Calibration and field standardization of Foss Grainspec analyzers for corn and soybeans

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Introduction

United States grain handling and trade has traditionally been done on a commodity basis. Most grain quality tests under the present grain standards are measures of physical condition. Though these tests serve as a basis for trading of generic commodity grain, their relationship to end-use value is hard to identify. Near infrared (NIR) technology has introduced a fast and economical method of measuring intrinsic qualities important to both processor and consumer.

The use of near infrared spectroscopy for measurement of moisture content in grain and seeds was introduced by Norris and Hart.[1] It is now used to measure a wide variety of composition characteristics in biological materials. Near infrared reflectance technology allowed food processors and feed analysts to determine the nutritional composition of ground samples in a few minutes. Though economical and useful for quality control in processing, NIR has not been widely accepted by grain handlers because of the time and labor required for grinding and sample preparation.

Successful segregation of grains for intrinsic qualities depends on the ability of grain handlers to identify characteristics important to the end-user. Because intrinsic quality variation in a given geographic location is caused mainly by varietal and agronomic factors, the maximum differentiation exists as farmers deliver to country elevators. The economic importance of composition has been shown for both corn[2] and soybeans.[3] Because soybean protein and oil contents have a large impact on the amount of products derived from solvent extraction, the Federal Grain Inspection Service (FGIS) introduced soybean oil and protein (basis 13% moisture) as official criteria on September 4, 1989.[4] Country elevators have recently attempted segregation of high-value soybeans[3] and genetically modified corn.[5]

Recent developments in both near infrared reflectance and transmittance analyzers have created the ability to analyze whole grain samples for moisture, crude protein, starch, fiber and oil content in less than one minute in an elevator setting. Measurement of additional constituents may also be possible. Whole-grain near infrared technology is widely used for similar measurements in worldwide trade of wheat and other small grains. Corn and soybeans are more difficult to analyze because of their irregular shape, larger size, and range of color variation.

Although near infrared analyzers are manufactured to rigorous specifications, no two instruments have exactly the same spectral characteristics, nor can they use the same calibration without being adjusted (standardized) for each grain being tested. The issue of uniformity in testing is a major concern of grain handlers. Consistency in trade is often more critical than absolute accuracy.

This study developed a calibration and calibration transfer (for standardization) protocol for the Foss Grainspec (Foss Food Technology, Eden Prairie, MN) to predict moisture, protein, oil, starch, and density in whole corn and moisture, protein, oil and fiber in whole soybeans.

Materials and methods

Foss Grainspec

The Grainspec is a near infrared transmission analyzer manufactured by Foss Electric Development, Wheldrake, York, UK. The analytical results are shown on a display and the information can be transmitted to a printer. Data can also be transmitted to a serially connected computer via the proprietary Tracker data collection software and/or to a distant host computer connected with a modem.

Each Grainspec spectrum consists of 33 wavelengths generated from 11 primary filters and a "tuning wheel", which produce three measurement points at approximately 8 nm intervals from each filter. This produces a spectrum from 810.5 nm to 1075 nm. The sample is poured into the hopper and taken by a conveyor belt in discreet subsamples, which are flushed automatically after being scanned. The purpose of the multiple subsample measurements is to reduce sampling error. We used the average of 10 subsamples for calibration purposes and the average of 5 subsamples for field predictions. Ten subsamples require 400–500 g of grain, 5 subsamples only 200–250 g. The choice of subsample numbers is a compromise between precision and speed.

Samples and scan data

Samples of corn and soybeans were collected at harvest by the Grain Quality Laboratory, Iowa State University (ISUGQL), Ames, Iowa, during the years 1987–1993, and were stored in sealed glass jars at 2°C. These samples represented variations in genetics, geography, growing conditions and physical characteristics. The master Grainspec (serial no. 3549) arrived at our laboratory in the summer of 1992. Samples from years 1987–1991 were scanned at that time. These samples had all been dried to below 15% moisture. Thereafter, samples were scanned at harvest moistures in the master Grainspec (3549). Stabilization co-master Grainspecs (serial nos. 5174, 5487, RD02) were added before the 1993 harvest. Moisture content of each sample was determined by the air-oven method[6] at the time each scan was taken. Reference values on selected samples for each of the measured constituents, were obtained by the following analyses:

Corn moisture (air-oven), done by ISUGQL[6]
Soybean moisture (AOCS AC 2-41), done by ISUGQL[7]
Corn protein (AOCS Ba 4d-90) done by Woodson–Tenent, Des Moines, IA[8]
Soybean protein (AOCS AC 4-91), done by Woodson–Tenent, Des Moines, IA[9]
Corn oil (AOCS Ba 3-38), done by Woodson–Tenent , Des Moines, IA[10]
Soybean oil (AOCS AC 3-44), done by Woodson–Tenent, Des Moines, IA[11]
Soybean fiber (AOCS Ba 6-84), done by Woodson–Tenent, Des Moines, IA[12]
Corn starch (CRA G-28), done by Woodson–Tenent, Des Moines, IA[13]
Corn density, done by ISUGQL[14]

All samples collected from 1987–1991 had been previously chosen for wet chemistry analysis solely by the combination of constituents matrix (CCM). These samples had been collected to calibrate ground-grain near infrared analyzers. Reference chemical analysis (corn moisture,

protein, oil and starch; soybean moisture, protein, oil and fiber) was redone on the 1987–1991 samples. In 1993, principal component analysis (PCA) of spectral data was used to select calibration samples for chemical analysis. Scans from 1987–1993 samples with chemistry were then pooled and subjected to PCA, which yielded 95 spectrally significant corn samples and 69 spectrally significant soybean samples.

The CCM, a factorial combination of constituent values,[15] was used to augment the spectrally selected samples. Each constituent was scored to have a high, medium, or low value on a constant-moisture basis. The three ranges are given in Table 1, along with the percentage of cells filled with at least one sample. A calibration with four constituents would have an 81-cell grid (3^4 = 81). Some of the combinations were biologically or practically impossible to find (e.g. high in all constituents). In our calibrations, 90% of the cells had one or more calibration samples in them. Spectral selection filled most, but not all, possible cells, which is why the manual CCM evaluation after the spectral sample selection was necessary.

Calibration development

The calibration process started with the base calibration, using only scans taken on the master Grainspec 3549, and was completed with a final calibration that included the base calibration set plus "stabilization samples" and "temperature samples". Both calibrations were derived by Partial Least Squares analysis (PLS), using the Tracker calibration software. Records were kept to

Table 1. Combination of constituents matrix.

Constituent	Defining range, by category (number of samples in base calibration)		
	High	Medium	Low
	Corn[a]		
Moisture (%)	>22%	12%–22%	<12%
Protein (%)	>9%	7%–9%	<7%
Oil (%)	>5%	3.5%–5%	<3.5%
Starch (%)	>62%	58%–62%	<58%
Density (g cm^{-3})[b]	>1.300	1.240–1.300	<1.240
	Soybeans[a]		
Moisture (%)	>13%	9%–13%	<9%
Protein (%)	>37%	33%–37%	<33%
Oil (%)	>19.5%	16.5%–19.5%	<16.5%
Fiber (%)	>5.5%	4.0%–5.5%	<4.0%

[a]Corn basis 15% moisture; soybean basis 13% moisture.
[b]Not included in the sample selection process.

document outlier rejections. Less than 5% of the samples were rejected in either the base or the final calibrations.

Stabilization samples (20–30) were scanned on the co-master Grainspecs (5174, 5487, RD02) at room temperature. Temperature samples (eight) were scanned on the master Grainspec (3549) at various sample/instrument temperature combinations. The base calibration was used to generate "reference" values for the stabilization and temperature samples in the final calibration sample set. The purpose of the stabilization and temperature sets was to compensate for these effects of varying temperatures and instrument differences, not to add more chemical data. This process has the same purpose as the stability file concept of Shenk and Westerhaus,[16] in which master scans are subtracted from modified condition scans with the difference scan given zero reference values. An independent set of samples were scanned on the Grainspec 3549 to be used as a validation set for both the base and final calibrations.

The 20–30 stabilization samples were manually selected to have low standard error when predicted on the base calibration and to represent a variety of optical and intrinsic characteristics. The instrument temperature/sample temperature variation was added to the calibration by including master scans of eight samples of each grain at factorial combinations of three instrument temperatures (5°C, 25°C and 45°C) and three sample temperatures (5°C, 25°C and 45°C). Reference values were predicted by the base calibration using the 25°C scans on the master Grainspec. The final calibration sample set contained the base calibration master scans, the stabilization

Table 2. Foss Grainspec 1993 calibrations for moisture, protein, oil and starch in whole corn. Instrument 3549.

Statistic	Constituent (range)			
	Moisture (5.0–40.0)	Protein[b] (5.0–14.0)	Oil[b] (2.5–8.5)	Starch[b] (48.0–68.0)
I. Calibration $n = 363$ all constituents				
R^2	98.8	91.6	94.0	96.5
SEC (%)	0.53	0.39	0.23	0.66
Mean	12.70	8.12	3.62	62.75
II. Validation[a] $n = 892$ moisture, $n = 392$ protein, oil, starch				
R^2	99.2	91.5	70.2	90.2
SEP (%)	0.64	0.38	0.27	1.15
Bias	0.14	0.05	0.09	–0.18
Mean	15.96	8.45	3.70	62.47
Slope	1.02	1.01	0.87	0.96
Intercept	–0.24	–0.10	0.57	2.06

[a] Includes temperature samples.
[b] 15% moisture basis.

co-master scans and the temperature master scans excluding the room–room temperature combination.

Both the base and final calibration data sets were developed using PLS regression, which groups spectral data into "factors". Factors are multiplicative combinations of independent variables (e.g. absorbance values) that are generated progressively and independently of one another. Factors were added until the next additional factor explained very little of the remaining variability in the model. Generally, one less factor was then used for the calibration. A practical objective when choosing factors was to have the standard error of calibration (SEC) and the standard error of prediction (SEP) close in value. Overfitting produced low SEC but deteriorated the SEP on samples not included in the calibration and magnified the effect of instrument differences when calibrations were transferred.

Transferability

The manufacturer's recommended procedure for the transfer of calibrations to slave units uses a "standardization map" developed specifically for each grain. The map is a set of 33 linear-regression derived equations relating the absorbance of the slave to the absorbance of the master at each wavelength. Standardization maps are supplied by Multispec based on wheat and

Table 3. Foss Grainspec 1993 calibrations for moisture, protein, oil and fiber in whole soybeans. Instrument 3549.

Statistic	Constituent (range)			
	Moisture (4.5–24.0)	Protein[b] (30.0–46.0)	Oil[b] (14.5–22.5)	Fiber[b] (3.4–7.3)
I. Calibration $n = 306$				
R^2	98.0	96.4	95.3	84.7
SEC (%)	0.34	0.42	0.36	0.22
Mean	10.14	36.94	17.74	5.22
II. Validation[a] $n = 474$ moisture, $n = 392$ protein, oil, fiber				
R^2	98.1	98.7	90.4	40.3
SEP (%)	0.44	0.54	0.47	0.44
Bias	–0.08	–0.01	0.00	–0.21
Mean	9.38	37.63	18.38	5.51
Slope	1.01	1.01	0.98	0.75
Intercept	–0.17	–0.33	0.29	1.34

[a] Includes temperature samples.
[b] 13% moisture basis.

Table 4. Field performance of 15 Grainspecs. 1993 calibration, 1994 corn and soybean crops.

Calibration ID	Constituent	Average bias (% pts)	Range (% pts)	Standard deviation[c]
0281411590	Corn moisture	–0.16	6.9–23.0	0.35
0281414146	Corn protein[a]	0.08	5.7–11.2	0.28
0281416195	Corn oil[a]	0.07	2.1– 9.0	0.26
0281417731	Corn starch[a]	–0.08	55.7–63.2	0.38
0281346037	Soybean moisture	–0.05	5.9–16.6	0.22
0281348355	Soybean protein[b]	0.00	32.3–39.5	0.27
0281350403	Soybean oil[b]	–0.01	15.6–20.3	0.25
0281360900	Soybean fiber[b]	0.07	3.8–5.9	0.20

[a]15% moisture basis.
[b]13% moisture basis.
[c]Relative to master Grainspec.

barley samples. We developed grain-specific maps for corn and soybeans. The Grainspec has one available map-storage position for each commodity.

Corn and soybean maps were generated by running 30 standardization samples in each Grainspec. The scans (optical data) were compared, slave to master, by using a linear regression program developed by Multispec. This program generates a slope and intercept adjustment for each wavelength of the slave instrument. A set of 10 check samples was run after loading the standardization map into a slave instrument to check the accuracy of the map.

The standardization sample set contained 30 samples each for corn and soybeans. These samples were chosen to represent diverse chemical and optical properties. Up to 10 additional soybean samples and 20 additional corn samples were added to the core set to extend the moisture range, if higher moisture grain was available. Optical diversity of the standardization set is critical for successful transfer.

Slave units, in which 1993 corn and soybean calibrations had been inserted, were located in country elevators during the 1994 harvest and post-harvest seasons. Several operators recorded data and sent samples to Iowa State. All were sent a set of 10 samples, which had been pretested in the master. The predicted values collected on each slave unit were returned to Iowa State. The key evaluation parameters for each crop and constituent were the mean and range of bias between slave and master and the standard deviation of differences. Samples used to compare on-site performance of slave units to the master did not have chemical data.

Calibration updates

Updates of corn and soybean calibrations were made after the 1994 harvest to include new varietal and environmental effects, as well as to expand the range of temperature compensation.

Table 5. Foss Grainspec 1994 calibrations for moisture, protein, oil, starch and density in whole corn. Instrument 3549.

Statistic	Constituent (range)				
	Moisture (5.0–50.0)	Protein[a] (5.0–14.0)	Oil[a] (2.5–9.0)	Starch[a] (35.0 – 68.0)	Density (1.18 – 1.35)
I. Calibration	$n = 354$ moisture, $n = 293$ all other constituents				
R^2	99.5	94.4	95.4	97.4	88.6
SEC (%)	0.52	0.40	0.28	1.01	0.013
Mean	15.40	8.18	3.88	59.82	1.27
II. Validation	$n = 1176$ moisture, $n = 462$ protein, oil, starch, $n = 431$ density				
R^2	99.5	90.1	83.1	92.5	71.6
SEP (%)	0.50	0.45	0.27	1.36	0.02
Bias	0.05	–0.01	0.10	–0.46	0.00
Mean	16.39	8.42	3.68	61.75	1.29
Slope	1.010	1.00	0.94	0.92	0.92
Intercept	–0.10	0.03	0.30	4.38	0.10
III. Temperature validation	$n = 300$				
SEC (%)	0.64	0.45	0.33	0.70	0.01

[a]15% moisture basis.

PCA was used to select approximately 100 new samples per crop for chemical analyses. From these, 45 corn and 40 soybean samples were added to the 1993 base calibration sample sets and new base calibrations were derived for each constituent.

Final calibrations were derived by using the new base sample sets with new (more diverse) stabilization samples and new temperature samples added. Six 1994 samples were chosen to replace six of the original stabilization samples. Temperature sample scans were carried out as described, with the addition of 31 new corn samples and 25 new soybean samples scanned at four sample temperatures (–15°C, 5°C, 25°C and 45°C). These samples had a much wider range of moisture than the original temperature set. Frozen samples were not included at corn moistures above 20% because they decreased the transferability of the calibrations (wavelength coefficients became large and unstable). A separate temperature validation set was formed from scans on the master and co-master Grainspecs at different instrument and sample temperatures not used in the final calibration.

Table 6. Foss Grainspec 1994 calibrations for moisture, protein, oil and fiber in whole soybeans. Instrument 3549.

Statistic	Constituent (range)			
	Moisture (5.0–23.5)	Protein[a] (30.0–45.5)	Oil[a] (13.5–22.0)	Fiber[a] (3.5–7.5)
I. Calibration $n = 363$ moisture, $n = 225$ protein, oil, fiber				
R^2	98.7	98.4	96.7	82.8
SEC (%)	0.34	0.36	0.31	0.25
Mean	10.85	36.9	18.03	5.03
II. Validation $n = 625$ moisture, $n = 329$ protein, oil, fiber				
R^2	98.5	97.3	88.1	41.3
SEP (%)	0.38	0.56	0.56	0.45
Bias	–0.12	–0.12	0.00	–0.05
Mean	9.97	37.65	18.34	5.33
Slope	1.02	0.97	0.96	0.75
Intercept	–0.35	0.86	0.67	1.32
III. Temperature validation $n = 277$, $n = 205$ protein, oil, fiber				
SEP (%)	0.36	0.38	0.31	0.23

[a] 13% moisture basis.

Results and discussion

Tables 2 and 3 contain summaries of the most pertinent descriptive data for the 1993 calibrations. The calibration data are reported on an as-is moisture basis. Moisture correction is done by the Grainspec, using the measured moisture.

The performance of the calibrations was acceptable when compared with previous values reported in the literature for other near infrared analyzers, although SEP's were approximately 0.1% point larger than those of monochromator-based whole-grain analyzers. The corn moisture calibration was clearly superior to the reported results for capacitance moisture meters[17,18] because variability did not change appreciably over the entire moisture range.

Calibration transfer data for the optically-standardized slave units are contained in Table 4. The optical-equation transfer procedure worked well, with only small and usually statistically insignificant bias differences between slaves and master. All slave data were collected by operators at elevators without direct supervision from either Iowa State or Foss Food Technology. Standard deviations between master and slave data were approximately half those between master and

reference chemical data, which demonstrates that consistency can be achieved in field situations without the calibration-specific slope-bias of predicted values that is widely used in the US Official Grain Inspection system.[19] Slope-bias of predicted values must be redone when calibrations are updated; optical standardization need not.

Although the fit of the 1993 calibrations was good within their range, a new base calibration was needed to extend the range, as was a wider range for the temperature and stabilization sets. Tables 5 and 6 show the statistics for the updated 1994 final calibrations.

Constituent and sample temperature ranges were broadened without a loss in accuracy. The 1994 calibrations include both normal and specialty varieties available or soon-to-be-available for production. There was no detectable difference in accuracy between the white, yellow, waxy and flint corn hybrids in the same calibration. The 1994 moisture calibrations (both corn and soybeans) have been approved as the legal-for-trade manufacturer original equipment by the United States National Type Evaluation Program.[20]

Conclusions

1. The step-by-step calibration protocol produced accurate calibrations while incorporating known influences in an organized manner.
2. Grainspec achieved standard errors of prediction for corn moisture, protein, oil and starch, of 0.50%, 0.45%, 0.27% and 1.36%, respectively and 0.02 g cm^{-3} for density. Grainspec achieved standard errors of prediction for soybean moisture, protein, oil and fiber of 0.38%, 0.56%, 0.56% and 0.45%.
3. The optical equation transfer process limited standard errors relative to the master to about half the calibration standard errors of prediction for samples tested at elevators by elevator personnel.
4. Instrument stability and temperature compensation (from -5°C to $+45^{\circ}$C) was achieved over the full range of constituent values, except for frozen corn above 20% moisture.

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