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Equivalency of near infrared transmission instruments for grain analyzers

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Equivalency of near infrared transmission instruments for grain analyzers

by

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Abstract

The Grain Inspection Packers and Stockyard Administration (GIPSA) has one approved near infrared transmission (NIRT) instrument for the official inspection of grains. This is believed to be more accurate multiple makes and models used. This study focused on determining if more than one make and model of transmission instruments can be used. Three National Type Evaluation Program (NTEP) approved NIRT instruments coded A, B, and C were evaluated for equivalence. To be equivalent, the variation of results of all the instruments would be less than or equal to that of one instrument make and model on a population of samples. The study used 5 copies of each instrument, for a total of 15 machines. The number of samples used were, 250 wheat, 100 barley, 145 soybeans, and 149 corn. The samples were passed through all 15 machines three times each. Results for wheat (protein), barley (protein), soybean (protein and oil), and corn (protein and oil) were collected using the NTEP approved calibrations for each unit, with and without slope-bias standardization. A least mean squares analysis partitioned the variance by A-A, A-B, B-B, B-C, and C-C. The instruments as set up were not equivalent because A-B, A-C, and B-C were significantly larger than the within brand comparisons A-A, B-B, and C-C ($p<0.0001$).

CHAPTER 1: GENERAL INTRODUCTION

Thesis Organization

This thesis is organized into three chapters. Chapter 1 contains the general introduction and a review of relevant literature on this topic of research. Chapter 2 is in the publication format of Cereal Chemistry is titled “Equivalency of near infrared transmission instruments as grain analyzers”. It outlines the methods and results of evaluating near infrared transmission instruments of different makes and models for equivalency. Chapter 3 then gives general conclusions and future recommendations. The study was supported by the United States Department of Agriculture, Grain Inspection Packers and Stockyards Administration.

Literature Review

Near infrared spectroscopy background

Near infrared spectroscopy is technology that uses near infrared light to determine composition of organic products. Organic molecule bonds naturally vibrate. When exposed to near infrared light, compounds exhibit selective response to a higher excitation level. Specific molecules reflect, transmit, or absorb the light, in relative amounts unique to different molecules. The wavelength at which the light is reflected or transmitted is detected by sensors. The near infrared light region lies between 750 nm and 2600 nm (Murrary & Williams, 1987). Figure 1 shows the position of near infrared light on the electromagnetic spectrum.

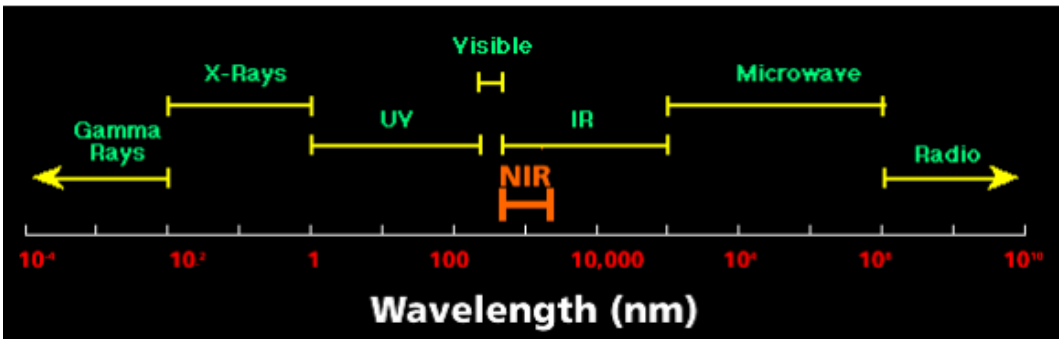

Figure 1: Near infrared light in the electromagnetic spectrum.

Source: (Analytical 2005)

A general setup of a near infrared spectroscopy instrument consists of a lamp, wavelength isolator, sample, and detector (Figure 2).

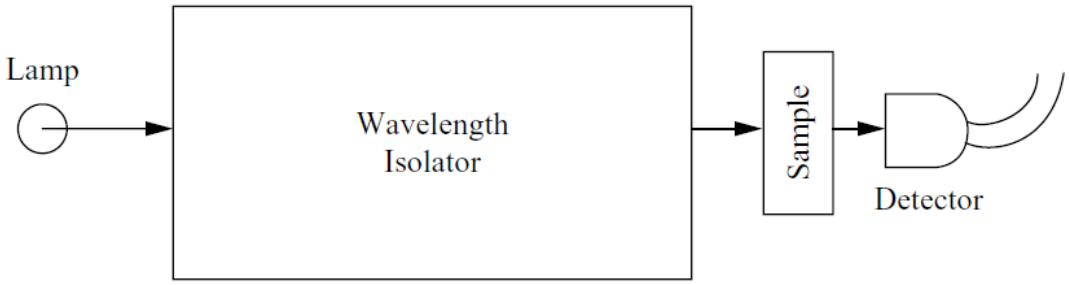

Figure 2: A general setup of a transmission NIRS instrument.

Source: (Ozaki, McClure, & Christy, 2006)

The lamp provides the light then there is a wavelength isolator that separates the light source from the sample at a fixed length. The sensor or detector is then positioned right behind the sample for a NIRT instrument. NIRS is used in many industries from agriculture (constituents in grain) to medical (hemoglobin).

There are many advantages to using near infrared spectroscopy when analyzing food or other products. Some advantages is include that it is a quick and non-invasive method. Some disadvantages of using NIRS technology is creating calibration equations tend to use large sets. When analyzing organic material this is especially important in the food industry a sample can be analyzed in either a ground or whole sample state. Disadvantages are the reliance on reference chemistry and calibration equations. Developing calibrations is a timely process and can introduce lots of error.

The worldwide market for near infrared spectroscopy instruments has been estimated at between $US 100 and $US 200 million annually(Ozaki et al., 2006). There are two types of NIRS instruments reflectance and transmittance (Figure 3).

NIR transmission instruments

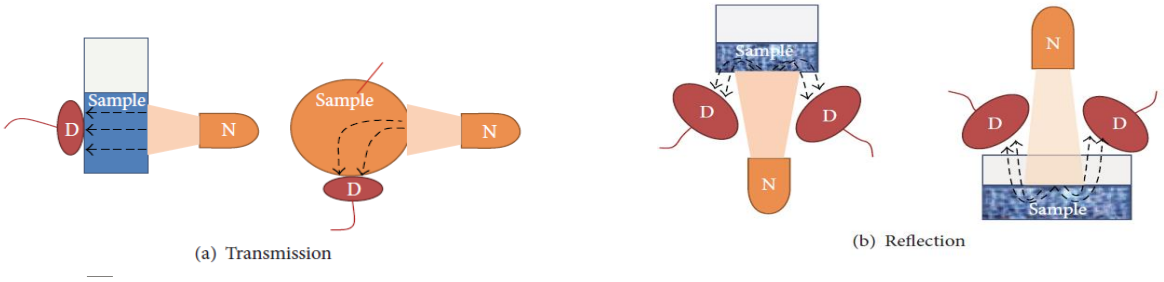

Figure 3: (a) NIR transmission (NIRT) (b) NIR reflection (NIRR)

N is the light source

D is the detection sensor

Source (Alander, Bochko, Martinkauppi, Saranwong, & Mantere, 2013)

The basic principle of NIRT is to have the light source on one side and the sensor on the other side across a fixed gap of sample. Reflection instruments have the sensor and light source on the same side

at a variable angle determined by sample surface. When reflection instruments analyze irregular samples such as whole grain samples the light reflecting off of the sample might not be picked up by the sensor. This is especially true for bigger grains such as soybeans or corn that might not be able to have a smooth surface area as smaller grains. Transmission instruments detect the light that is transmitted through the sample which is easier to contain and detect.

Calibration

A calibration for each grain and constituent is required for each model of NIRS. To develop a calibration, representative samples with a range of reference (lab) values are ran through the instrument. Spectral data taken from one sample that was ran through a Infratec 1241 (Foss Instruments NA, Eden Prairie, MN) is shown in Figure 4 with the x-axis being wavelength of near infrared light that is being measured compared to the y-axis which is absorption.

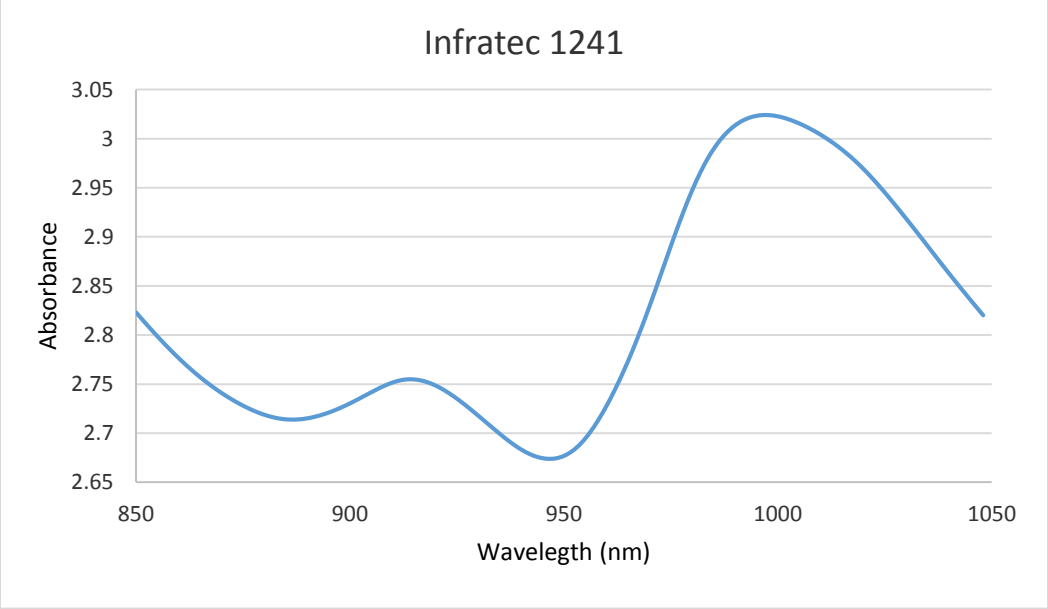

Figure 4: Spectra data for one sample run on the Infratec 1241.

Spectra are used to compare to reference values (usually laboratory chemistry tests) that give the "real" results. Multivariate statistics are used to develop the calibration equation that will be used in the instrument for measuring future samples. Samples are tested at near infrared wavelengths every 0.5 to 2.0 nm. This requires many coefficients used in the calibration equation. A large and broad range of samples is needed to make an accurate and robust calibration. Multiple linear regression (MLR), principal component regression (PCR), partial least squares (PLS), and artificial neural networks (ANN) are a few algorithms used to develop calibrations and need a minimum of 100 samples. Figure 5 is a graph of the reference verses the output of a NIRT instrument showing the wide range and amount of samples used to develop a calibration equation.

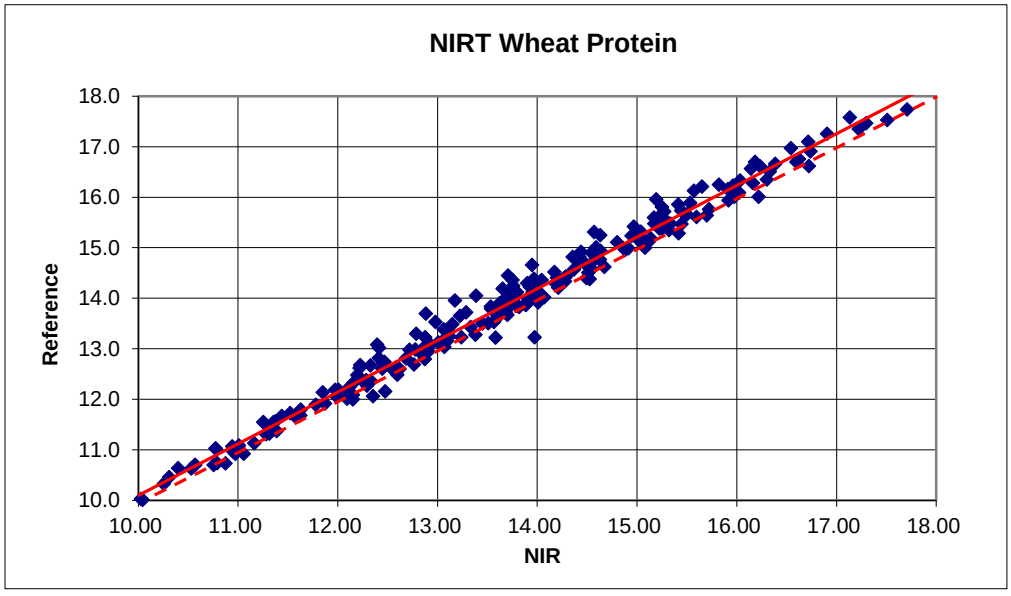

Figure 5: Example of near infrared transmission instrument wheat protein results. (12% MB)

Standardization is done to each calibration to compensate for small optical differences among copies of like instruments. This is done by running samples through the test instrument copy, and comparing by linear regression, values given by the instrument and the reference values The slope and

bias of standardization are then put into the test instrument to make the instrument more accurate. Standardization parameters are instrument copy specific.

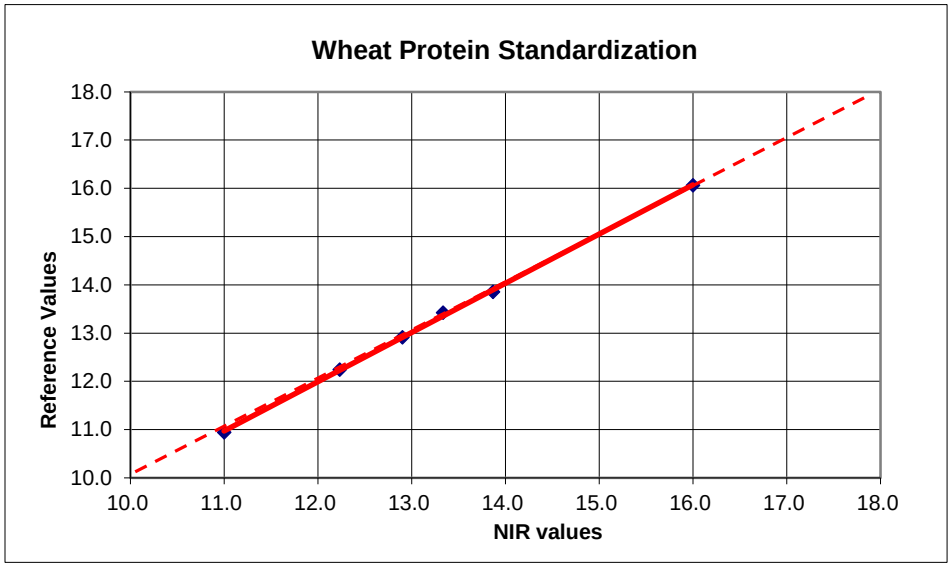

Figure 6: Example of NIRT instrument standardization for wheat samples

GIPSA background

Grain Inspection Packers and Stockyard Administration (GIPSA) is an agency of the United States Department of Agriculture (USDA). GIPSA is split into two parts; the Federal Grain Inspection Service (FGIS) and the Packers and Stockyards Program (PSP). FGIS focuses on fair facilitation of grain trade in domestic and international markets. FGIS offers weighing and inspection services, for all grain traded in and out of the United States, and operates under the U.S. Grain Standards Act (USGSA).

All grain that is exported has to be Officially inspected for all US Grade Factors for example protein levels (wheat only), moisture, foreign material, and test weight. The only mandatory Official test carried out by near infrared transmission instruments is protein in export wheat. For corn, soybean, and barley, the composition test is not mandatory for either exported or domestically traded grains. Voluntary services

are offered by FGIS to determine constituents in wheat, barley, soybeans, and corn. The only constituents that can be analyzed Officially are wheat (protein), barley (protein), soybean (protein and oil), and corn (protein and oil).

GIPSA uses the Foss Infratec Grain Analyzer, models 1225, 1226, 1227, 1229, and 1241 for Official testing. The Foss Infratec and two other transmission instruments are approved by the National Type Evaluation Program (NTEP). NTEP is under the direction of the National Conference of Weights and Measures. NTEP provides assurance that instruments are accurate, and eligible to be used for state-regulated trade that is outside inspections done by GIPSA Table 1 shows the acceptance and maintenance tolerances for NTEP for NIR grain analyzers.

Table 1: Acceptance and Maintenance Tolerances for NIR Grain Analyzers

Acceptance and Maintenance Tolerances for NIR Grain Analyzers				
Type of Grain	Constituent	Individual Samples (%)	Average for Five Samples (%)	Range of Five Retests (%)
Wheat	Protein	0.60	0.40	0.40
Barley	Protein	0.70	0.50	0.50
Soybeans	Protein	0.80	0.60	0.60
	Oil	0.70	0.50	0.50
Corn	Protein	0.80	0.60	0.60
	Oil	0.70	0.50	0.50

Source: (NIST 2015)

CHAPTER 2: EQUIVALENCY OF NEAR INFRARED TRANSMISSION INTRUMENTS AS GRAIN ANALYZERS

Introduction

The United States of America is the world leader in grain production with 80.8 million acres of corn, 81.8 million acres of soybeans, 47 million acres of wheat, and 3 million acres of barley(USDA National Agricultural Statistics Service, 2016). The Grain Inspection, Packers and Stockyards Administration (GIPSA) oversees the Federal Grain Inspection Services (FGIS). FGIS provides inspection services for domestic and international marketing of grains. FGIS also provides services mandated by the United States Grain Standards Act for export grains, including Official weighing of exported grain, inspection of exported grain, and testing exported corn for aflatoxin. For domestic trade, FGIS offers voluntary (on-demand) inspection and weighing services for wheat, barley, soybeans, and corn.

Around 30 million metric tons of wheat is exported annually from the United States every year (see Figure 7). The only grain constituent test mandated for export trade is protein in wheat. Wheat protein is important because it determines the best end use of wheat. There is a premium for certain amounts of protein; the premium varies with the different wheat classes. The Spring Wheat protein scale is +0.04 each (1/5) premium over 14%, +0.25 additional kicker at 15%, and -0.04 each (1/5) below 14% (Growers, 2011). Winter Wheat protein scale is +0.04 each (1/5) above 12% and -0.04 each (1/5) below 12% (Growers, 2011). GIPSA-FGIS uses near infrared transmission (NIRT) spectroscopy to measure protein in wheat. On an optional basis, FGIS will measure barley (protein), soybean (protein and oil), and corn (protein and oil)(GIPSA 2006).

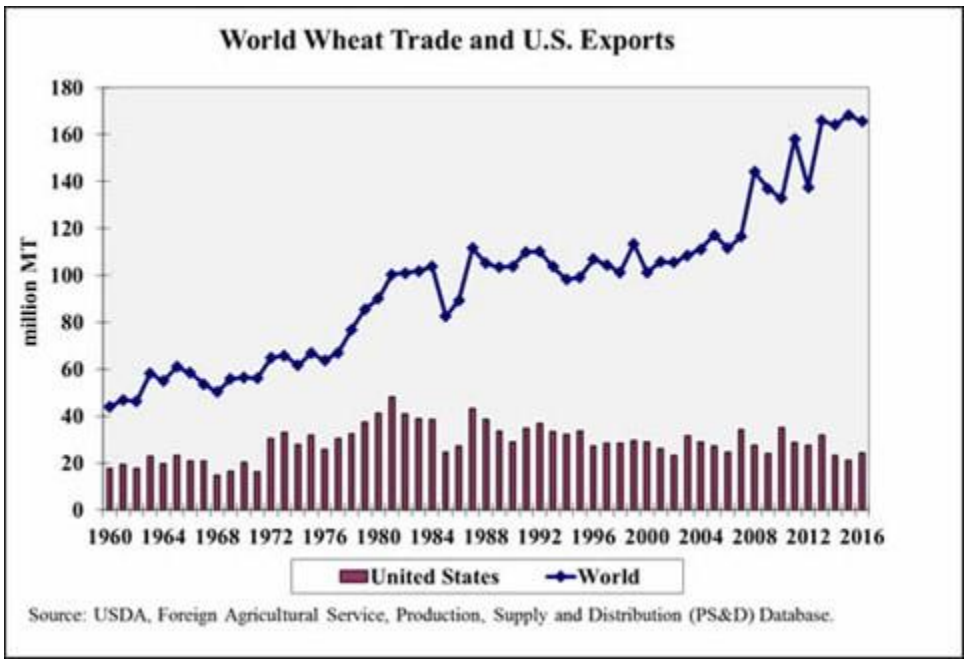

Figure 7: World wheat trade and United States wheat exports(USDA, 2016).

Near infrared spectroscopy is technology that uses near infrared light and detection sensors to determine organic material components. There are two types of NIRS instruments transmission and reflection instruments. Transmission units have light pass through a fixed path length of sample with sensors on the opposite side to capture the light that is not absorbed or reflected. Reflection instruments have the light source and the detectors on the same side to capture light reflected off the sample. NIRS technology is widely used because it is a quick and non-invasive procedure. There is a calibration made for each constituent/grain for a given brand of instrument. To establish a calibration, spectral data from selected samples are calibrated against the laboratory reference values. The reference values are usually determined from wet chemistry done in a lab. Instruments even with the same make and model can have subtle differences in optics. This will also affect the calibration equation that is the same for a make and model therefore standardization plays a key part in making instruments more accurate and equivalent(AACC 1999).

Currently, FGIS has one approved NIRT instrument to evaluate grain in the Official system for export and domestic markets. The approved models are 1225, 1226, 1227, 1229, and 1241 (GIPSA, 2006). It is assumed that using only one instrument make and calibration decreases variability in results across inspection points. Weaknesses of one approved instrument are lack of market competitiveness, slowed technology advances, and inability to make use of "the best instrument for the job" should there be others identified.

GIPSA also supports the general market (non-Official) approval of NIRS instruments operating National Type Evaluation Program (NTEP) (NTEP, 2016). NTEP has a program of the National Conference on Weight and Measurements (NCWM) (NTEP, 2016), which regulates many measuring devices for trade in states. The instrument used for Official testing is one of the three approved by the NTEP. The GIPSA National Grains Center Laboratory does the evaluation work for NCWM. NTEP evaluates calibrations for wheat (protein), barley (protein), soybean (protein and oil), and corn (protein and oil). These are the same product-constituent combinations that GIPSA offers in the Official System(GIPSA 2006).

Expanding the instrument pool would allow for more competitive markets for NIRS instrumentation. The objective of this study was to evaluate equivalency among the three NTEP approved models of NIRT instruments. The three instruments used only calibrations approved on the NTEP process. Wheat, barley, soybean, and corn samples were used to evaluate the performance of the instruments. Equivalency is defined as the variance of more than one instrument is less than or equal to that of instrument.

Materials and methods

Instruments

Three near-infrared transmission spectroscopy instruments were compared in this study. The three instrument brands were NTEP approved with five copies of each instrument brand, provided by the respective manufacturers. To maintain confidentiality, the instrument brands are referred to as A, B, and C. Table 2 shows specifications for instrument brands A, B, and C. All are monochromator-based units that pass the grain through the sample cell in a series of discreet steps (subsamples), in a fixed path length (cell width).

Table 2: Properties of the study instruments

	Spectral Data Range (nm)/ Calibration range	Increment (nm)	Path length used for Wheat and Barley (mm)	Path length for Soybeans and Corn (mm)
Instrument A	730-1100	0.5	18	30
Instrument B	850-1048	2.0	18	30
Instrument C	570-1100	0.5	18	25

The spectral data range is different for each instrument. Instrument C includes wavelengths in the visible light range.

Standardization samples

Standardization samples were provided by GIPSA and the Iowa State University Grain Quality Lab (ISU GQL) to determine slope and bias prior to analysis of evaluation samples. Slope and bias is the method used by all three manufacturers to do final alignment of instrument copies. There were six samples of wheat, five samples of barley, 20 soybean samples, and 30 corn samples. Moisture and protein were evaluated for wheat and barley; moisture, protein, oil, and fiber were evaluated for soybeans; moisture,

protein, oil, starch, and density were evaluated for corn. For the equivalence test, however, only the Official factors. Reference values were provided by the respective sample providers. Standardized and not standardized results were compared in the evaluation of equivalence.

Evaluation samples

GIPSA provided wheat and barley evaluation samples for which protein had been analyzed by the official reference method. There were 250 wheat samples of five classes; 75 Hard Red Winter, 75 Hard Red Spring, 30 Soft White, 20 Hard White, and 50 Durum. There were 100 Barley samples of two classes; 75 six-row and 25 two-row.

Soybean and corn evaluation samples were from the ISU GQL. There were 145 soybean samples (collected from 2006-2014) and 149 corn samples (collected from 1997-2014). The reference data for these samples was generated by Eurofins Lab (Des Moines, IA) (protein, oil, and starch) and ISU (density). The equivalency evaluation for corn and soybeans was done for protein and oil.

Study design

The 15 machines were arranged in randomized order on the lab benches to limit structural errors that could result from having machines of the same brand grouped together. Two capacitance moisture meters (GAC 2500 and Perten AM5200) used by GIPSA were included in the rotation to obtain a moisture reference although moisture was not the focus of this study(GIPSA 2016). Moisture data provided a backup check against lab errors and sample mix ups. Each sample was first run through the moisture meters. Then, a number was drawn to determine at which bench location the sample would start. The sample

was run three times through the starting instrument and then moved to the instrument on its right through the order sequentially, being processed through each instrument three times. After the sample was analyzed 45 times, three times by all 15 instruments, it was again run through both moisture meters three times each. For the standardization samples, the same analysis procedure was used with one exception; these samples were always analyzed first by machine 1, and continued sequentially through machine 15. This was done to facilitate data recording for determination of slope and bias values for each instrument.

Data cleaning procedure

NIRS analysis of each of the standardization and evaluation samples generated spectral data and predicted composition data. The predicted and spectral data for all grains were purged of duplicate entries, and of those that were incorrectly labeled. The data were graphed by instrument (reference values compared to the predicted values) for each grain/constituent combination, and outliers in the data set (that were deemed a result of operator error) were identified and removed or corrected as appropriate. Overall there were approximately 29,700 individual sample tests with 29,033 tests used for analysis; points were removed due to operator and labeling errors.

Statistical procedure

Accuracy, precision, reproducibility, and equivalence of all instruments for all grains and constituents were determined. Using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). A blocked variance design was used to assess equivalence among brands as well as within brands. The blocked analysis model computed averaged squared difference for these comparisons (A vs. A, B vs. B, C vs. C, B vs. A, A vs. C, and B vs. C) on a sample by sample basis, after averaging the three replicates. These comparisons were then tested statistically to determine equivalence, or lack there of.

Results

Standardization samples

The slope and bias calculations were applied to the evaluation samples in the data spreadsheets. The minimum, average, and maximum values for standardization sample characteristics are reported in Table 3.

Table 3: Standardization sample reference values are these instrumental or reference data.

Grain	Factor	N	Minimum (%)	Average (%)	Maximum (%)
Wheat	Protein	6	10.60	13.19	16.41
Barley	Protein	5	11.80	13.24	14.37
Soybean	Protein	20	31.00	36.80	49.87
Soybean	Oil	20	10.65	18.22	21.97
Corn	Protein	30	5.63	9.70	15.10
Corn	Oil	30	2.92	4.93	10.80

Evaluation samples

Table 4 (wheat, barley, soybeans, and corn) contains the summary statistics for the evaluation sample results across the 15 machines.

Table 4: Evaluation sample properties measured on a moisture basis of 12% (wheat), 0% (barley), 13% (soybean), and 15% (corn).

	Wheat	Barley	**Soybean**		**Corn**	
	Protein (%)	**Protein (%)**	**Protein (%)**	**Oil (%)**	**Protein (%)**	**Oil (%)**
Minimum	7.97	10.92	24.72	10.40	5.53	2.63
Average	13.65	13.14	37.48	18.06	9.51	5.78
Maximum	18.69	15.25	52.08	22.36	15.96	16.06
Standard Deviation	2.11	0.87	5.22	2.61	2.30	2.14

Table 5 - 10, show overall results for each grain (wheat, barley, soybean, and corn) from the evaluation sample set with and without standardization. The following will explain each value in the tables. Overall accuracy for each brand is the standard deviation of differences from the reference value for over all samples. Precision for each brand is the average of the standard deviation across the three replicates for all the samples. Reproducibility for each brand is the average of the standard deviation. The equivalence estimate is the average of the standard deviation for each sample across all 15 machines.

Table 5: Wheat for protein before and after standardization.

Wheat	Protein Unstandardized			Protein Standardized		
	A	B	C	A	B	C
Overall Accuracy (% pts)	0.28	0.21	0.24	0.27	0.21	0.24
Precision (% pts)	0.06	0.07	0.06	0.06	0.07	0.06
Reproducibility Across Copies (% pts)	0.12	0.05	0.09	0.06	0.05	0.09
Equivalence Estimate (% pts)	0.15			0.14		

Table 6: Barley protein before and after standardization

Barley	Protein Unstandardized		Protein Standardized	
	B	C	B	C
Overall Accuracy (% pts)	0.23	0.26	0.23	0.26
Precision (% pts)	0.15	0.09	0.20	0.22
Reproducibility Across Copies (% pts)	0.12	0.10	0.23	0.26
Equivalence Estimate (% pts)	0.14		0.16	

Table 7: Soybean protein before and after standardization.

Soybeans	Protein Unstandardized			Protein Standardized		
	A	B	C	A	B	C
Overall Accuracy (% pts)	0.65	0.71	0.56	0.64	0.72	0.56
Precision (% pts)	0.19	0.14	0.15	0.19	0.14	0.15
Reproducibility Across Copies (% pts)	0.22	0.14	0.30	0.23	0.16	0.29
Equivalence Estimate (% pts)	0.36			0.40		

Table 8: Soybean oil before and after standardization.

Soybean	Oil Unstandardized			Oil Standardized		
	A	B	C	A	B	C
Overall Accuracy (% pts)	0.36	0.30	0.32	0.40	0.30	0.32
Precision (% pts)	0.10	0.07	0.08	0.10	0.07	0.08
Reproducibility Across Copies (% pts)	0.19	0.06	0.11	0.21	0.06	0.11
Equivalence Estimate (% pts)	0.45			0.23		

Table 9: Corn protein before and after standardization.

Corn	Protein Unstandardized			Protein Standardized		
	A	B	C	A	B	C
Overall Accuracy (% pts)	0.47	0.47	0.42	0.45	0.47	0.42
Precision (% pts)	0.14	0.12	0.12	0.14	0.12	0.12
Reproducibility Across Copies (% pts)	0.14	0.12	0.11	0.15	0.12	0.11
Equivalence Estimate (% pts)	0.20			0.21		

Table 10: Corn oil before and after standardization.

Corn	Oil Unstandardized			Oil Standardized		
	A	B	C	A	B	C
Overall Accuracy (% pts)	0.51	0.48	0.45	0.54	0.48	0.45
Precision (% pts)	0.14	0.11	0.12	0.14	0.11	0.12
Reproducibility Across Copies (% pts)	0.17	0.07	0.16	0.22	0.07	0.17
Equivalence Estimate (% pts)	0.24			0.25		

In all cases, the equivalence estimate was larger than the individual instrument reproducibility. The blocked variance analysis (Table 11) determined that the instrument brands were not equivalent. Among the three instrument brands included in the analyses, there was a significant difference in the values obtained for wheat, barley, soybean, and corn protein as well as soybean and corn oil (P<0.0001 for all). This meant the variation across copies of a brand was significantly smaller than the variation across all 15 units as a group.

Table 11: Blocked variance analysis model results.

		Unstandardized		Standardized	
Grain	Constituent	AB vs. BB	CB vs. BB	AB vs. BB	CB vs. BB
Wheat	Protein	P <0.0001	P <0.0001	P <0.0001	P <0.0001
Barley	Protein	N/A	P <0.0001	N/A	P <0.0001
Soybean	Protein	P <0.0001	P <0.0001	P <0.0001	P <0.0001
Soybean	Oil	P <0.0001	P <0.0001	P <0.0001	P <0.0001
Corn	Protein	P <0.0001	P <0.0001	P <0.0001	P <0.0001
Corn	Oil	P <0.0001	P <0.0001	P <0.0001	P <0.0001

Discussion

In this study equivalence is achieved when the overall variance of using more than one instrument is less than or equal to that of using one instrument make and model. This would mean that the variability of the results would not change due to using more than one instrument. This was found to not be the case for the instruments used in this study.

A low standard deviation of repeatability for the repetitions for each instrument was found for all constituents and grains. This indicated that all instrument hardware was performing well and giving consistent results. The standard deviation within instrument brands which is the reproducibility among copies was low but different when looking at Table 5 – Table 10. In all cases product- constituent the

reproducibility across all units was significantly larger than the reproducibility across copies of the same unit. The blocked variance model also supported that the instrument brands as equipped were not equivalent for any constituent.

The standardization sample sets were used to determine slopes and biases for each instrument. The objective of this study was to evaluate the instruments for equivalency and standardization included optimizing all instruments to the same starting point. In the case of these instruments and calibration, the standardization process did not improve equivalence. If standardization sample sets were larger there may have been more of an improvement seen with adding standardization values of slope and bias.

The calibrations, while all NTEP approved, however each used different databases, and different lab sources. It is unknown what sample sets were used in developing calibration equations. This could introduce some error and show that the instrument brands may have had different ranges of data that could be determined by their calibration equations. Data demonstrated that parallel use of multiple units with uncontrolled calibrations is unlikely to meet an equivalence test. Variation among units is likely to be greater across the multiple units than across copies of individual units. Equivalence test worked in both ways we did it. The next logical step would be calibration process improvements, and better criteria for deciding how much loss in equation can be tolerated for the other benefits that might occur. The instruments A, B, and C for wheat and barley had a combined variance of less than 0.15 % points this shows that for those calibrations equivalence may not have been achieved but when evaluating grain the variance was small enough that it wouldn't have mattered. This was noticed when using the blocked variance analysis model, the analysis did show the instruments not being equivalent but the machines had small variance values.

Conclusion

Ensuring that NIRS instruments are equivalent could mean more instrument makes and models could be introduced to official inspection which would create some diversity in the market. There is also an opportunity to have more accurate readings with more instrument brands in the pool of available instruments. Results from three different instrument brands A, B, and C show that they are not equivalent ($p<0.0001$). However, if achieving equivalency is the goal than controlling some calibration variables would be of interest.

CHAPTER 3: GENERAL CONCLUSIONS

NIRS instruments provide a quick and reliable method of determining constituents in grain. This is helpful when determining constituents for trade and official inspections done by GIPSA. Only one make of NIRT is being used for Official inspections. There is opportunity to determine whether more than one make and model could be introduced. Results from three different instrument brands A, B, and C show that they are not equivalent ($p<0.0001$). However, if achieving equivalency is the goal than controlling some calibration variables could be of interest.

Although these instruments are not equivalent to the Official NIRT instrument Foss Infratec 1241 there may be instruments in the future that may be equivalent. There could be a future study that focuses on creating a test that can evaluate new instruments to be brought in as Official NIRT instruments. This could give opportunity for NIRS instrument companies to create more accurate instruments that could benefit the inspection of grain. There can also be work done in determining if calibration variables are controlled equivalency can be achieved for this study. This may help with determining a proper procedure for future instruments to be evaluated and to be able to achieve equivalence.

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APPENDIX A: BLOCKED VARIANCE ANALYSIS RESULTS

Table A1- Table A5 are results from the blocked variance analysis ran on the evaluation sample values. To obtain the following results first the averaged squared difference was taken for all combinations of AA, BB, CC, AB, AC, and CB. These results were then run through a least mean squares analysis comparing the combinations to each other. The tables show the first two columns are the comparisons, then the Estimate, standard error, degrees of freedom, t value, and p-value. The hypothesis was whether the combinations are equal to each other or not. If the p-value is below 0.05 the results are significantly different.

Table A1: Wheat Protein Results from blocked variance analysis

Comparisons		Estimate	Standard Error	Degrees of Freedom	T Value	P-value
AA	AB	-0.2570	0.07333	1241	-3.50	0.0005
AA	AC	-0.5355	0.07333	1241	-7.30	<0.0001
AA	BB	1.8227	0.07329	1241	24.87	<0.0001
AA	CB	-0.0731	0.07329	1241	-1.00	0.3191
AA	CC	0.8073	0.07333	1241	11.01	<0.0001
AB	AC	-0.2785	0.07333	1241	-3.80	0.0002
AB	BB	2.0797	0.07329	1241	28.38	<0.0001
AB	CB	0.1839	0.07329	1241	2.51	0.0122
AB	CC	1.0643	0.07333	1241	14.51	<0.0001
AC	BB	2.3581	0.07329	1241	32.18	<0.0001
AC	CB	0.4624	0.07329	1241	6.31	<0.0001
AC	CC	1.3428	0.07333	1241	18.31	<0.0001
BB	CB	-1.8957	0.07318	1241	-25.90	<0.0001
BB	CC	-1.0154	0.07329	1241	-13.85	<0.0001
CB	CC	0.8804	0.07329	1241	12.01	<0.0001

Table A2: Barley protein results from blocked variance analysis

		Estimate	Standard Error	DF	T Value	p-value
BB	CB	-0.8620	0.1113	198	-7.74	<.0001
BB	CC	0.02454	0.1113	198	0.22	0.8258
CB	CC	0.8865	0.1113	198	7.96	<.0001

Table A3: Soybean protein results for blocked variance analysis.

Comparisons		Estimate	Standard Deviation	Degrees of Freedom	T Value	p-value
AA	AB	-0.7191	0.09243	580	-7.78	<.0001
AA	BB	1.0532	0.09243	580	11.40	<.0001
AA	CB	-1.1883	0.09243	580	-12.86	<.0001
AA	CC	-0.6832	0.09243	580	-7.39	<.0001
AB	BB	1.7723	0.09243	580	19.18	<.0001
AB	CB	-0.4692	0.09243	580	-5.08	<.0001
AB	CC	0.03588	0.09243	580	0.39	0.6980
BB	CB	-2.2415	0.09243	580	-24.25	<.0001
BB	CC	-1.7364	0.09243	580	-18.79	<.0001
CB	CC	0.5051	0.09243	580	5.47	<.0001

Table A4: Soybean oil results for blocked variance analysis

Comparisons		Estimate	Standard Deviation	Degrees of Freedom	T value	p-value
AA	AB	-2.2914	0.08999	580	-25.46	<0.0001
AA	BB	2.2750	0.08999	580	25.28	<0.0001
AA	CB	0.6999	0.08999	580	7.78	<0.0001
AA	CC	1.1926	0.08999	580	13.25	<0.0001
AB	BB	4.5663	0.08999	580	50.74	<0.0001
AB	CB	2.9913	0.08999	580	33.24	<0.0001
AB	CC	3.4840	0.08999	580	38.71	<0.0001
BB	CB	-1.5750	0.08999	580	-17.50	<0.0001
BB	CC	-1.0824	0.08999	580	-12.03	<0.0001
CB	CC	0.4927	0.08999	580	5.47	<0.0001

Table A5: Corn protein results for blocked variance analysis

Comparisons		Estimate	Standard Deviation	Degrees of Freedom	T value	p-value
AA	AB	-0.8413	0.09843	572	-8.55	<0.0001
AA	BB	0.1878	0.09843	572	1.91	0.0569
AA	CB	-0.7797	0.09843	572	-7.92	<0.0001
AA	CC	0.5629	0.09843	572	5.72	<0.0001
AB	BB	1.0291	0.09843	572	10.46	<0.0001
AB	CB	0.06159	0.09843	572	0.63	0.5317
AB	CC	1.4042	0.09843	572	14.72	<0.0001
BB	CB	-0.9675	0.09843	572	-9.83	<0.0001
BB	CC	0.3751	0.09843	572	3.81	0.0002
CB	CC	1.3426	0.09843	572	13.64	<0.0001

Table A6: Corn oil results from blocked variance analysis:

Comparisons		Estimate	Standard Deviation	Degrees of Freedom	T value	p-value
AA	AB	-0.4076	0.09062	572	-4.50	<0.0001
AA	BB	1.7250	0.09062	572	19.04	<0.0001
AA	CB	-0.7184	0.09062	572	-7.93	<0.0001
AA	CC	0.05297	0.09062	572	0.58	0.5591
AB	BB	2.1326	0.09062	572	23.53	<0.0001
AB	CB	-0.3107	0.09062	572	-3.43	0.0006
AB	CC	0.4606	0.09062	572	5.08	<0.0001
BB	CB	-2.4434	0.09062	572	-26.96	<0.0001
BB	CC	-1.6720	0.09062	572	-18.45	<0.0001
CB	CC	0.7713	0.09062	572	8.51	<0.0001

APPEDNIX B: STATISTICS FOR INSTRUMENTS UNSTANDARDIZED AND STANDARDIZED

Table B1-B4 display results for wheat, barley, soybean, and corn evaluation samples. Results are displayed for accuracy, precision, reproducibility across copies, and equivalence estimate.

Table B1: Wheat results for unstandardized and standardized instrument brand A, B, and C for moisture and protein.

	Unstandardized				Standardized		
	A	B	C		A	B	C
Moisture							
Accuracy - SEP - Overall	0.34	0.18	0.16		0.34	0.18	0.20
Precision	0.02	0.01	0.01		0.02	0.01	0.01
Reproducibility Across Copies	0.14	0.06	0.07		0.11	0.05	0.15
Equivalence Estimate	0.18				0.24		
Protein							
Accuracy - SEP - Overall	0.28	0.21	0.24		0.27	0.21	0.24
Precision	0.06	0.07	0.06		0.06	0.07	0.06
Reproducibility Across Copies	0.12	0.05	0.09		0.06	0.05	0.09
Equivalence Estimate	0.15				0.14		

Table B2: Barley results for unstandardized and standardized instrument brand A, B, and C for moisture and protein.

	Unstandardized				Standardized		
	A	B	C		A	B	C
Barley Moisture							
Accuracy - SEP - Overall	0.24	0.16	0.17		0.22	0.17	0.21
Precision	0.06	0.04	0.02		0.06	0.04	0.02
Reproducibility Across Copies	0.14	0.09	0.10		0.12	0.10	0.15
Equivalence	0.18				0.15		
Barley Protein							
Accuracy - SEP - Overall		0.23	0.26			0.23	0.26
Precision		0.15	0.09			0.15	0.09
Reproducibility Across Copies		0.12	0.10			0.13	0.10
Equivalence Estimate	0.14				0.16		

Table B3: Soybean results for unstandardized and standardized instrument brand A, B, and C for moisture, protein, and oil.

Soybean	Unstandardized				Standardized		
	A	B	C		A	B	C
Moisture							
Accuracy - SEP - Overall	0.30	0.32	0.22		0.30	0.32	0.22
Precision	0.05	0.06	0.04		0.05	0.06	0.04
Reproducibility Across Copies	0.09	0.09	0.10		0.09	0.09	0.10
Equivalence Estimate	0.17				0.22		
Protein							
Accuracy - SEP - Overall	0.65	0.71	0.56		0.64	0.72	0.56
Precision	0.19	0.14	0.15		0.19	0.14	0.15
Reproducibility Across Copies	0.22	0.14	0.30		0.23	0.16	0.29
Equivalence Estimate	0.36				0.40		
Oil							
Accuracy - SEP - Overall	0.36	0.30	0.32		0.40	0.30	0.32
Precision	0.10	0.07	0.08		0.10	0.07	0.08
Reproducibility Across Copies	0.19	0.06	0.11		0.21	0.06	0.11
Equivalence Estimate	0.45				0.23		

Table B4: Corn results for unstandardized and standardized instrument brand A, B, and C for moisture, protein, and oil.

Corn	Unstandardized				Standardized		
	A	B	C		A	B	C
Moisture							
Accuracy - SEP - Overall	0.50	0.51	0.46		0.50	0.51	0.46
Precision	0.12	0.10	0.06		0.12	0.10	0.06
Reproducibility Across Copies	0.24	0.11	0.18		0.24	0.11	0.18
Equivalence Estimate	0.30				0.30		
Protein							
Accuracy - SEP - Overall	0.47	0.47	0.42		0.45	0.47	0.42
Precision	0.14	0.12	0.12		0.14	0.12	0.12
Reproducibility Across Copies	0.14	0.12	0.11		0.15	0.12	0.11
Equivalence Estimate	0.20				0.21		
Oil							
Accuracy - SEP - Overall	0.51	0.48	0.45		0.54	0.48	0.45
Precision	0.14	0.11	0.12		0.14	0.11	0.12
Reproducibility Across Copies	0.17	0.07	0.16		0.22	0.07	0.17
Equivalence Estimate	0.24				0.25		