons in 2016). The United States is also the world's fourth largest oat producer, and it imports more than 75% of the world's shipments. The United States generally uses about one-third of its total yearly oat supply for food or industrial uses and about two-thirds for feed. There is interest among U.S. millers in expanding the domestic supply of oat grain. U.S. oat production in 2016 was 939,121 tons (26). As the leading oat-producing state, South Dakota produced 193,050 tons in 2016. An increased emphasis on oat production has prompted the need for effective and efficient tools to monitor the baseline data on quality traits for this cereal grain. Quality evaluation of oats needs to encompass both food and feed quality to ensure a sustainable crop that has diversified functionality and end-use applications. The existence of analytical methods for rapid analysis, therefore, is important for the long-term tracking of oat quality parameters.

In addition to its health benefits, oat crops present advantages for soil health and agricultural sustainability. Since 2013, farmers have shown increased interest in including oats in their crop rotations and, thereby, increasing the diversity of the crops grown in the region. Oat production can be used to break weed and pest cycles in corn–soybean rotations, and it is a low input crop. The focus of U.S. oat breeding programs is to develop new adapted oat varieties with improved agronomic characteristics and improved milling and nutritional qualities. Further improvements for milling and nutritional qualities are necessary to ensure that new varieties meet the needs of the developing food market and provide as many health benefits for consumers as possible.

BACKGROUND

NIRS employs wavelengths in the near-infrared region (850–2,500 nm) of the energy spectrum to scan samples. Chemical constituents within samples, such as proteins and lipids, absorb some of this energy while reflecting the rest. The reflected energy is detected and correlated to the amount of the compound present in the sample. Reliable and accurate reference chemical measurements, thus, are indispensable to good calibration development. NIRS methods have been used in agriculture as screening tools for fast-throughput breeding program selection. Advancements in spectroscopy and refinements in reference analytical methods have improved NIRS analysis significantly over the years to advance the use of the technology as an analytical tool and not merely as a screening protocol. NIRS can rapidly (within seconds) and simultaneously measure the protein, oil, and moisture contents in oats with accuracy and precision (4,16). Krishnan et al. (15) used NIRS to measure oat grain properties. NIRS fatty acid calibrations were shown to be effective as a screening tool in ground oat groat samples from the South Dakota oat breeding program (16). NIRS was used to determine the β-glucan content in barley (7,10,14,24), naked barley (22), and naked oats (4). Schmidt et al. (22) used a Fourier-transformation NIRS instrument to obtain a correlation coefficient of 0.96 to 0.98 between their prediction equation and enzymatically determined barley grain β-glucan content. Osborne (18,19) has provided several reviews of NIRS use.

NIR technologies offer the potential for nondestructive, high-throughput sampling with a low cost per sample once calibrations are in place. These are correlative techniques, however, that rely on the skillful performance of wet chemistry analyses for calibration and validation to generate accurate and precise output.

Although previous studies have reported on NIRS estimation of single constituents, the ability to measure multiple components simultaneously and nondestructively in a single run has enormous advantages for data interpretation. Oat groats represent the edible portion of the oat seed after the hull is removed. NIR calibrations reported in the literature were developed using ground oat groats. NIR calibrations for whole oat groats, however, would remove the time-consuming step of sample grinding. In addition, whole oat groat calibrations do not harm the seed, which remains intact and viable and can be planted to obtain a new generation. Samples can be cleaned, dehulled, and read directly in the NIR spectrometer. This represents a significant technological advancement in oat quality measurement. NIRS, thus, may potentially be used to obtain comprehensive compositional information that can be used effectively for product innovation.

The purpose of this study was to develop predictive calibration models for estimating β-glucan, protein, and oil contents of U.S. oat cultivars using NIRS and validated AACC International (AACCI) reference methods and an AOCS standard procedure (1,3). A rapid, nondestructive (whole oat groat) NIRS method was also developed to estimate β-glucan, protein, and oil contents based on the standard reference analyses procedures approved by AACCI (1). This represents a single analytical platform for reliable and nondestructive multiple-component analysis that provides decision-making tools for producers, plant breeders, and food processors. The influence of genetics and growing environments on the variability of multiple oat constituents was also a research objective.

METHODS AND MATERIALS

General Approach

A schematic of the experiment design used in NIRS calibration development and the validation of those methods is provided in Figure 1. More than 500 oat samples grown in diverse growing regions in the United States and representing two growing years (2015 and 2016) were collected, cleaned, and dehulled to yield oat groats. Sampling included cultivars grown in Washington, Iowa, Wisconsin, Minnesota, and North Dakota, as well as samples from the South Dakota oat breeding program. Dehulling was accomplished using a huller (Codema LLC). A subset of the samples was ground using a mill (Ultra Centrifugal mill, Retsch) equipped with a 0.5 mm screen to yield ground oat groats. Both whole and ground oat groat samples were scanned in an NIRS feed analyzer (DS2500, FOSS NA) to yield spectra from 250 to 2,500 nm. All samples were subjected to reference chemical and enzymatic analyses using officially accepted methods for moisture, protein, oil, and β-glucan contents. Predictive calibration equations were developed for whole and ground oat groat samples using a modified partial least squares (MPLS) regression technique. Calibration and validation sample sets were

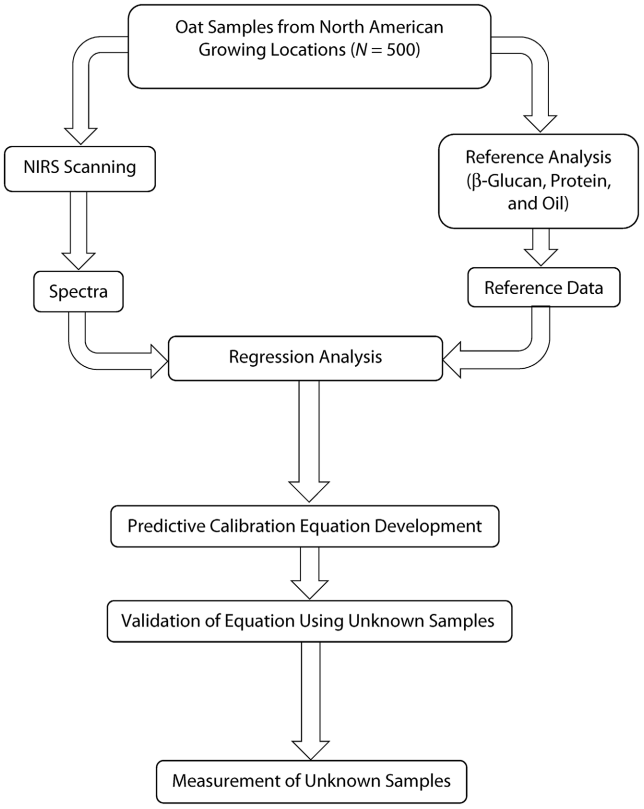

Fig. 1. Flow diagram summarizing experimental methodology for near-infrared reflectance spectroscopy (NIRS) calibration development and validation.

identified for each of the constituents using NIRS software. The validation sample sets, which were not part of the calibration development, were used as independent checks on the accuracy and precision of NIRS predictive equations for the target constituents. A fixed sample set containing varieties (16 cultivars) common to the two growing years and grown in the same locations (4 locations) each year was evaluated by both NIRS and reference analyses to determine the influence of genetics and environmental effects on the variability of protein, oil, and β-glucan contents. A total of 128 samples, thus, were used in the variety by environment study. The effectiveness of NIRS was evaluated both as a screening and analytical tool relative to chemical and enzymatic analytical methods. Ranking of varieties was done to determine if NIRS and reference analyses provided essentially the same information on oat quality.

Reference Analyses

Analytical Methods. Moisture content analysis was performed using the standard air-oven method approved by AACCI (AACCI Method 44-15.02) (1). Crude protein content was measured using the Dumas combustion method (AACCI Method 46-30.01) (1) and employing a protein analyzer (FlashEA 1112, Thermo Fisher Scientific). Nitrogen content measured by this procedure was expressed as percent protein using a conversion factor of 5.83 (% N × 5.83). Crude oil content was measured using AOCS Standard Procedure Am 5-04 (3). Enzymatic analysis of β-glucan content was performed using AACCI Method 32-23.01 (1) with a mixed β-glucan linkage kit (Megazyme International Ireland Limited). Standard barley flour and oat flour samples with known levels of β-glucan provided with commercial kits were used as controls and checks on analytical accuracy. All data were expressed on a moisture-free basis for ease of data interpretation.

NIRS Calibration Development. Oat samples from different North American growing locations and breeding programs were scanned on the NIR spectrometer (N = 500). Based on spectral analyses, and depending on the target constituent, calibration sample sets were identified for each of the constituents: N = 308 for β-glucan content; N = 394 for protein content; and N = 385 for oil content. Separate sample sets, designated as the validation set (N = 104 for β-glucan content; N = 134 for protein content; and N = 133 for oil content), that were independent of those used in the calibration set were employed to test the accuracy and precision of NIRS measurements. The effectiveness of the calibrations was evaluated by statistical comparison of NIRS estimations against true values measured using official reference procedures. High correlation coefficients and low standard error terms provided a good estimate of accuracy and precision, respectively, of NIRS methods. NIRS spectral data are multivariate in nature because they contain large numbers of data points, i.e., one data point in each wavelength for each sample. In addition, differences between samples are discerned only through small spectral variations. For this reason, for multivariate data analysis it is important to obtain accurate information from the spectra. Multivariate data analysis consists of two processes: spectral preprocessing and calibration model development (17). Different calibration models, such as principal component regression (PCR) (25), partial least squares (PLS) (9,12), artificial neural network (ANN) (8), and support vector regression (SVR) (5), are used in quantitative calibration analysis. In a population of 200–300 samples, a small subset of samples may be identified for reference analysis and calibration development. Such samples are selected by the software based purely on spectral information. This sample set is designated as the calibration sample set.

RESULTS

Reference analyses of oat and barley flour standards employing the β-glucan assay kit yielded values of 4.1 and 7.98%, respectively, and a coefficient of variation values of 1.82 and 1.64%, respectively, based on repeated analyses (N = 49). The reported values for the control oat and barley flours were 4.1 and 8.0%, respectively. Our results showed good accuracy and precision in laboratory implementation of the AACCI procedure for β-glucan content. The inclusion of barley and oat flour standards in the day-to- day analyses provided an excellent tool for quality assurance and served as a measure of confidence in minimized laboratory error.

NIRS Calibration Development Using Ground and Whole Oat Groat Samples

The graphs in Figures 2–4 illustrate the accuracy of the NIRS calibrations for estimation of β-glucan, protein, and oil contents in ground oat groats. Reference values for these constituents were plotted against their corresponding NIRS estimates. Both calibration sample sets (Figs. 2A, 3A, and 4A) and validation sample sets (Figs. 2B, 3B, and 4B) for each of the three oat constituents showed strong correlations between reference and predicted values. Validation sample set plots effectively showed how NIRS estimates were close to the true values, even in unknown samples. The graphs in Figures 5–7 illustrate the accuracy of the NIRS calibrations for estimation of β-glucan, protein, and oil contents in

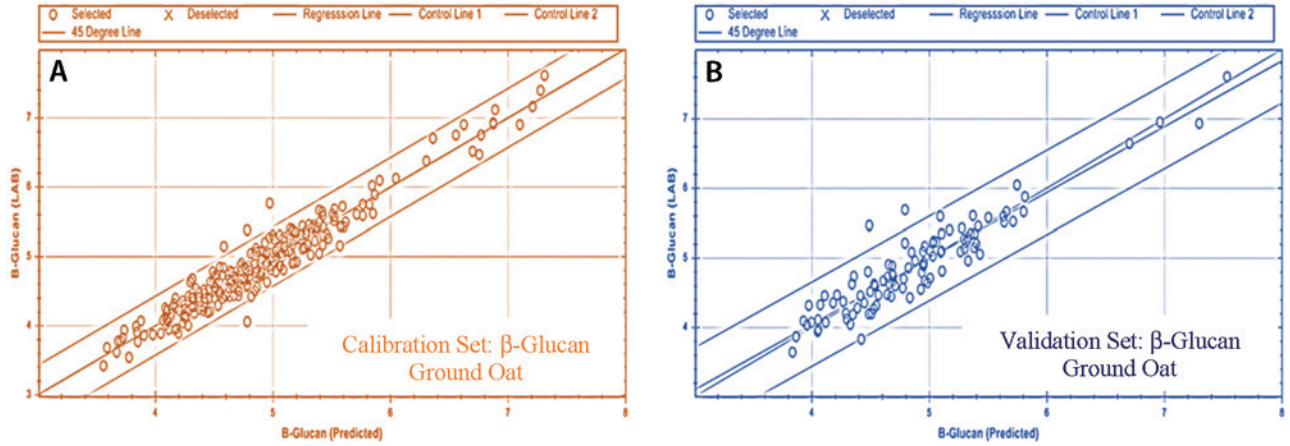

Fig. 2. Ground oat groat β-glucan calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of β-glucan content.

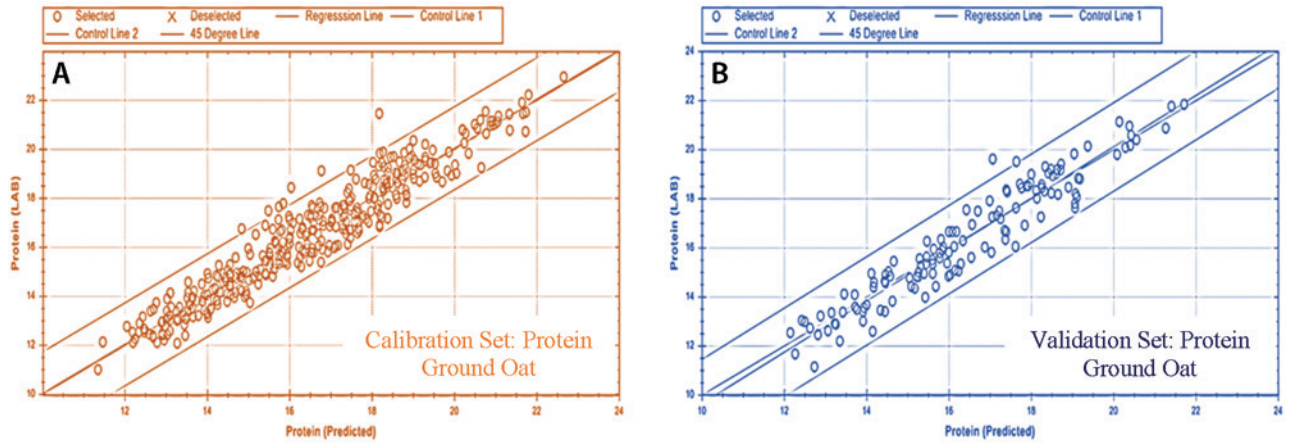

Fig. 3. Ground oat groat protein calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of protein content.

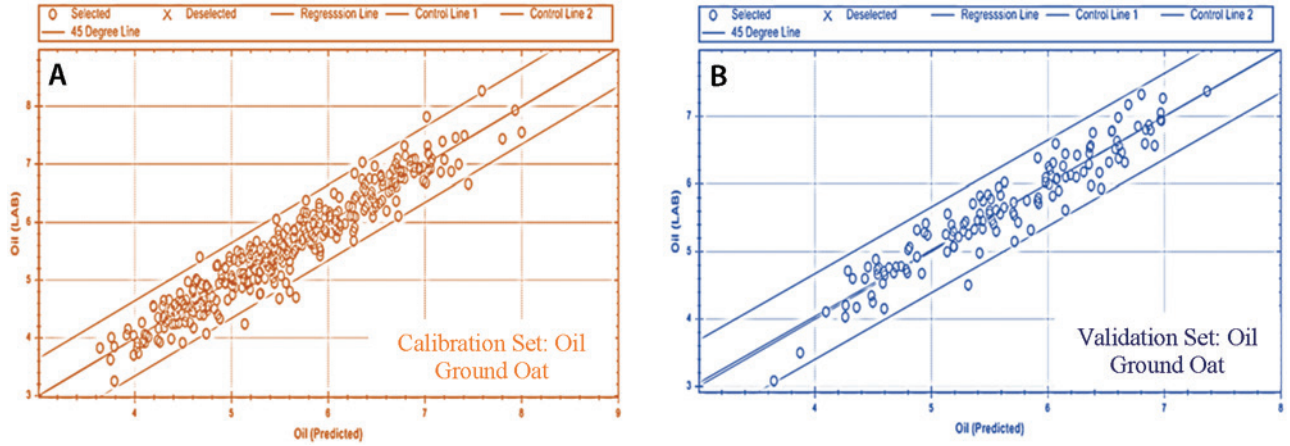

Fig. 4. Ground oat groat oil calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of oil content.

whole oat groat samples. Reference values for these constituents were plotted against their corresponding NIRS estimates for both the calibration (Figs. 5A, 6A, and 7A) and validation (Figs. 5B, 6B, and 7B) sample sets. The graphs in Figures 2–7 also provide an idea of the range of occurrence of the three analytes. Strong predictive NIRS calibration equations require the existence of a large range of target nutrient concentrations that encompass diverse growing environments and broad genetic variability. Robust and effective NIRS calibrations, thus, may require longitudinal evaluation of crop composition and large sample numbers, which in turn, require painstaking reference analysis. All samples in our study, whether they were used in calibration development or in validation of the methods, were analyzed chemically or enzymatically as well as by NIRS methods.

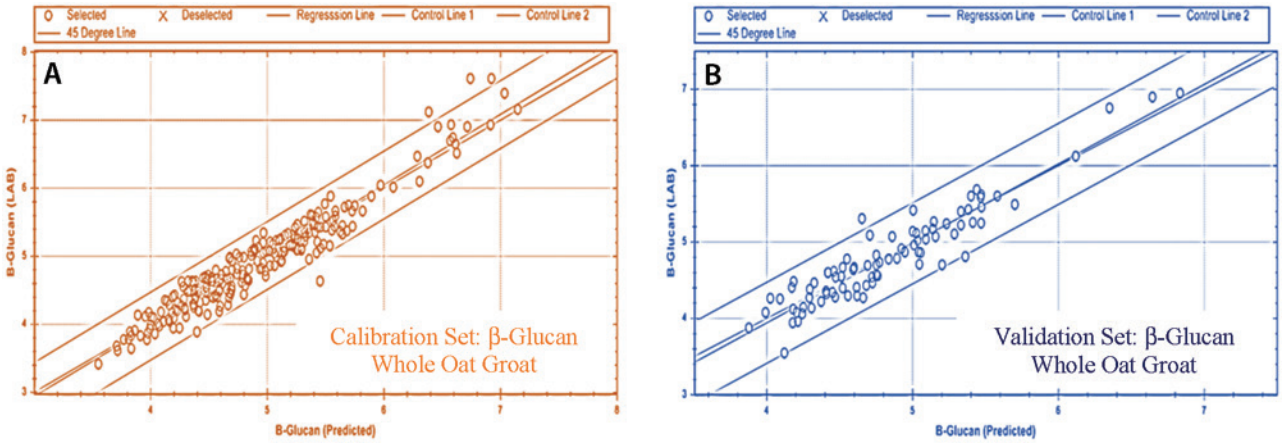

Fig. 5. Whole oat groat β-glucan calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of β-glucan content.

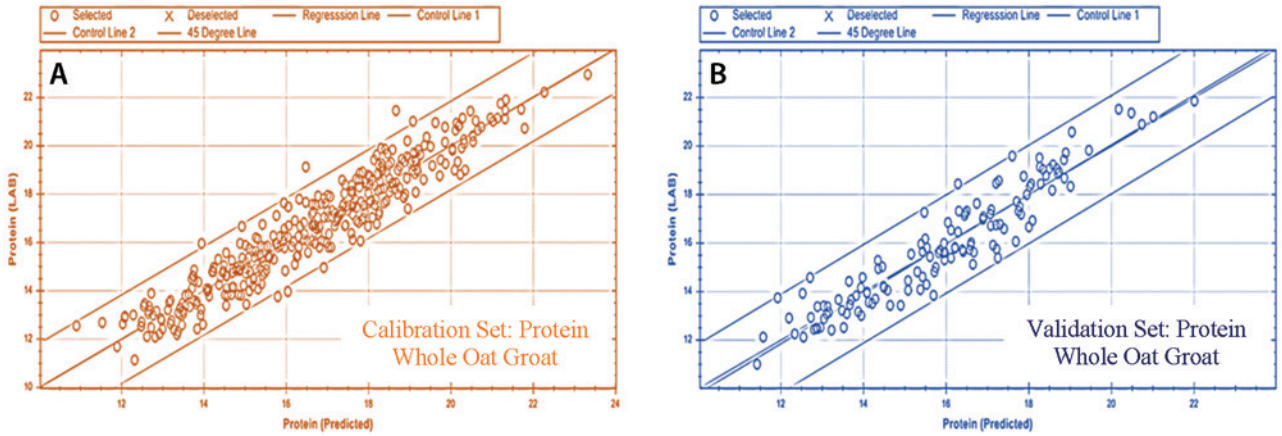

Fig. 6. Whole oat groat protein calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of protein content.

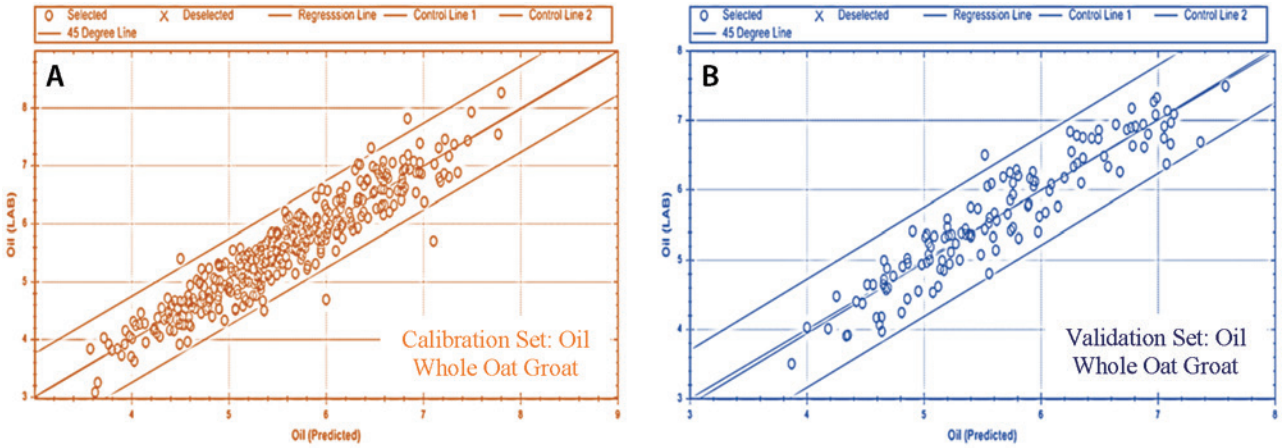

Fig. 7. Whole oat groat oil calibration (**A**) and validation (**B**) sample sets—correlation between reference and near-infrared reflectance spectroscopy (NIRS) estimates of oil content.

NIRS calibration and validation statistics for β-glucan, protein, and oil content estimation were computed using PLS analysis (Tables I–III). The NIRS terms define the effectiveness of the calibration as well as the validation of the calibration equations using sample sets that were independent of calibration development. Good predictive equations were developed for the three constituents in both ground and whole oat groat matrices.

The accuracy of NIRS predictive equations was based on coefficients of determination (R^2), standard errors of calibration (SEC), standard errors of cross-validation (SECV), standard error of prediction (SEP), and one minus variance ratio (1-VR, where VR is explained by variance divided by total variance). Ideal R^2 and 1-VR values approaching 1.0 and low standard errors are indicative of good calibrations. An ideal R^2 of 1.0 in-

Table I. Near-infrared reflectance spectroscopy (NIRS) calibration and validation statistics on β-glucan content in oats[a]

	Calibration Set						Validation Set				
Constituent	***N***	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	***N***	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Ground oat	308	4.84	0.94	0.16	0.22	0.88	104	4.89	0.87	0.001	0.24
Whole oat	247	4.87	0.93	0.18	0.23	0.89	85	4.82	0.89	–0.025	0.21

[a] RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross-validation; 1-VR: one minus variance ratio; SEP: standard error of prediction; RSQ_{val}: coefficient of correlation.

Table II. Near-infrared reflectance spectroscopy (NIRS) calibration and validation statistics on protein content in oats[a]

	Calibration Set						Validation Set				
Constituent	***N***	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	***N***	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Ground oat	394	16.35	0.94	0.61	0.64	0.93	134	16.40	0.91	–0.052	0.72
Whole oat	394	16.59	0.92	0.70	0.80	0.89	133	15.88	0.89	–0.045	0.81

[a] RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross-validation; 1-VR: one minus variance ratio; SEP: standard error of prediction; RSQ_{val}: coefficient of correlation.

Table III. Near-infrared reflectance spectroscopy (NIRS) calibration and validation statistics on oil content in oats[a]

	Calibration Set						Validation Set				
Constituent	***N***	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	***N***	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Ground oat	385	5.52	0.93	0.23	0.26	0.92	133	5.64	0.91	0.018	0.25
Whole oat	394	5.52	0.90	0.27	0.30	0.88	133	5.62	0.88	–0.016	0.31

[a] RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross-validation; 1-VR: one minus variance ratio; SEP: standard error of prediction; RSQ_{val}: coefficient of correlation.

Table IV. Comparison of AACCI method and near-infrared reflectance spectroscopy (NIRS) prediction for β-glucan content in calibration and validation samples of ground and whole oats

		AACCI Method (%)		NIRS Prediction (%)	
Constituent	***N***	**Mean**	**SD**	**Mean**	**SD**
Calibration set					
Ground oat	313	4.84	0.66	4.84	0.64
Whole oat	251	4.90	0.69	4.90	0.74
Validation set					
Ground oat	104	4.90	0.67	4.90	0.66
Whole oat	85	4.82	0.63	4.84	0.57

Table V. Comparison of AACCI method and near-infrared reflectance spectroscopy (NIRS) prediction for protein content in calibration and validation samples of ground and whole oat groats

		AACCI Method (%)		NIRS Prediction (%)	
Constituent	***N***	**Mean**	**SD**	**Mean**	**SD**
Calibration set					
Ground oat	401	16.40	2.42	16.36	2.32
Whole oat	402	16.60	2.41	16.59	2.29
Validation set					
Ground oat	134	16.40	2.44	16.46	2.23
Whole oat	133	15.89	2.40	15.92	2.20

Table VI. Comparison of AOCS procedure and near-infrared reflectance spectroscopy (NIRS) prediction of oil content in calibration and validation samples of ground and whole oat groats

		AACCI Procedure (%)		NIRS Prediction (%)	
Constituents	***N***	**Mean**	**SD**	**Mean**	**SD**
Calibration Set					
Ground oat	402	5.53	0.91	5.53	0.88
Whole oat	402	5.53	0.89	5.53	0.84
Validation Set					
Ground oat	133	5.64	0.84	5.63	0.81
Whole oat	133	5.62	0.90	5.6	0.83

dicates highly correlated methods. The SEC is computed as the standard error of the difference between the true values of the constituents and their corresponding estimated values. Low SEC values are indicative of low errors in NIRS estimation. Validation sample sets are designed to test how accurately the models predict unknown values. Standard errors computed between true values and NIRS estimates in the validation sample set were designated as the SEP. SEC and SEP, thus, are parallel statistical terms corresponding to standard errors in the calibration sets and validation sets, respectively. High R^2 and coefficients of cross-validation along with low SEC or SEP are hallmarks of good NIRS predictive models.

Calibration for the estimation of β-glucan content in ground oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.94, 0.16, 0.22, and 0.88, respectively. Calibrations for estimated β-glucan content in whole oat groats yielded excellent R^2, SEC, SECV, and 1-VR values of 0.93, 0.18, 0.23, and 0.89, respectively. Calibration for the estimation of protein content in ground oat groats yielded R^2, 1-VR, SEC, and SECV values of 0.93, 0.93, 0.61, and 0.64, respectively. Calibration for the estimation of protein content in whole oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.92, 0.70, 0.80, and 0.89, respectively. Calibration for the estimation of oil content in ground oat groats yielded higher R^2 and 1-VR values of 0.93 and 0.92, respectively, and lower SEC and SECV values of 0.23 and 0.26, respectively. Calibration for the estimation of oil content in whole oat groats yielded R^2, SEC, SECV, and 1-VR values of 0.90, 0.27, 0.30, and 0.88, respectively.

Table VII. Analyses of variance in β-glucan, protein, and oil contents of oats grown in South Dakota in 2015 and 2016

		Official Method[a]			NIRS Method (Ground Oat Groat)[b]			NIRS Method (Whole Oat Groat)[c]		
Constituent	df	Mean Square	*F* Ratio	Significance Level[d]	Mean Square	*F* Ratio	Significance Level[d]	Mean Square	*F* Ratio	Significance Level[d]
β-Glucan										
Cultivar	15	0.188745	104.458	***	5.6659	160.095	***	4.7967	199.306	***
Location	3	0.054769	30.311	***	1.2325	34.826	***	0.913	37.9369	***
Year	1	0.039736	21.9911	***	0.7165	20.2459	***	0.3503	14.5535	***
Cultivar × location	45	0.003021	1.6718	*	0.0632	1.7868	*	0.0656	2.7246	***
Location × year	3	0.020693	11.4521	***	0.4456	12.59	***	0.4076	16.9376	***
Cultivar × year	15	0.003602	1.9937	*	0.0554	1.5656		0.0575	2.3904	**
Cultivar × location × year	43	0.001341	0.742		0.0372	1.0506		0.0365	1.5151	
Protein										
Cultivar	15	0.000117	10.699	***	6.22	8.0097	***	5.53	17.4145	***
Location	3	0.00027	24.7656	***	19.49	2.51E+01	***	25.13	79.0605	***
Year	1	0.006655	611.067	***	506.52	6.53E+02	***	451.43	1,420.47	***
Cultivar × location	45	1.91E-05	1.7496	*	0.87	1.1177		0.72	2.2603	**
Location × year	3	0.000725	66.6074	***	28.01	3.61E+01	***	36.53	114.933	***
Cultivar × year	15	1.56E-05	1.4308		0.63	0.8102		0.58	1.8297	
Cultivar × location × year	43	1.26E-05	1.1577		0.56	0.72		0.64	2.0073	**
Oil										
Cultivar	15	9.3077	155.1	***	8.738	335.7736	***	7.2278	99.1164	***
Location	3	8.9094	148.461	***	6.6824	256.7857	***	7.8262	107.323	***
Year	1	0.2936	4.8916	*	0.3722	14.3011	***	0.6265	8.5914	**
Cultivar × location	45	0.1368	2.2789	**	0.1216	4.6743	***	0.135	1.8515	*
Location × year	3	6.8996	114.971	***	6	230.561	***	4.4507	61.0341	***
Cultivar × year	15	0.2544	4.2385	***	0.0819	3.1464	***	0.0721	0.9888	
Cultivar × location × year	43	0.111	1.8497	*	0.0721	2.7721	***	0.0907	1.2441	

[a] AACCI Approved Methods 32-23.01 for β-glucan analysis and 46-30.01 for protein analysis (1); AOCS Standard Procedure Am 5-04 for oil analysis (3).
[b] Near-infrared reflectance spectroscopy method for ground oat groats.
[c] Near-infrared reflectance spectroscopy method for whole oat groats.
[d] ***, **, and * indicate significant at $P = 0.001$, 0.01, and 0.05, respectively.

High R^2 and 1-VR and low SEC and SECV values, thus, provide evidence supporting the accuracy and precision of calibration models developed for estimation of β-glucan, protein, and oil contents in oats. The study shows that NIRS is an efficient technology for oat quality measurement for high-throughput breeding programs and in oat processing.

Side-by-side comparisons of overall means for β-glucan, protein, and oil contents in all oat samples used in the study are provided in Tables IV–VI. They show how well NIRS estimations of β-glucan, protein, and oil contents matched AACCI and AOCS methods of analysis (or true values) for the same samples. These results show that NIRS-derived β-glucan, protein, and oil content estimations were not statistically different from their corresponding chemically determined values for the same samples. In our study, almost all of the samples were used in both NIRS method development and in reference analysis.

Analysis of variance (ANOVA) statistics, employing chemical reference data and showing significant effects of cultivar (genetics), growing location, and growing year on the variability of β-glucan, protein, and oil contents of oats grown in the South Dakota oat breeding program during the 2015 and 2016 crop years, are provided in Tables VII. All three factors showed a statistically significant influence on data variability. Similar ANOVA tables generated with NIRS methods showed the same results for the influence of cultivar, growing location, and growing years (not included in this article).

Oat cultivar rankings based on β-glucan, protein, and oil contents, as measured by AACCI and NIRS methods (whole oat groat and ground oat groat) are provided in Tables VIII–X. The rankings visibly demonstrate the effectiveness of the NIRS ground whole oat groat predictive calibrations in discriminating between named cultivars grown in South Dakota in two years on the basis of β-glucan, protein, and oil contents.

CONCLUSIONS

- **Calibrations** – High R^2 and 1-VR and low SEC and SECV values indicate high accuracy and precision of calibration models developed for estimation of oat β-glucan, protein, and oil contents.
- **Validation** – Similarly, high R^2 and low SEP values in the validation data set show the calibration models were effective in estimating the β-glucan, protein, and oil contents of oat samples that were not part of the calibration set.
- **Cost-Effectiveness** – NIRS is cost-effective for evaluating large numbers of samples in view of the reduced need for labor-intensive reference analyses. At a cost of $330 per enzyme assay kit, and with 50 samples/kit for duplicate determinations, more than 500 determinations were made in this study for β-glucan content alone. This translates to $3,300 in enzyme assay costs for β-glucan analysis, not including analyst costs and investments in time and effort.
- **Robustness** – The inclusion of a large number of samples from varied growing regions and with a varied range of analyte concentrations strengthened the predictive power of NIRS for oat quality determination.
- **Single Analytical Platform** – This study demonstrates that NIRS can serve as a single platform for rapid multicomponent analysis of oat quality determinants.

Table VIII. Ranking of oat cultivars based on β-glucan content in 2015 and 2016 South Dakota samples[a]

AACCI Reference Analysis		NIRS[b] Analysis (Ground Oat Groat Calibration)		NIRS[b] Analysis (Whole Oat Groat Calibration)	
GMI423	6.93 a	GMI423	6.86 a	GMI423	6.60 a
Newburg	5.35 b	Newburg	5.37 b	Newburg	5.35 b
Jury	5.20 bc	Horsepower	5.24 bc	Jury	5.30 b
Horsepower	5.14 c	Jury	5.14 cd	Horsepower	5.14 c
Rockford	5.13 c	Rockford	4.98 de	Rockford	5.06 c
Goliath	4.92 d	Goliath	4.93 e	Souris	4.87 d
Souris	4.89 de	Souris	4.88 e	Hayden	4.87 d
Hayden	4.75 ef	Hayden	4.86 e	Goliath	4.77 de
Deon	4.65 fg	Deon	4.62 f	Deon	4.68 e
Shelby427	4.53 gh	Shelby427	4.49 fg	Streaker	4.46 f
Streaker	4.38 hi	Streaker	4.36 gh	Shelby427	4.42 f
Stallion	4.32 ij	Stallion	4.31 hi	Stallion	4.35 fg
Jerry	4.18 j	Jerry	4.24 hi	Jerry	4.25 gh
SD110466	4.17 jk	SD110466	4.16 ij	SD110466	4.17 h
Colt	4.00 kl	Colt	4.02 jk	Colt	4.13 h
Natty	3.90 l	Natty	3.99 k	Natty	3.97 i

[a] Means followed by the same letter within each column are not significantly different from each other ($P < 0.05$).
[b] NIRS: near-infrared reflectance spectroscopy.

Table IX. Ranking of oat cultivars based on protein content in 2015 and 2016 South Dakota samples[a]

AACCI Reference Analysis		NIRS[b] Analysis (Ground Oat Groat Calibration)		NIRS[b] Analysis (Whole Oat Groat Calibration)	
SD110466	17.24 a	SD110466	17.47 a	SD110466	17.53 a
Stallion	16.88 ab	Stallion	17.23 ab	Jerry	16.90 b
Jerry	16.79 ab	Jerry	17.20 ab	Streaker	16.83 b
Streaker	16.79 ab	Streaker	16.59 bc	GMI423	16.78 b
GMI423	16.49 b	GMI423	16.58 bc	Stallion	16.71 b
Goliath	15.91 c	Shelby427	16.20 cd	Goliath	16.06 c
Deon	15.87 c	Deon	16.08 cde	Deon	16.04 c
Shelby427	15.83 c	Goliath	15.82 cdef	Jury	15.90 cd
Horsepower	15.68 cd	Horsepower	15.80 cdef	Shelby427	15.86 cd
Natty	15.42 cd	Jury	15.79 cdef	Rockford	15.82 cde
Rockford	15.41 cd	Rockford	15.67 def	Natty	15.61 cdef
Jury	15.40 cde	Newburg	15.48 def	Hayden	15.43 def
Colt	15.37 cde	Souris	15.48 def	Colt	15.42 def
Hayden	15.15 de	Natty	15.42 def	Horsepower	15.42 def
Newburg	15.15 de	Hayden	15.33 ef	Souris	15.34 ef
Souris	14.77 e	Colt	15.24 f	Newburg	15.31 f

[a] Means followed by the same letter within each column are not significantly different from each other ($P < 0.05$).
[b] NIRS: near-infrared reflectance spectroscopy.

Table X. Ranking of oat cultivars based on oil content in 2015 and 2016 South Dakota samples[a]

AOCS Reference Analysis		NIRS[b] Analysis (Ground Oat Groat Calibration)		NIRS[b] Analysis (Whole Oat Groat Calibration)	
Rockford	6.91 a	Rockford	6.89 a	Rockford	6.77 A
Stallion	6.49 b	Stallion	6.54 b	Stallion	6.45 b
GMI423	6.46 b	Streaker	6.29 c	GMI423	6.37 bc
Streaker	6.36 bc	GMI423	6.27 c	Hayden	6.29 bcd
Hayden	6.23 cd	Hayden	6.26 cd	Streaker	6.12 cde
Jury	6.20 cd	Jury	6.22 cd	Jury	6.06 def
Newburg	6.10 d	Newburg	6.11 de	Newburg	6.03 efg
Shelby427	6.07 d	Horsepower	6.00 e	Shelby427	5.85 fg
Horsepower	5.78 e	Shelby427	5.98 e	Horsepower	5.82 g
Deon	5.40 f	Deon	5.55 f	Deon	5.42 h
Goliath	5.26 fg	Goliath	5.29 g	Goliath	5.31 hi
Souris	5.07 g	Souris	5.07 h	Souris	5.08 ij
Jerry	4.66 h	Jerry	4.74 i	Jerry	4.83 jk
Colt	4.54 h	Colt	4.40 j	Colt	4.82 k
SD110466	4.25 i	SD110466	4.35 j	SD110466	4.39 l
Natty	3.99 j	Natty	4.10 k	Natty	4.10 m

[a] Means followed by the same letter within each column are not significantly different from each other ($P < 0.05$).
[b] NIRS: near-infrared reflectance spectroscopy.

- **Efficiency** – Effective NIRS calibrations for whole oat groats represent a significant advancement in view of the elimination of the need to grind samples.

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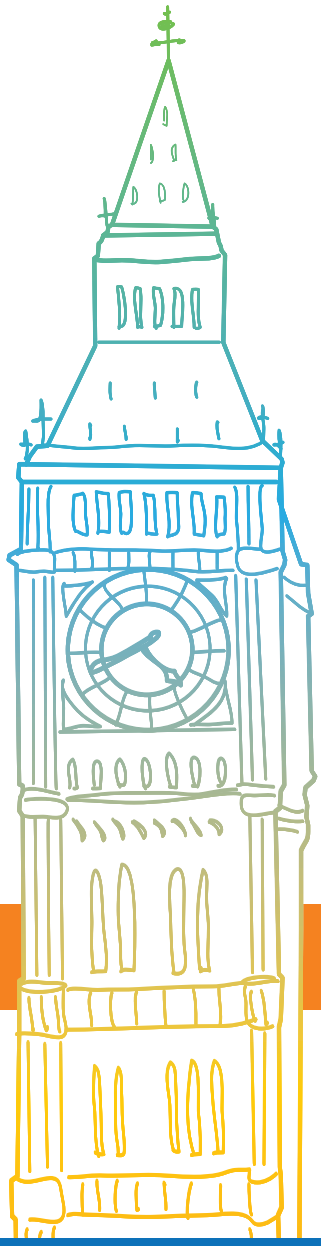
CEREALS & GRAINS 18



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