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Development of a Near Infrared Reflectance Spectroscopy (NIRS) Platform for Rapid Wheat Quality Analysis

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DEVELOPMENT OF A NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) PLATFORM FOR RAPID WHEAT QUALITY ANALYSIS

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

DEVELOPMENT OF A NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) PLATFORM FOR RAPID WHEAT QUALITY ANALYSIS

LILY SCHIMKE

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Wheat is a valuable cereal grain in terms of its growability, versatility, and multifunctional nutritional components. Research into the genetic characteristics and growing conditions of the grain is advantageous to wheat breeders, farmers, food scientists, food processors, and consumers. Optimizing the quality of the wheat grain is important to yielding a crop with the most desirable traits. Analytically obtaining data on the quality attributes of wheat is a lengthy and resource intensive process. Near infrared reflectance spectroscopy (NIRS) technology is rapid, cost-effective, and a powerful analytical tool that can be harnessed to create predictive calibrations for estimations of wheat parameters.

This study looked to analytically obtain Total Dietary Fiber reference values, as well as relate these values to genetic and environmental variability. Ninety-nine hard red spring wheat samples, including 33 varieties grown in three locations (Brookings, Miller, Groton) in 2018 were analyzed in duplicates. It was determined that both variety and growing location were significant in influencing variability of TDF residue at the 0.001 level of significance. A Duncan Multiple Range Test was conducted at the 0.05 level of significance to identify the rankings of the growing locations, which indicated that Brookings and Miller were statistically the same and both were better than Groton in

terms of the TDF residue %. Similarly, a Duncan Multiple Range Test was used to identify that 13 of the 33 varieties were ranked the highest, and statistically the same, including SURPASS, SD4740, FOREFRONT, SD4719, SD4707, SD4816, LCS-TRIGGER, SD4720, SD4721, BRICK, SD4711, SD4775, and ADVANCE.

Predictive NIRS calibration estimations for TDF and other selected wheat constituents, and mixing and baking parameters were created for 2018, 2019, and combined 2018/2019 data. A good calibration will have a high coefficient of determination (RSQ), high variance ratio (1-VR), low standard error of calibration (SEC), low standard error of cross validation (SECV), and a low standard error of prediction (SEP). TDF did not yield a good calibration (RSQ 0.07, SEC 1.52, SECV 1.70, 1-VR -0.18). owing to a small range of occurrence and lack of homogeneity of the residue. Furthermore, milling of wheat grains involves grinding and sifting, removing bran, a significant source of fiber. For the 2018 dataset, the parameters with an RSQ>0.6 included single kernel hardness index (0.87), dry gluten (0.82), farinograph water absorption% (0.91), water absorption capacity (0.91), NIR grain moisture (0.84), NIR grain protein (0.99), NIR grain ash (0.87), flour protein (0.88), flour ash (0.85), mixograph's mid-line peak value (0.85), total gluten (0.70), good wet gluten (0.78), wet gluten (0.70), WAM% (0.64), kernel protein (0.78), kernel ash (0.72), and flour extraction (0.63). For the 2019 dataset, the parameters with an RSQ>0.6 included the farinograph moisture% (0.81), water absorption% (0.92), WAC% (0.92), WAM% (0.84), NIR protein (0.99), NIR ash (0.88), and NIR moisture (0.64). For the combination 2018/2019 calibration model, parameters with RSQ> 0.60 included the Farinograph's dough development time (0.95), water absorption (0.90), WAC% (0.88), WAM% (0.87), NIR moisture (0.92), NIR protein (0.99), NIR ash (0.90),

farinograph moisture (0.77), mixing tolerance index (0.60), dry gluten (0.66), and time to breakdown (0.62). The accuracy of these calibrations was validated with a validation subset of data in which the reference values were known, but not included within the calibration development. A paired t-test showed that the NIRS predictions were not statistically different than the known reference values at a 95% confidence level for all tested parameters except for 2018 NIR ash, flour ash, and kernel ash.

Correlations between wheat constituents, mixing parameters, and baking parameters were generated to determine their relationships. Pearson's correlations coefficients indicated strong correlations among gluten parameters, water binding of flour, and mixograph/farinograph measurement values.

This study shows that growing location and wheat variety have a statistical impact on dietary fiber variability, and that dietary fiber is poorly predicted by NIRS calibrations. NIRS predictive calibrations for other constituents (gluten, protein, moisture, mixograph/farinograph parameters), were able to be established with high RSQ, 1-VR, and low SEC, SECV, SEP, and bias. Many quality parameters of wheat were found to be correlated with one another, further increasing the predictive potential of desirable wheat quality traits.

Chapter 1:

Introduction

Wheat is a cereal crop that is grown mainly in the Great Plains region of the United States, as well as throughout the world (Wheat Foundation, 2019). Wheat was domesticated about 10,000 years ago and as technology continues to progress, wheat breeders are interested in new efforts to maximize the efficiencies of wheat production to meet the nutritional, economic, and social requirements of our society.

Wheat is a valuable cereal grain in terms of its growability, versatility, and multifunctional nutritional components. Scientific literature gives evidence that wheat is of nutritional importance for both humans and animals. Wheat is a multifunctional food comprised of important nutrients such as carbohydrates, protein, fiber, fat, vitamins, minerals, and phytochemicals—all of which may contribute to a healthy human diet (Shewry, 2015). The composition of wheat makes it ideal for many uses in terms of human and animal nutrition, palatability, functionality, and long-term storage. The main reported health benefits for humans include the prevention of heart disease, regulation of blood lipid levels, and the reduction of cancer risk (McKee, 2000). There is strong evidence supporting that dietary fiber provides preventative measures against many chronic diseases including type 2 diabetes and cardiovascular disease (Anderson, 2009; Buttress, 2008; Shewry, 2015). In wheat, the dietary fiber is heavily localized in the endosperm, and is primarily composed of the polysaccharide arabinoxylan, which is a hemicellulose consisting of repeating copolymers of arabinose and xylose (Lu, 2000;

Mitchell, 2015). The increased public awareness of the potential benefits of a high fiber, disease-preventative, whole-food diets has promoted a growing interest for the consumption of whole-grain products, and thus, the need for research into the topic.

Knowing quantitative values for wheat constituents is valuable for wheat breeders and end-product quality analysis. Traditional analytical methods for determination of these constituents are time-consuming, costly, and use many harsh reagents. Thus, a rapid, all-inclusive scanning technique would be useful for the evaluation of a large number of wheat samples. This may be achieved using an NIRS instrument. Prior to being useful, the development of calibration equations is necessary to output reliable data. This, in conjunction with a statistical relationship between wheat constituents and its baking functionality, gives insight into optimizing and maximizing growing efficiencies of wheat.

Near infrared reflectance spectroscopy (NIRS), since the turn of the century, has taken hold as an innovative technology that can be useful in determining the physical or chemical composition of a material. It is a rapid, non-invasive, and comparatively inexpensive means of measuring the quality parameters of a material. This is an alternative to traditional, time-consuming, costly individual analytical techniques, and has the benefit of being an all-inclusive test to determine the constituents of a sample. However, the NIRS conclusions can only be as reliable as the reference data that is used to create the calibration model. The principle behind NIRS is based on absorption of the electromagnetic wavelength range of 800-2500nm. When a sample is scanned with NIR radiation, an absorbance spectrum is produced which is based on the varying energy levels that it takes to bend or stretch each type of chemical bond. The absorbance spectra

that is outputted is based upon the O-H, N-H, and C-H chemical bonds and stretching vibrations (Teye, 2013). The trends can be analyzed and modeled to produce meaningful information about the material that is being tested. Since the early 90s, thousands of papers have been published detailing the works and discoveries made using NIRS technology, which demonstrates its vast application and reliability within the field of science (Osborne, 2006).

Objectives

Dr. Krishnan's Cereal Quality laboratory is working to determine nutritional constituents of wheat through the use of Near Infrared Reflectance Spectroscopy (NIRS). For my thesis project, I have analyzed the Total Dietary Fiber (TDF) content of hard red spring wheat grown in South Dakota in 2018, and this data was used to develop and create a calibration model for NIRS. My objectives included:

- Development and validation of predictive NIRS calibration equations for the estimation of dietary fiber content in South Dakota wheat.
- Evaluation of predictive calibrations for selected wheat constituents (protein, gluten), dough mixing parameters (absorption, mixing time, stability, peak mixing time), and baking output parameters (loaf volume)
- Study of variability of dietary fiber content across growing locations of South Dakota wheat.

The hypotheses tested in this study included:

- The NIRS prediction values for TDF of wheat samples are statistically the same as reference TDF analysis values for those samples.
- Accurate and precise predictive calibrations, judged by high R squared (RSQ) values and low standard of error (SEP) and standard error of prediction (SEP) values, can be developed to estimate selected wheat constituents, dough mixing parameters, and baking output parameters.
- Growing location and variety have statistically significant effects on the variability of dietary fiber content of wheat grown in South Dakota.

Chapter 2:

Literature Review

History and Origin of Wheat

Wheat is a staple food worldwide and is one of the three globally produced cereal grains, along with barley and maize. According to archeological records, wheat was domesticated about 10,000 years ago in the Fertile Crescent (Balfourier, 2019). Since then, the generic term "wheat" has undergone many adaptions through natural spread and intentional genetic modifications by humans. The first and most notable domestication trait of wheat was the selection of larger grains and kernels that stuck inside, rather than fall off the plant prior to harvest (Hughes 2019; Copper, 2015). In the wild, this is not a favorable trait, as it inhibits the natural spread of the seeds. As the world transitioned from a hunter-gatherer to an agricultural based way of living, the rise of bread wheat was crucial for civilizations, as populations grew during the Babylonian and Assyrian empires. Through DNA analysis, the first bread wheat that was identified to have enough gluten for use in yeasted breads was discovered in Macedonia at about 1350 BCE (Sheffield University, 2011). The Egyptians were the first to develop bread and use an oven for large scale production (Cabalerro, 2003). Wheat took about 100 years to reach China. From there, wheat continued to spread across Europe and wheat straw was used as roofing material during the Bronze Age until the 19th century (Belderok, 2000; Cauvain, 2003). Beginning in the 1940s and peaking in the 60s, the Green Revolution increased worldwide food production, through an advancement in agricultural technologies. The

Green Revolution adopted higher yielding cereal varieties, the use of chemical fertilizers, expanded irrigation, and improved mechanization of cultivation. Norman Borlaug, the "Father of the Green Revolution", was a key leader in this initiative and received a Nobel Peace Prize in 1970 as well as being credited with saving billions of people from starvation (Farmer, 1986). Today, technology has continued to expand, and wheat breeders are continuing efforts to maximize efficiencies to meet the nutritional, economic, and social requirements of our society.

Importance of Wheat Crop

Wheat is a versatile crop, with hard red spring wheat most commonly grown in North Dakota. It is also prominent in South Dakota, Montana, and Minnesota (Wheat Foundation, 2019). It is used domestically and exported to foreign countries, many of which also produce wheat. The composition of wheat makes it ideal for many uses in terms of human and animal nutrition, palatability, functionality, and long-term storage. As of late, dietary fiber has been in the spotlight for its potential health benefits, and wheat is touted as a common dietary source for it (Shewry, 2015). Beyond the nutritional importance, wheat also has a cultural impact. Wheat is centralized to social gatherings, rituals, provided structure to early civilizations, and gives a framework for society in terms of field workers, bakers, consumers, and storage management (Bjornstad, 2016).

Nutritional Importance for Humans

Approximately 20% of energy intake by humans comes from wheat (Shewry, 2015). Wheat is commonly considered a source of energy in the form of carbohydrates. However, it is multifunctional food comprised of other important nutrients such as protein, fiber, fat, vitamins, minerals, and phytochemicals—all of which may contribute to a healthy human diet (Shewry, 2015). Due to the widespread growability, availability, and functionality of wheat, it is of human nutritional importance. At maturity, the wheat grain consists of roughly 70% carbohydrate, 10-18% protein, and about 1.5% fat (Sramkova, 2009). Resistant starch, a component of dietary fiber, is the starch that is not absorbed in the small intestine. Resistant starch has been shown to have health benefits as a substrate to fermentation in the colon, which may reduce colon cancer. The fermentation process lowers the colons pH, which may lead to less accumulation or production of harmful, cancerous components (Fassler, 2006). Phytochemicals have been in the spotlight as of late for their strong antioxidant capacity and potential for synergistic health benefits (Adom, 2003). Phenolic acids are the main group of phytochemicals in wheat. There is increasing evidence that phenolic compounds may improve vascular function in both humans and animals (Katz, 2001). There have been numerous studies that show the beneficial impact of wheat consumption in relation to preventing heart disease, regulating blood lipids, reducing cancer risk, and diverticular disease (McKee, 2000). The increased public awareness of the potential benefits of high fiber, disease-preventative, whole-food diets has promoted a growing interest for the consumption of whole-grain products.

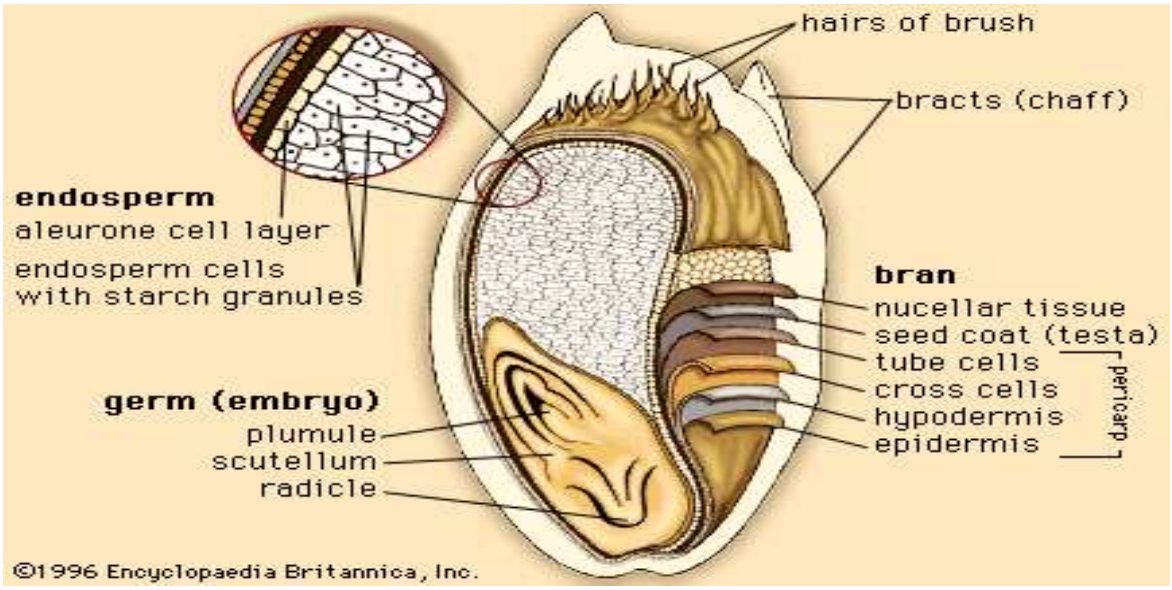

Figure 1. Structure of Whole Wheat Kernel (Image source: Encyclopedia Britannica)

Dietary Fiber Source

It is estimated that less than 5% of most age and gender groups meet the adequate intake of 25-38g/day of dietary fiber, a measure established by the Institute of Medicine (Mobley, 2014). Wheat products contain about 1-10g of dietary fiber per 100g serving (USDA National Nutrient Database, 2013). Raw wheat contains 12-15% fiber, mainly concentrated in the bran, which is not present in traditional milled flour (USDA National Nutrient Database, 2013). Dietary fiber is the edible part of the plant that is resistant to digestion and absorption in the small intestine and partially digestible in the large intestine following ingestion (AACCI, 2001). Dietary fiber is not digested in the upper GI tract and many of the health benefits come from its fermentation in the colon. In the GI tract, insoluble fiber aids in fecal bulk and speeds transit time and soluble fiber increases the viscosity of the digestion and lowers the glycemic load (Mitchell, 2015). The fiber content of a whole wheat grain ranges from 11.6% to 12.7% on a dry weight basis (Carson, 2009). White flour has been reported at TDF% levels of 1.94- 6.27 % (Shewry, 2015). When wheat is milled into white flour, much of the bran is removed. The dietary fiber is heavily centralized in the endosperm, and primarily composed of the polysaccharide arabinoxylan (approximately 70%) (Mitchell, 2015). Arabinoxylan is a hemicellulose that is found primarily in plant cell walls and consists of repeating copolymers of arabinose and xylose (Lu, 2000). Arabinoxylans have been found to occur at 1.5-2.5% in wheat flour (Courtin and Delcour, 2002). They have an impact on flour functionality, and have both water soluble and water insoluble components. It has been stated that water soluble arabinose is beneficial to bread baking, while water insoluble arabinose has negative effects (Courtin and Delcour, 2002). There is strong evidence that

the dietary fiber provides preventative measures against many chronic diseases including type 2 diabetes and cardiovascular disease (Anderson, 2009; Buttress, 2008; Shewry, 2015). Dietary fiber is also beneficial to wheat quality for end-use functionality. In bread making, the presence of fiber causes an increased water hydration for the flour (Dhingra, 2011).

Dietary fiber is analytically measured in a laboratory setting using AOAC Method 991.43. This method has been in existence since 1985 (Megazyme, 2019). This is an enzymatic-gravimetric method. It mimics the digestive process of the human body to determine the total dietary fiber quantity. The enzymes used are amylase, protease, and amylogluosidase. Amylase is heat stable and requires requires a pH of 8.2 to activate. Protease requires a pH of 7.5 to activate, and amylogluosidase requires a pH of 4.5 to activate. The digestible carbohydrates are broken down into simple sugars through enzymatic digestion, then removed via precipitation and filtration, thus mimicking the absorption of these constituents into the body. The precipitate contains the non-digestible portion, the dietary fiber, as well as protein and inorganic material. Thus, the sample must be further analyzed for protein and ash content to correct the calculated value and determine the true dietary fiber content. Another method of dietary fiber analysis is an enzymatic-chemical approach. This method first removes the available carbohydrates then chemically characterizes fiber content. It involves an acid reflux then HPLC or gas chromatography to measure the fiber components (Institute of Medicine Panel, 2001).

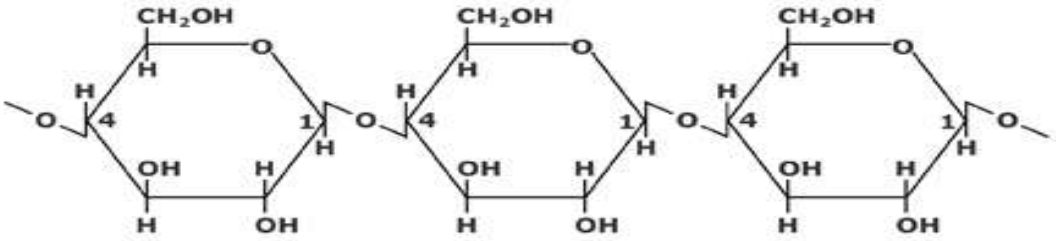

Figure 2. Structure of Dietary Fiber (cellulose) in Wheat (Image source: www.sciencelearn.org.nz)

Wheat as a Fodder Crop

While wheat is a substantial contributor to human health and nutrition, it can also be a useful source for animal consumption. Cereal plants, which include wheat, are noted to produce nutritious feed to maintain livestock over the winter months, and wheat forage is as valuable as oat forage (Cash, 2007; Shuja, 2009). Wheat forage is valuable because it can be grazed or cut for hay (Bruening, 2007). In temperate climates, winter wheat is the wheat of choice for forage, while spring wheat is preferred in the tropical and subtropical regions of the world (Mannetje, 2000). Wheat is often preferred over barley or oats because it can withstand wetter soils (MSU, 2012). Further, wheat could be considered a low-risk crop, because there are many paths for its end use, depending on the growing conditions. For example, a substantially wet season, which is not uncommon in many regions of the world, may cause the wheat to sprout prior to harvest, thus rendering it unfit for human utilization in the food industry (NDSU, 2017). Historical research at NDSU has shown that sprouted hard red spring wheat supports higher levels of performance in swine, compared with barley-soybean diets. It is notable that the nutrient levels in sprouted wheat are greater than non-sprouted wheat, but just because of the concentration change that occurs as the present starch is used for the germination process (NDSU, 2017).

Economic Importance

Wheat is the world's most abundant food grain, as it provides over 20% of the calories and protein in the overall diet. It is the most consumed food for about 35% of the world's population, and in more than 40 countries (Bushuck, 1998). The widespread nature of the crop, the ease of processability and storage, and the countless end uses of wheat, make it an ideal and valuable subject of study. Annually, the United States uses about 12.5 million acres to produce 500 million bushels. Specifically, for hard red spring wheat, the United States yields about 45 bushels per planted acre. Approximately half of each hard spring wheat crop is used domestically, and the other half is exported to about 50 different countries around the world, mainly to Asian and South American countries (ND Wheat Commission, 2019).

Variability Factors

Varieties and Growing Condition

There are several varieties of wheat that are grown throughout the world. Wheat is primarily classified by its growing season—winter wheat and spring wheat. Further classification includes hardness (soft/hard), color (red/white), and kernel shape. For food purposes, protein content is the main constituent that determines its end use. Hard red winter and hard red spring typically contain the greatest percentage of protein (10-13% and 12-15%, respectively) and are often used in breads and other products that require a strong internal structure (NDSU 2018). The environment, genotype, and their interaction may play a strong role in determining the yield and quality of the crop. Hard red spring

wheat (the focus of this study) is grown mainly in the northern United States, about 95% in SD, ND, MN, MT, and where the summers are mild. (ND Wheat Commission, 2019). Even within this relatively small growing region, wheat crop constituents vary, likely based on soil constituents and environmental factors encountered throughout the growth cycle. Other studies have shown that environmental conditions can influence gluten composition in bread wheat, this emphasizes the importance of consideration in growing location. Further, the nitrogen fertilization strategy can influence the grain protein content (Dubois, 2018). It was also noticed that the extent of the impact of nitrogen fertilization on protein quantity is dependent on the variety of wheat grown (Wieser, 1998). Thus, the genetic makeup and the environmental factors are separate, but interrelated. Soil wetness and composition, rainfall, drought, pests, fertilization quantity and composition, temperature, and humidity are environmental factors that are often out of our control, but impactful to wheat quality (Torbica, 2008).

Analytical Approaches

Near-Infrared Reflectance Spectroscopy (NIRS)

Near-infrared spectroscopy (NIRS) is an innovative technology that can be used as a tool for determining the physical or chemical composition of a material. It is a rapid, non-invasive, and comparatively inexpensive means of measuring the quality parameters of a material. One of the key benefits of using NIRS over other analytical techniques is that it can be an all-inclusive test to determine the constituents of a sample. For example, rather than individually testing for protein, moisture, fat, etc. of foodstuff, the machine can

output all of this information with a simple scan, once it is calibrated to do so. The calibration process involves the acquisition of spectral data for known samples, chemical reference data for known samples, the creation of a calibration model, and validation of the calibration using known samples. The reference data is arguably the most crucial piece of information because the NIRS conclusions can only be as reliable as the reference data used to create the calibration model.

The principle behind NIRS is based on absorption of the electromagnetic wavelength range of 800-2500nm. When a food is scanned with NIR radiation, the radiant energy is absorbed, reflected, or transmitted. A spectrum of the absorbance can be plotted, and the trends can be analyzed to determine the chemical or physical composition of the sample. The absorbance spectra that is outputted is based upon the O-H, N-H, and C-H chemical bonds and stretching vibrations (Teye, 2013). Organic biological compounds are readily able to be tested due to their presence of these bonds. The absorbance spectrum is based on the varying energy levels that it takes to bend or stretch each type of chemical bond. A fairly complex spectra is produced due to the broad nature of the overlapping and combination bands. The patterns within the spectra can be statistically analyzed and modeled to deduce meaningful information, such as the chemical constituents, about the material that is tested.

NIR has been used successfully to assess the baking quality parameters of wheat. A study done in 2017 demonstrated the use of NIR calibrations for predicting bread loaf volume. This expanded upon the model that protein content could predict baking quality, because protein content predictions became much less reliable at protein contents above 12%. Protein content is not and should not be the only indicator of baking quality (Gabriel et.

al, 2017). This example demonstrates that NIR can be a powerful analytical tool in terms of predictive powers for overall wheat quality and that additional calibration models are needed.

History of NIRS

NIRS was first discovered in 1800 by Frederick William Hershel, a British astronomer (Davies, 2000). He discovered the near-infrared region of the electromagnetic spectrum by questioning whether visible colors had an associated temperature within white sunlight. He continued to test the temperature effect beyond that of visible light and discovered the non-visible region—now called the near-infrared region (Braz, 2003). Nearly 100 years later, in 1903, William W. Coblentz was the first to accurately measure and obtain the spectra for inorganic and organic compounds within the infrared range. By the 1930s, IR spectrometers were built, which rapidly expanded the use of using absorbance spectrums to determine the chemical composition of a sample substance. Technological advances over the last century have made the process quicker, simpler, and more reliable (Derrick, 1999). NIR has been used for the quality testing of crossbred material from wheat breeding programs since the late 1970s. The wheat breeders can use the hardness and moisture values of a kernel to determine a plan of action for planting their fields, while protein and moisture are valuable measures of the quality of a flour, especially since the selling price of wheat is often based on its protein content. Since the early 90s, thousands of papers have been published detailing the works and discoveries

made using NIRS technology, which demonstrates its vast application and reliability within the field of science (Osborne, 2006).

NIRS in Wheat Breeding Programs

Grain end-use quality traits are among the most important in wheat breeding (Hayes et. al, 2017). Wheat breeders require knowledge on the functionality and compositional data of wheat. Using NIRS in wheat breeding programs is a means of accelerating the progress towards improving end-use quality. These end-use quality traits can be difficult and expensive to due to their assays being limited to flour quantities that are only available late in the breeding cycle (Hayes et. al, 2017). A single NIRS scan has the ability to simultaneously predict multiple components of the sample, once calibration equations are created for the desired characteristics. NIRS scanning is rapid, only requires a small sample of flour, and has successful been implemented into many plant breeding programs (Font et. al, 2006; Sissons et. al 2006). Because plant breeders are often faced with limited sample quantity, the use of NIRS to obtain useful data from their sampling of a single grain kernel is extremely valuable (Pojic et. al, 2012).

NIRS has successfully been implemented into wheat breeding programs. NIRS was used to analyze the evolution patterns from 1800 to 2000 protein, minerals, fatty acids, and carbohydrates. Using this data allowed for concentration comparisons of biochemical components and indicated links to temporal trends over the years (Roussel et. al, 2005). NIRS has had agronomic impact in predicting water soluble carbohydrates, with an $R^2 > 0.97$ (Wang et. al, 2011). NIRS has shown the potential for predicting protein content,

moisture content, and flour b* values at a level that is suitable for quality control purposes (R^2 > 0.97) (Dowell et. al, 2006). Other parameters have also been successfully predicted and used for quality screening including SDS sedimentation volume, color values, gluten content, mixograph, farinograph, loaf volume, and water absorption and mixing time values (Dowell et. al, 2006). Similar results were seen in both hard red winder wheat and hard red spring wheat varieties to predict quality using spectra. Another example of early NIRS models that have been developed include models for glutenin content, gliadin content, SDS sedimentation volume, and mixograph peak resistance values, at accuracy levels suitable for screen purposes in breeding programs (Delwiche et. al, 1998). However, it is important to note that the majority of relationships are correlated heavily with protein content, which can influence the accuracy of these predictions. Removing the influence of protein content from the analysis found that only a limited number of factors could be predicted with NIRS with an R^2 > 0.70, and most constituents were reduced to an R^2 < 0.20 (Dowell et. al, 2006). This shows that NIRS can be used to predict constituent and functionality parameters, but are heavily tied to protein content itself. When datasets consist of pure breeding lines, it is hypothesized that the removal of protein influence is less negatively impactful (Sissons et. al, 2006). Protein content on its own is not a reliable for predicting baking quality, as protein levels exceed 12%, the accuracy of predictions is reduced, as the R^2 for predicting bread loaf volume falls to 0.15 (Gabriel et. al, 2017). Hence, functionality and baking quality parameters could benefit from additional modeling, including NIRS calibrations.

Calibration Development

To create the calibration, spectral measurements from the NIRS scan are related to the reference data that is gathered via chemical methods using calibration models that are based on multiple variable based regression models. The data are centered with modified partial least squares (PLS) and the outliers are identified and removed. The spectra are processed with multiplicative scatter correction to partially correct baseline differences. Cross validation is used to identify outliers, choose the number of PLS or principle component analysis (PCA) factors in the calibration model, as well as provide an estimation of the performance of the calibration model when used to predict unknown samples (Bellato, 2011).

Wheat Quality Testing

The composition of the wheat itself may impact the functionality of wheat in a flour for food applications such as bread baking. Grain, flour, dough tests, as well as the final baked product are all indicative of overall quality. There are many individual analytical tests that can be conducted to determine the various factors relating to wheat quality including protein, ash, moisture, rheology, and dough mixing analysis. There are instruments available to gather quantitative data, which makes analysis less subjective, and measurable.

A farinograph is an instrument that is used to give insight about the water absorption, gluten content, stability to overmixing, and rheological mixing properties of a dough (Brabender, 2020). It records the torque experienced by the mixing blades on the dough

during mixing. The output is a mixing curve of the water absorption, arrival time, stability time, peak time, departure time, and mixing tolerance index. The vertical axis is typically in Brabender units (BU), and the horizontal axis is time. These are important values for a dough in terms of its mixing properties. The arrival time is marked as the point at which the top of the curve first intersects the 500BU line. The 500BU line is considered the baking industry standard for the ideal flour to water ratio for baking quality. Too much water will result in the curve failing to reach the 500BU line, and too little water will exceed the line. The peak time is the highest point on the curve and represents when the dough has reached maximum viscosity before the gluten begins to break down. The mixing tolerance index (MTI) is a measure of the dough's resistance to overmixing. It is measured as the difference in BU between peak time and 5 minutes after peak time has been reached. The departure time is the point where the top of the curve falls back below the 500BU line. Mixing stability indicates how long a dough can be mixed before breaking down, and is measured as the time between arrival time and departure time.

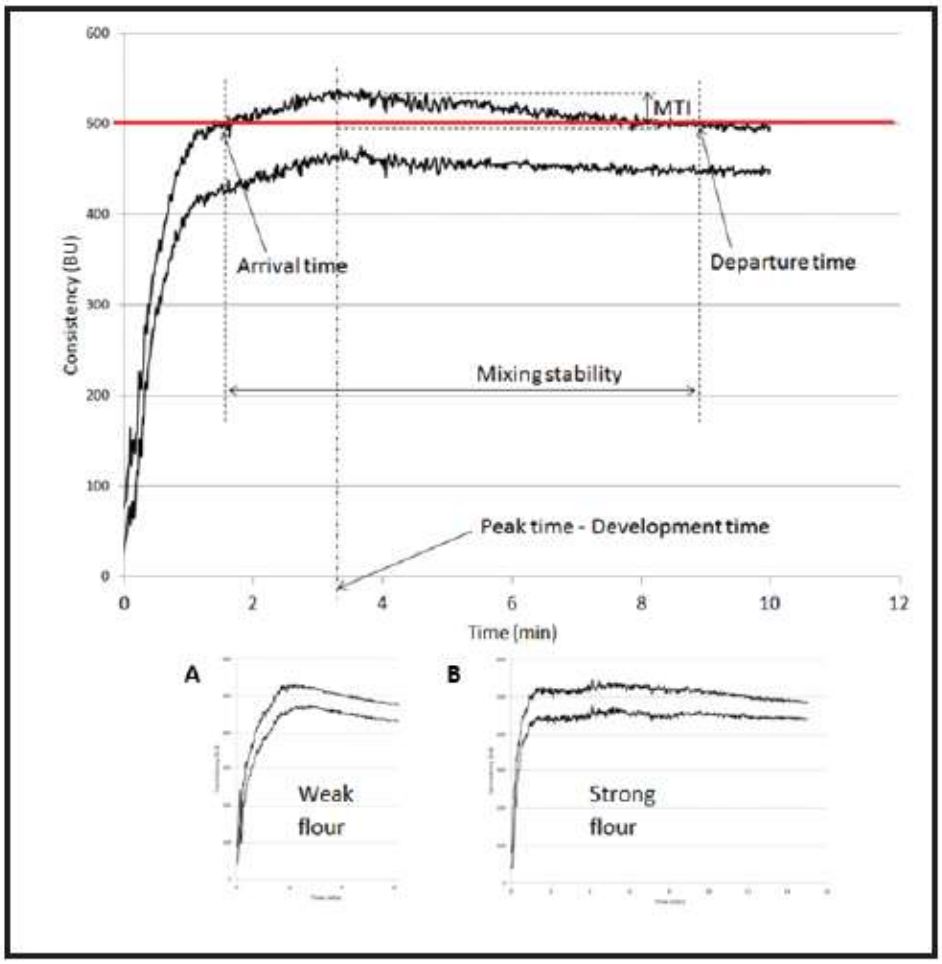

Figure 3. Typical Farinograph Output (Image Source: Chapter 45, Food Product Innovations Using Soy Ingredients).

A Glutomatic is an instrument used to gather qualitative gluten data. Gluten is a protein that forms a network that contributes to viscosity and extensibility to a dough (Shewry, 2002). This gluten network traps air pockets in the bread baking process, thus showing the importance of gluten content on bread baking quality, and therefore wheat quality. Gluten is comprised of glutenin and gliadin, connected via a disulfide bridge in a dough. It is important to the retention of gases during fermentation and baking to allow for expansion and retention of the bread loaf. During the bread baking process, the gluten content is correlated with the quality of the finished product. Thus, the Glutomatic is indicative of the baking quality of the grain. A Glutomatic is an automatic gluten washing system that gives qualitative values on wet gluten, dry gluten, and water binding capacity. The wet gluten is washed from flour with water or a salt solution (1% sodium chloride) then centrifuged on a sieve, weighted, dried, and weighted again. The difference in the wet and dry gluten content indicates the water that is bound in the gluten, thus the water binding capacity. The gluten index represents the ratio of wet gluten that remains on the sieve after centrifugation to the total wet gluten. The gluten index has been used as an indicator of dough and bread quality, with lower GI values exhibiting good quality (Bonfil et al, 2012). However, GI values do not always correlate with other accepted quality parameters, such as loaf volume, so caution should always be used when drawing conclusions.

Loaf volume is a quality characteristic that can be accurately, quantitatively measured. Due to the irregular shape of a loaf of bread, loaf volume is traditionally measured by a rapeseed displacement method (AACC Method 10-05.01). In this method, a container is filled with a known volume of rapeseeds. The majority of these seeds are then removed,

the loaf of bread is placed in the container, and the container is filled with the removed seeds. The seeds that remain represent the displaced volume that the loaf of bread took up in the container and are measured in a graduated cylinder to quantify loaf volume. The specific volume of the loaf can be determined with the ratio of loaf weight to loaf volume. A small volume typically indicates a weaker flour, or a stronger flour that required a longer fermentation period to enable the gluten to become more extensible (Fellows, 1995).

Bread loaf volume is a reliable indicator of bread quality, but it is an end-use product, thus the need for other predictive parameters that can be measured prior to bread baking. The protein content, falling number, and dough extensibility are useful to predicting the breadcrumb structure, loaf volume, and texture of the resulting bread (Rozyto et al., 2011). Mixolab is a useful test that can indicate pasting properties and mixing properties in one test. However, the pasting properties have not been found to be linked directly to baking quality. Thus, Mixolab results may not be any more useful than a Farinograph test in terms of baking quality indicators. The Mixolab parameters that are most related to loaf volume are stability and water absorption, however not single-handedly (Caffe-Treml et al., 2010). Dough systems are complex and multifactored. A combination of parameters is often required in order to gain a reliable prediction of baking quality. In this study, we are looking to add dietary fiber into the mix of potential quality indicators.

Chapter 3:

Materials and Methods

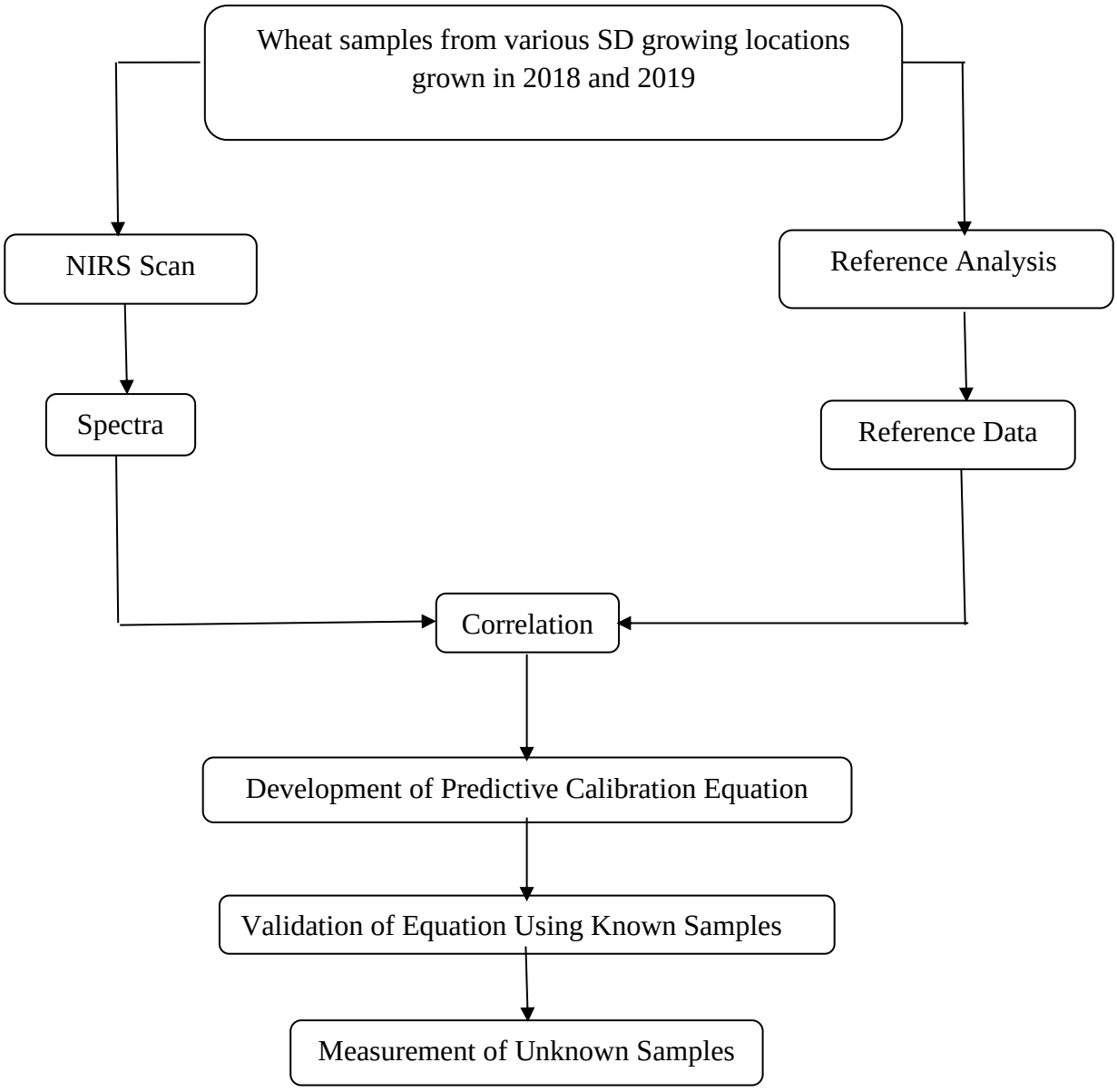

Figure 4. Experimental design for the calibration of NIRS instrument for estimation of wheat constituents.

YEAR	ENTRY	LOC	NAME		YEAR	ENTRY	LOC	NAME
2018	1	BRK	ADVANCE		2018	18	GRO	SD4707
2018	2	BRK	BOOST		2018	19	GRO	SD4708
2018	3	BRK	BRICK		2018	20	GRO	SD4711
2018	4	BRK	BRIGGS		2018	21	GRO	SD4719
2018	5	BRK	FALLER		2018	22	GRO	SD4720
2018	6	BRK	FOCUS		2018	23	GRO	SD4721
2018	7	BRK	FOREFRONT		2018	24	GRO	SD4740
2018	8	BRK	LCS-TRIGGER		2018	25	GRO	SD4745
2018	9	BRK	OXEN		2018	26	GRO	SD4746
2018	10	BRK	PREVAIL		2018	27	GRO	SD4748
2018	11	BRK	SELECT		2018	28	GRO	SD4752
2018	12	BRK	STEELE-ND		2018	36	GRO	SD4771
2018	13	BRK	SURPASS		2018	38	GRO	SD4773
2018	14	BRK	TRAVERSE		2018	39	GRO	SD4775
2018	15	BRK	SD4539		2018	43	GRO	SD4792
2018	16	BRK	SD4625		2018	47	GRO	SD4816
2018	17	BRK	SD4706		2018	1	MIL	ADVANCE
2018	18	BRK	SD4707		2018	2	MIL	BOOST
2018	19	BRK	SD4708		2018	3	MIL	BRICK
2018	20	BRK	SD4711		2018	4	MIL	BRIGGS
2018	21	BRK	SD4719		2018	5	MIL	FALLER
2018	22	BRK	SD4720		2018	6	MIL	FOCUS
2018	23	BRK	SD4721		2018	7	MIL	FOREFRONT
2018	24	BRK	SD4740		2018	8	MIL	LCS-TRIGGER
2018	25	BRK	SD4745		2018	9	MIL	OXEN
2018	26	BRK	SD4746		2018	10	MIL	PREVAIL
2018	27	BRK	SD4748		2018	11	MIL	SELECT
2018	28	BRK	SD4752		2018	12	MIL	STEELE-ND
2018	36	BRK	SD4771		2018	13	MIL	SURPASS
2018	38	BRK	SD4773		2018	14	MIL	TRAVERSE
2018	39	BRK	SD4775		2018	15	MIL	SD4539
2018	43	BRK	SD4792		2018	16	MIL	SD4625
2018	47	BRK	SD4816		2018	17	MIL	SD4706
2018	1	GRO	ADVANCE		2018	18	MIL	SD4707
2018	2	GRO	BOOST		2018	19	MIL	SD4708
2018	3	GRO	BRICK		2018	20	MIL	SD4711
2018	4	GRO	BRIGGS		2018	21	MIL	SD4719
2018	5	GRO	FALLER		2018	22	MIL	SD4720
2018	6	GRO	FOCUS		2018	23	MIL	SD4721
2018	7	GRO	FOREFRONT		2018	24	MIL	SD4740
2018	8	GRO	LCS-TRIGGER		2018	25	MIL	SD4745
2018	9	GRO	OXEN		2018	26	MIL	SD4746
2018	10	GRO	PREVAIL		2018	27	MIL	SD4748
2018	11	GRO	SELECT		2018	28	MIL	SD4752
2018	12	GRO	STEELE-ND		2018	36	MIL	SD4771
2018	13	GRO	SURPASS		2018	38	MIL	SD4773
2018	14	GRO	TRAVERSE		2018	39	MIL	SD4775
2018	15	GRO	SD4539		2018	43	MIL	SD4792
2018	16	GRO	SD4625		2018	47	MIL	SD4816
2018	17	GRO	SD4706					

Figure 5. Statistical sampling of 2018 Hard Red Spring Wheat (N-99).

YEAR	ENTRY	LOC	NAME		YEAR	ENTRY	LOC	NAME
2019	1	GRO	ADVANCE		2019	21	SEL	SD4773
2019	2	GRO	BOOST		2019	22	SEL	SD4775
2019	3	GRO	BRICK		2019	25	SEL	SD4840
2019	4	GRO	BRIGGS		2019	26	SEL	SD4842
2019	5	GRO	FALLER		2019	27	SEL	SD4843
2019	6	GRO	FOCUS		2019	28	SEL	SD4844
2019	7	GRO	FOREFRONT		2019	30	SEL	SD4848
2019	8	GRO	LCS-TRIGGER		2019	31	SEL	SD4849
2019	9	GRO	OXEN		2019	32	SEL	SD4852
2019	10	GRO	PREVAIL		2019	33	SEL	SD4854
2019	11	GRO	SELECT		2019	34	SEL	SD4855
2019	12	GRO	SURPASS		2019	39	SEL	SD4870
2019	13	GRO	SY-VALDA		2019	40	SEL	SD4871
2019	14	GRO	TRAVERSE		2019	41	SEL	SD4873
2019	15	GRO	SD4625		2019	42	SEL	SD4874
2019	19	GRO	SD4771		2019	43	SEL	SD4876
2019	20	GRO	SD4772		2019	1	WAT	ADVANCE
2019	21	GRO	SD4773		2019	2	WAT	BOOST
2019	22	GRO	SD4775		2019	3	WAT	BRICK
2019	25	GRO	SD4840		2019	4	WAT	BRIGGS
2019	26	GRO	SD4842		2019	5	WAT	FALLER
2019	27	GRO	SD4843		2019	6	WAT	FOCUS
2019	28	GRO	SD4844		2019	7	WAT	FOREFRONT
2019	30	GRO	SD4848		2019	8	WAT	LCS-TRIGGER
2019	31	GRO	SD4849		2019	9	WAT	OXEN
2019	32	GRO	SD4852		2019	10	WAT	PREVAIL
2019	33	GRO	SD4854		2019	11	WAT	SELECT
2019	34	GRO	SD4855		2019	12	WAT	SURPASS
2019	39	GRO	SD4870		2019	13	WAT	SY-VALDA
2019	40	GRO	SD4871		2019	14	WAT	TRAVERSE
2019	41	GRO	SD4873		2019	15	WAT	SD4625
2019	42	GRO	SD4874		2019	19	WAT	SD4771
2019	43	GRO	SD4876		2019	20	WAT	SD4772
2019	1	SEL	ADVANCE		2019	21	WAT	SD4773
2019	2	SEL	BOOST		2019	22	WAT	SD4775
2019	3	SEL	BRICK		2019	25	WAT	SD4840
2019	4	SEL	BRIGGS		2019	26	WAT	SD4842
2019	5	SEL	FALLER		2019	27	WAT	SD4843
2019	6	SEL	FOCUS		2019	28	WAT	SD4844
2019	7	SEL	FOREFRONT		2019	30	WAT	SD4848
2019	8	SEL	LCS-TRIGGER		2019	31	WAT	SD4849
2019	9	SEL	OXEN		2019	32	WAT	SD4852
2019	10	SEL	PREVAIL		2019	33	WAT	SD4854
2019	11	SEL	SELECT		2019	34	WAT	SD4855
2019	12	SEL	SURPASS		2019	39	WAT	SD4870
2019	13	SEL	SY-VALDA		2019	40	WAT	SD4871
2019	14	SEL	TRAVERSE		2019	41	WAT	SD4873
2019	15	SEL	SD4625		2019	42	WAT	SD4874
2019	19	SEL	SD4771		2019	43	WAT	SD4876
2019	20	SEL	SD4772					

Figure 6. Statistical sampling of 2019 Hard Red Spring Wheat (N-99).

Sample preparation:

The whole data set had 99 wheat samples from different locations of South Dakota during the 2018 growing season. About 100 g of subsamples was tempered and milled on a Quadrumat Junior mill to yield flour to be used for analysis, milled to a particle size of 250 micrometers. Additional reference data (another 99 samples) was obtained for the 2019 growing season and used for statistical quality analysis.

Chemical analysis of Total Dietary Fiber:

The level of dietary fiber in the 2018 wheat samples was determined by using AACC method 991.43 by using an ANKOM Automated Total Dietary Fiber Analyzer.

This procedure began with sample preparation of the wheat samples and reagents needed throughout the procedure. The wheat samples were pre-milled with a particle size less than 0.5mm. A 95% ethanol solution was prepared by mixing 950mL of ethanol with 50mL of distilled water. Similarly, a 78% ethanol solution was prepared by mixing 780mL of ethanol with 120mL of distilled water. An enzyme solution of alpha-amylase was prepared by diluting 5mL of the enzyme to 25mL, with distilled water. This process was repeated for the other two enzymes, protease, and amyloglucosidase (AMG). A MES-TRIS buffer solution was prepared by dissolving 19.52g of MES and 12.2g TRIS into 1.7L of distilled water. The pH was adjusted to 8.2 using 6N NaOH, then the solution was diluted to 2L with distilled water. A 0.561N HCL solution was prepared by diluting 93.5mL of 6N HCl to 1L with distilled water.

This procedure began with the numbering of all of the empty filter bags using a solvent resistant marker. The bags were weighed on an electronic balance using a tared bag weight holder to get their initial empty weight. Each of the 8 chemical containers (HCl, amylase, protease, AMG, distilled water, 78% ethanol, 95% ethanol, and buffer) were filled to the minimum line or above. Approximately 1g of diatomaceous earth was weighed in a tared metal tin, and this was repeated six times, one for each of the six filter bags. Aliquots (0.5+/- 0.05g) of the sample were weighted in a tared metal tin and repeated to get six total samples. The clamp bars were removed from the instrument, nitrogen was turned on to 90psi, filter bags put in place on the bottom portion of the instrument, and the diatomaceous earth was added to each of the six bags. Flow through bags were placed on the top portion of the instrument, clamp bars put in place, and the samples were added to each of the six bags. Then the machine started its automated process. The process involved three enzyme digestions, rinsing, and precipitation. The pH was manually checked before the protease digestion phase, using HCl to adjust the pH to 4.0-4.7. After the automated process was complete, the filter bags were removed from the instrument and rinsed twice with acetone. After the acetone evaporates, a heat sealer was used to seal the bags closed above the filter portion of the bag. The filter bags were placed in an oven set at 105˚C for 90 minutes to remove the moisture. The bags were then removed from the oven and placed into a desiccator to cool. Once cool, the bags were weighed to obtain a final weight. In order to obtain the total dietary fiber values, the values had to be adjusted for ash and protein content. For ash determination, the final samples were placed into weighed crucibles, and put into a muffle furnace at 550 ºC for 22 hours. The crucibles were weighed again following this procedure to obtain the

difference in weight, thus the ash content. For protein determination, the samples were analyzed with an Elementar Rapid N Exceed protein analyzer, which follows a Dumas protein analysis procedure. This is a combustion method that outputs % nitrogen, which was converted to protein using a conversion factor of 5.8, the conversion standard for wheat.

Calculations (all weights in grams)

$$\%\ TDF = \frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \times 100$$

$$= \frac{[((f_{F1} - f_{S1} - D_1) + (f_{F2} - f_{S2} - D_2))/2] - P - (A_2 - D_2) - B}{(M_1 + M_2)/2} \times 100$$

Where:

M_1, M_2	=	Original wt for duplicate samples adjusted for pre-treatment fat and sugar losses (g)
R_1, R_2	=	Residue for duplicate samples (g)
f_F	=	Final Filter Bag (g)
f_S	=	Initial Filter Bag (g)
D	=	Original wt of Diatomaceous Earth (g)
P	=	Protein of residue and bag (g)
A	=	Ash of residue and bag (g)
B	=	Blank (g)
	=	$[(BR_1 + BR_2)/2] - P_B - (A_B - D_B)$
	=	$[((f_{BF1} - f_{BS1} - D_{B1}) + (f_{BF2} - f_{BS2} - D_{B2}))/2] - P_{B1} - (A_{B2} - D_{B2})$
BR_1, BR_2	=	Residue for duplicate blanks (g)
f_{BF}	=	Final Blank Filter Bag (g)
f_{BS}	=	Initial Blank Filter Bag (g)
P_B	=	Protein of Blank Filter Bag (g)
A_B	=	Ash of Blank Filter Bag (g)
D_B	=	Original wt of Diatomaceous Earth in Blank Filter Bag (g)

Figure 7. Total Dietary Fiber Determination Calculations (Image source: ANKOM TDF Automated Procedure Manual)

Moisture Analysis:

Moisture analysis was conducted on the samples so the data could be reported on a dry basis. All samples were determined in duplicates. For each sample, two aluminum dishes were labeled and placed in a 130ºC oven for 15 minutes. They were removed and placed in a desiccator to cool for 10 minutes, and then weighed. 1-2 grams of sample were placed into each dish, and the exact weight was recorded. These dishes were placed in the 130ºC oven for 1 hour, then removed and placed in a desiccator to cool for 10 minutes. Final weights were recorded for the dry samples. The percent moisture content was calculated by:

$$\frac{(Initial\ weight\ of\ aluminum\ dish+sample)-(Final\ weight\ of\ aluminum\ dish+sample)}{Inital\ weight\ of\ the\ sample} * 100$$

Spectroscopic analysis:

A near-infrared reflectance spectra was obtained for 2018 and 2019 ground wheat samples through scanning of the samples on an NIRS DS2500 analyzer with a spectral range of 800 to 2500nm.

Calibration Development:

To create the calibration, spectral measurements from the NIRS scan were related to the reference data that was gathered via chemical methods using calibration models that are

based on multiple variable based regression models. The data was centered with modified partial least squares (PLS) and the outliers were identified and removed. The spectra were processed with multiplicative scatter correction to partially correct baseline differences. Cross validation is used to identify outliers, choose the number of PLS or principle component analysis (PCA) factors in the calibration model, as well as provide an estimation of the performance of the calibration model when used to predict unknown samples (Bellato, 2011). A validation sample set containing samples of known constituent values was set aside for testing the performance of the calibration. These samples were not used in the creation of the calibration. About 25% of the total samples were used for this validation sample set. The Coefficient of determination of calibration (RSQ), standard error of calibration (SEC), standard error of cross validation (SECV), standard error of prediction (SEP), and variance ratio (1-VR) were the statistical terms that were used to determine the accuracy of the NIRS calibration. Bias was a statistical term used to describe the validation set in terms of the difference in results from reference analysis verses the NIRS prediction.

Predictive calibrations were created for key grain, flour, and dough parameters, for which analytical data were available. Predictive calibrations for gluten, grain, and flour protein content estimations were created to estimate predictive values for the constituents. Dough mixing properties such as peak mixing time, flour water absorption, dough stability, and mixing tolerance index were similarly explored. Final bread baking trial data was used to create estimations and determine the predictability of baking properties through spectral data of the flour.

Software:

ISIscan Nova version 8.0.6.2 was used to collect the spectra of the sample scans on the NIRS DS2500 machine. The spectra were auto synchronized with FOSS Mosaic Solo version 8.0.4.12. The scanned data was exported and utilized with WinISI Project Manager version 4.12.0.15440.

Statistical Analysis:

The reference analysis of total dietary fiber was done in duplicate for each sample. All laboratory values for total dietary fiber, protein, and gluten were expressed on a dry weight basis. A correlation analysis was employed to validate the NIRS prediction through a calibration equation. Duncan's Multiple Range Test was utilized to determine difference between the means at a 95% confidence interval ($p < 0.05$). Conclusions about the difference amongst the growing locations and various samples in terms of total dietary fiber content was drawn from analysis of variance (ANOVA). Effects of growing location and variability was statistically determined. The relationship between wheat constituents and baking functionality parameters were statistically analyzed. This allowed for conclusions to be drawn about the genetic factors, environmental factors, and their interaction, in relation to wheat quality.

Chapter 4:

Results and Discussion

The determination of TDF residue content of hard red spring wheat grown in 2018 in Brookings, Miller, and Groton, South Dakota was calculated using officially accepted reference methods (AOAC 991.43). The reference data was processed against spectral data to create a predictive calibration equation that was then statistically validated with a validation subset of data. The validation sample subset was independent and different than the samples used to create the initial calibration model. They served as an additional check of the effectiveness of calibration model in predicting "unknown" samples. The statistical terms used for analysis included the coefficient of determination (RSQ), one minus variance ratio (1-VR), standard error of calibration (SEC), standard error of cross validation (SECV), the coefficient of determination of the validation (RSQval), standard error of prediction (SEP), and bias values. A paired t-test was used to determine whether the NIRS predicted values were statistically the same as the reference values. Additional predictive NIRS calibration equations were similarly developed for other wheat constituents, and mixing and baking parameters for 2018 and 2019 growing data. Combination calibration equations were developed for the combined data of 2018 and 2019, and compared to the singular year equations for robustness. These constituents and parameters were also analyzed for correlations and interrelationships among the parameters in order to draw conclusions about ties to how wheat constituents influence baking functionality.

Analysis of Total Dietary Fiber Content:

Reference Analysis

The TDF residue content that was calculated in the 99 wheat samples from the 2018 growing year was found through reference analysis methods (Table 1). There were 33 samples replicated in three growing locations (Brookings, Miller, Groton), and each was measured in duplicate on the ANKOM TDF machine to obtain two residue values for each sample. The measured dietary fiber residue had a mean of 4.96%, a range of 2.3-8.5%, and a standard deviation of 1.49. White flour has been reported at TDF% levels of 1.94- 6.27 % (Shewry, 2015). It makes sense that the TDF residue values that were obtained in this study fall within the upper end of the accepted TDF% range, as the residue still contains ash and protein contributing to the value. Reduced TDF values are expected in flour due to the milling of the grain that removes much of the bran, the concentrated location of dietary fiber in grains. The measured residue values are reported rather than the calculated TDF% values because the focus of this study was to analyze the impact of growing location and wheat variety on the TDF variability, rather than absolute TDF values to put on a nutrition label. The measured TDF residues are sufficient in determining these variability factors. Another advantage to using the measured residue values as opposed to the absolute TDF% values is that it is less time consuming to obtain these values, so future studies can conduct a larger sample study for less time and resources.

Control samples added confidence to the accuracy and precision of the results because the calculated TDF values of the controls were within the reported range, and had a low standard deviation and coefficient of variance (Table 2). The cereal control had a mean

TDF of 40.7% (expected 42.6 ± 2%) with a standard deviation of 0.92 and a CV of 2.3%. The whole wheat flour control had a mean TDF of 11.4% (expected 12.6 ± 1.5%) with a standard deviation of 0.46 and a CV of 4.1%.

Table 1. Total dietary fiber residual of 2018 wheat samples analyzed with ANKOM Total Dietary Fiber Analyzer

Constituent	N (# of samples)	Range %	Mean %	Standard Deviation
TDF residual	99	2.3-8.5	4.96	1.49

Table 2. Total dietary fiber of control samples analyzed with ANKOM Total Dietary Fiber Analyzer

Fiber Source	N	Mean	SD	CV	Reported Value
Cereal Control	2	40.7%	0.92	2.26%	42.6 ± 2%
Whole Wheat Flour Control	7	11.4%	0.46	4.05%	12.6 ± 1.5%

N: number of samples; SD: standard deviation; CV: coefficient of variance; Reported value: given total dietary fiber % for control

Location verses variety effect on total dietary fiber content variability:

Thirty-three varieties of hard red spring wheat grown across three locations in 2018, were analyzed in duplicates for growing location and variety effects on the dietary fiber content. Table 3 provides the variety verses location effects and indicates that both variety and growing location are significant in terms of TDF residue at the 0.001 level of significance. A Duncan Multiple Range Test was conducted at the 0.05 level of significance to identify the rankings of the growing locations (Table 4). This test indicated that Brookings and Miller were statistically the same and both were better than Groton in terms of the TDF residue %. Similarly, using the Duncan Multiple Range Test, the means of the wheat varieties were ranked (Table 5), and statistically significant effects were found for TDF residues based on the wheat variety. Thirteen varieties including SURPASS, SD4740, FOREFRONT, SD4719, SD4707, SD4816, LCS-TRIGGER, SD4720, SD4721, BRICK, SD4711, SD4775, and ADVANCE were shown to be the highest ranking, and not statistically different from one another. A larger dataset may further identify and expose differences among the top performing varieties.

Location and variety have been found to be statistically impactful by others, in wheat (Tolera et. al, 2008), and other low TDF sources such as potatoes (Mullin et. al, 1993). This shines light on the relevance and importance of obtaining data such as this so that breeders can make informed decisions on cultivar and growing location in relation to crop yield and quality.

Table 3. Analysis of Variance of Total Dietary Fiber residue of wheat grown in South Dakota in 2018

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Significance**
Location	2	35.6	17.780	8.616	0.000259	***
Residuals	195	402.4	2.064			
Variety	32	141.2	4.412	2.453	0.000127	***
Residuals	165	363.3	2.202			

Df: degrees of freedom; Sum Sq: sum of squares; Mean Sq: mean squares; Significance: significant code, 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4. Ranking of growing locations based on the mean of TDF residue at 0.05 level of significance

Location	**Mean**	***Groups**
Brookings	5.400	a
Miller	5.080	a
Groton	4.385	b

*Means with the same letter within each column are not statistically different from each other ($P<0.05$).

Table 5. Ranking of wheat variety based on the mean of TDF residue at 0.05 level of significance

Variety	Mean	*Groups
SURPASS	6.950000	a
SD4740	6.466667	ab
FOREFRONT	6.400000	ab
SD4719	6.183333	abc
SD4707	5.850000	abcd
SD4816	5.800000	abcd
LCS-TRIGGER	5.633333	abcde
SD4720	5.283333	abcdef
SD4721	5.216667	abcdef
BRICK	5.200000	abcdef
SD4711	5.183333	abcdef
SD4775	5.133333	abcdef
ADVANCE	5.116667	abcdef
SD4706	5.016667	bcdefg
SELECT	5.000000	bcdefg
SD4792	4.966667	bcdefg
FALLER	4.933333	bcdefg
FOCUS	4.900000	bcdefg
OXEN	4.866667	bcdefg
PREVAIL	4.866667	bcdefg
SD4539	4.833333	bcdefg
STEELE-ND	4.750000	bcdefg
TRAVERSE	4.733333	bcdefg
SD4708	4.650000	bcdefg
SD4748	4.616667	bcdefg
SD4771	4.616667	bcdefg
SD4625	4.416667	cdefg
BRIGGS	3.966667	defg
BOOST	3.883333	efg
SD4745	3.883333	efg
SD4746	3.550000	fg
SD4752	3.466667	fg
SD4773	3.183333	g

*Means with the same letter within each column are not statistically different from each other ($P<0.05$).

NIRS Analysis of Total Dietary Fiber

Of the 99 wheat samples from 2018, 56 were selected from the NIRS scanning to be used for calibration equation development. Of these 56 samples, 42 were used for calibration development, and the remaining 14 were used for validation of the model. The accuracy of the calibration was statistically assessed (Table 6). The mean, RSQ, SEC, SECV, and 1-VR were used to assess the calibration set, and the mean RSQval, bias, and SEP values were used to assess calibration with the validation set. The calibration statistics for TDF gave a mean of 4.80 with an RSQ of 0.07, SEC of 1.52, SECV of 1.70, and a 1-VR of -0.18. The validation set gave a mean of 5.23 with an RSQval of 0.00, a bias of 0.34, and a SEP of 1.45. For a good calibration model, the RSQ, 1-VR, and RSQval would have been high, and the SEC, SECV, SEP, and bias would have been low values. TDF is a particularly complex fiber matrix. Milled wheat flour is sifted to remove bran flakes which are good sources of dietary fiber. Physical removal of the bran, the low range of occurrence of fiber are thus plausible explanations for poor NIRS predictability.

Table 6. NIRS calibration and validation statistics of Total Dietary Fiber (TDF) residue of 2018 wheat samples

	Calibration Sample Data						Validation Sample Data				
Constituent	**N**	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	**N**	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
TDF residue	42	4.80	0.07	1.52	1.70	-0.18	14	5.23	0.00	0.34	1.45

N: number of samples; RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross validation; 1-VR: 1 minus variance ratio; RSQval: coefficient of determination of validation subset; SEP: standard error of prediction

Development of NIRS predictive calibrations of other wheat constituents and parameters:

NIRS predictive calibrations were successfully determined for selected wheat constituents, dough mixing parameters, and baking parameters. The accuracy of these calibrations was statistically assessed (Table 7 and 9). The mean, RSQ, SEC, SECV, and 1-VR were used to assess the calibration set, and the mean RSQval, bias, and SEP values were used to assess calibration with the validation set. For a good calibration model, the RSQ, 1-VR, and RSQval would have been high, and the SEC, SECV, SEP, and bias would have been low values.

For the 2018 data (Table 7a, Table 7b, Table 7c), the parameters with RSQ> 0.80 included the single kernel hardness index (0.87), dry gluten (0.82), farinograph water absorption% (0.91), water absorption capacity (0.91), NIR grain moisture (0.84), NIR grain protein (0.99), NIR grain ash (0.87), flour protein (0.88), flour ash (0.85), and the mixograph's mid-line peak value (0.85). A value of R^2 greater than 0.80 means that fit of the model is a statistically good fit. Wheat breeders are able to accept lower values, down to 0.60, due to the biology, the environment, and chance variability factors associated with growing crops. The additional parameters included with an RSQ>0.60 included total gluten (0.70), good wet gluten (0.78), wet gluten (0.70), WAM% (0.64), kernel protein (0.78), kernel ash (0.72), and flour extraction (0.63). For all parameters RSQ>0.60, except for NIR ash, flour ash, and kernel ash, no significant difference was found with a paired t-test that compared the means of the NIRS predicted values to the means of the analytical reference values, thus showing the effectiveness of the calibration models (Table 8).

Table 7a. NIRS calibration and validation statistics of selected wheat constituents and functionality parameters of samples from 2018 growing year

		Calibration Sample Set						Validation Sample Set				
Variable	**Source**	**N**	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	**N**	**Mean**	**RSQval**	**Bias**	**SEP**
Total Dietary Fiber Residue	ANKOM TDF Analyzer	42	4.80	0.07	1.52	1.70	-0.18	14	5.23	0.00	0.34	1.45
Hardness Index	Single Kernel Characterization System	42	168.1	0.87	4.39	5.70	0.77	14	74.6	0.56	2.15	5.26
Weight	Single Kernel Characterization System	41	31.33	0.25	1.44	1.53	0.135	14	31.95	0.002	0.491	2.00
Diameter	Single Kernel Characterization System	42	2.75	0.25	0.07	0.75	0.151	14	2.808	0.066	0.046	0.106
moisture(%)	NIR	42	13.28	0.84	0.196	0.213	0.801	14	13.4	0.83	0.11	0.28
Protein(%)	NIR	42	15.19	0.99	0.07	0.115	0.991	14	15.3	0.99	-0.018	0.11
Ash(%)	NIR	42	0.44	0.87	0.011	0.016	0.75	14	0.45	0.85	0.01	0.015
Wet bad gluten	Glutomatic	40	0.363	0.43	0.199	0.22	0.30	14	0.450	0.024	0.044	0.385
Total gluten	Glutomatic	42	4.35	0.70	0.208	0.226	0.634	14	4.39	0.59	-0.009	0.228
Dry gluten	Glutomatic	42	1.57	0.82	0.066	0.074	0.773	14	1.571	0.80	-0.026	0.058
Good wet gluten	Glutomatic	39	3.99	0.78	0.16	0.172	0.731	14	3.94	0.42	-0.086	0.250
Gluten index	Glutomatic	40	91.77	0.44	4.17	4.60	0.306	14	90.14	0.024	-0.76	8.15
Wet gluten	Glutomatic	42	43.5	0.70	2.08	2.26	0.63	14	43.90	0.59	-0.091	2.28
Water binding	Glutomatic	42	27.7	0.54	1.68	1.86	0.422	14	28.18	0.39	0.174	2.065

N: number of samples; RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross validation; 1-VR: 1 minus variance ratio; RSQval: coefficient of determination of validation subset; SEP: standard error of prediction

Table 7b. NIRS calibration and validation statistics of selected wheat constituents and functionality parameters of samples from 2018 growing year

		Calibration Sample Set						Validation Sample Set				
Variable	**Source**	**N**	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	**N**	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Loaf volume	USDA	42	215.7	0.57	15.5	17.6	0.43	14	222.4	0.00	9.57	20.33
Test Weight	USDA	42	60.6	0.33	1.15	1.22	0.23	14	59.98	0.23	-0.66	1.19
Kernel protein	USDA	42	16.26	0.78	0.57	0.64	0.72	14	16.4	0.72	0.064	0.35
Kernel Ash	USDA	42	1.62	0.72	0.077	0.098	0.529	14	1.725	0.62	0.06	0.10
Flour extraction	USDA	42	62.8	0.63	2.33	2.97	0.38	14	62.5	0.05	0.47	3.60
Flour protein	USDA	41	14.8	0.88	0.35	0.52	0.72	14	14.9	0.69	0.14	0.49
Flour ash	USDA	42	0.37	0.85	0.015	0.022	0.67	14	0.394	0.68	0.02	0.03
Mixogram pattern	USDA	40	5.85	0.46	0.39	0.41	0.40	14	5.86	0.04	-0.07	0.74
Bake mixing time	USDA	42	4.05	0.14	0.75	0.79	0.016	14	3.98	0.06	-0.13	0.92
Baking water absorption	USDA	42	56.9	0.37	1.54	1.64	0.278	14	57.7	0.16	0.56	1.24

N: number of samples; RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross validation; 1-VR: 1 minus variance ratio; RSQval: coefficient of determination of validation subset; SEP: standard error of prediction

Table 7c. NIRS calibration and validation statistics of selected wheat constituents and functionality parameters of samples from 2018 growing year

		Calibration Sample Set						Validation Sample Set				
Variable	**Source**	**N**	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	**N**	**Mean**	**RSQ_{val}**	**Bias**	**SEP**
Mid-line right value	Mixograph	42	47.99	0.517	3.81	4.04	0.444	14	48.43	0.326	-0.487	3.577
Mid-Line Right Slope Integral	Mixograph	42	163.3	0.115	34.11	35.12	0.039	14	163.1	0.00	-2.40	39.08
Mid-line peak time	Mixograph	42	4.69	0.25	0.94	1.04	0.05	14	4.57	0.47	0.21	1.08
Mid-line peak value	Mixograph	42	51.9	0.85	2.5	4.4	0.51	14	51.99	0.56	-1.61	4.11
Mid-line peak width	Mixograph	41	26.0	0.46	3.17	3.44	0.35	14	25.9	0.45	-0.38	2.87
Flour Moisture(%)	Farinograph	42	13.63	0.54	0.47	0.484	0.515	14	13.53	0.52	-0.127	0.64
Measuring time(min)	Farinograph	42	32.93	0.39	6.42	7.09	0.236	14	30.55	0.21	-1.46	5.35
Dough development time(min)	Farinograph	41	13.3	0.53	3.56	3.81	0.445	14	12.42	0.09	-1.27	3.85
Consistency	Farinograph	42	499.9	0.045	10.39	10.89	-0.07	14	500.5	0.008	0.022	9.47
Water absorption	Farinograph	42	64.5	0.91	0.77	1.48	0.666	14	65.73	0.68	-0.43	1.54
WAC(%)	Farinograph	42	64.5	0.91	0.78	1.49	0.662	14	65.8	0.72	-0.44	1.45
WAM(%)	Farinograph	42	64.11	0.64	1.64	1.85	0.527	14	65.2	0.62	0.24	1.72
Mixing tolerance index	Farinograph	42	20.8	0.50	8.34	10.22	0.224	14	19.5	0.06	-5.06	10.47
Time to breakdown	Farinograph	42	24.4	0.58	5.28	5.63	0.51	14	24.4	0.15	0.17	5.53

N: number of samples; RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross validation; 1-VR: 1 minus variance ratio; RSQval: coefficient of determination of validation subset; SEP: standard error of prediction

Table 8. Comparison of reference means and NIRS predicted means for parameters >0.6 RSQ of validation set 2018

Parameter	**Reference Mean (ANKOM)**	**Reference SD**	**Predicted Mean (NIRS)**	**Predicted SD**
*Kernel hardness index	71.61	7.32	72.46	6.54
*Total gluten	4.39	0.37	4.40	0.25
*Dry gluten	1.57	0.12	1.60	0.11
*Good gluten	3.94	0.32	4.04	0.20
*Wet gluten	43.90	3.66	43.99	2.49
*Water absorption (farinograph)	65.73	2.72	66.16	2.24
*WAC% (farinograph)	65.75	2.69	66.19	2.24
*WAM% (farinograph)	65.19	2.75	64.95	1.64
*NIR Moisture%	13.43	0.59	13.32	0.43
*NIR Protein %	15.32	1.01	15.34	1.00
NIR Ash %	0.447	0.028	0.437	0.024
*Kernel protein	16.44	0.62	16.37	0.67
Kernel ash	1.72	0.14	1.67	0.13
*Flour extraction	62.47	3.26	62.00	2.59
*Flour protein	14.94	0.88	14.80	0.72
Flour ash	0.394	0.042	0.374	0.029
*Mid-line peak time	51.99	5.16	53.60	5.76

*Reference mean and NIR predicted mean are statistically the same (paired t-test 95% confidence interval includes 0).

For the 2019 data (Table 9), the parameters with RSQ> 0.80 included the Farinograph moisture% (0.81), water absorption% (0.92), WAC% (0.92), WAM% (0.84), NIR protein (0.99), and NIR ash (0.88). The additional parameter included with an RSQ>0.60 was NIR moisture (0.64). For all parameters RSQ>0.60, no significant difference was found with a paired t-test that compared the means of the NIRS predicted values to the means of the analytical reference values, thus showing the effectiveness of the calibration models (Table 10).

Table 9. NIRS calibration and validation statistics of selected wheat constituents and functionality parameters from 2019 growing year

	Calibration Sample Set						Validation Sample Set				
Constituent	**N**	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**	**N**	**Mean**	**RSQval**	**Bias**	**SEP**
Moisture(%) (Farinograph)	75	14.14	0.81	0.38	0.69	0.39	24	13.98	0.37	-0.046	0.749
Measuring time(sec) (Farinograph)	74	1475.6	0.079	291.4	309.5	-0.053	24	1429.1	0.21	-65.3	187.2
Dough departure time(sec) (Farinograph)	73	1667.5	0.023	25.9	28.6	-0.2	24	1668	0.024	-1.16	27.4
Consistency(FU)(Farinograph)	74	499.8	0.08	9.6	10.32	-0.078	24	506.7	0.02	6.5	10
Water absorption(%) (Farinograph)	75	68.8	0.92	0.68	1.26	0.73	24	68.7	0.52	-0.74	1.94
WAC(%) (Farinograph)	74	68.7	0.92	0.7	1.23	0.74	24	68.9	0.58	-0.6	1.77
WAM(%) (Farinograph)	74	69	0.84	1.19	1.98	0.54	24	68.9	0.38	-0.63	2.65
Mixing tolerance index(FU) (Farinograph)	74	26.2	0.31	7.84	9.4	-0.0072	24	26.3	0.13	0.24	9.28
Time to breakdown (sec) (Farinograph)	75	1052	0.3	222.99	245.7	0.137	24	998.1	0.31	-66.5	183.2
NIR Moisture(%)	74	12.62	0.64	0.24	0.25	0.6	24	12.7	0.59	0.034	0.24
NIR Protein(%)	75	14.2	0.99	0.087	0.112	0.975	24	14.24	0.99	-0.017	0.134
NIR ASH(%)	75	0.48	0.88	0.011	0.013	0.83	24	0.495	0.78	0.005	0.013
Wet Bad Gluten	72	0.52	0.11	0.38	0.4	0.0017	24	0.53	0.001	0.047	0.374
Total Gluten	73	4.18	0.28	0.21	0.23	0.094	24	4.19	0.61	0.017	0.159
Dry Gluten	74	1.46	0.48	0.068	0.076	0.335	24	1.47	0.58	0.005	0.063
Good Gluten	74	3.66	0.39	0.25	0.27	0.29	24	3.66	0.21	-0.061	0.29
Gluten Index	72	87.8	0.12	8.53	9	0.0092	24	87.6	0.002	-1.12	8.4
Wet gluten	73	41.8	0.28	2.06	2.29	0.094	24	41.88	0.61	0.172	1.59
Water binding (glutomatic)	72	27.2	0.12	1.66	1.75	0.0038	24	27.17	0.6	-0.085	1.27

N: number of samples; RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross validation; 1-VR: 1 minus variance ratio; RSQval: coefficient of determination of validation subset; SEP: standard error of prediction

Table 10. Comparison of reference means and NIRS predicted means for parameters >0.6 RSQ of validation set 2019

Parameter	**Reference Mean (ANKOM)**	**Reference SD**	**Predicted Mean (NIRS)**	**Predicted SD**
*Moisture % (Farinograph)	13.98	0.88	14.03	0.85
*Water absorption% (Farinograph)	68.72	2.62	69.46	2.24
*WAC% (Farinograph)	68.90	2.60	69.50	2.14
*WAM% (Farinograph)	68.91	3.24	69.54	2.64
*NIR Protein	14.24	0.80	14.25	0.87
*NIR Moisture	12.68	0.37	12.64	0.34
*NIR Ash	0.50	0.027	0.49	0.024

*Reference mean and NIR predicted mean are statistically the same (paired t-test 95% confidence interval includes 0).

A calibration model was established for the combined data from 2018 and 2019 for all the constituent variables for which data was available for both 2018 and 2019. The same sample data that were used to generate the individual 2018 and 2019 were used to generate the overall combined predictive equations. This was done to generate the highest quality predictive capability. The combined predictive capability showed better predictive power than 2019, as 11 variables had an RSQ>0.80, compared to 7 in 2019.

For the combined data (Table 11), the parameters with RSQ> 0.80 included the Farinograph's dough development time (0.95), water absorption (0.90), WAC% (0.88), WAM% (0.87), NIR moisture (0.92), NIR protein (0.99), NIR ash (0.90), The additional parameters included with an RSQ>0.60 included Farinograph moisture (0.77), mixing tolerance index (0.60), dry gluten (0.66), and time to breakdown (0.62). For all parameters RSQ>0.60, no significant difference was found with a paired t-test that compared the means of the NIRS predicted values to the means of the analytical reference values, thus showing the effectiveness of the calibration models (Table 12). The effectiveness of these models can be seen visually in Figures 8-14, as plots of the reference values verses NIRS predicted values for the parameters with an RSQ>0.8.

This study found that the best predictors of wheat quality included protein content, gluten content and water holding capacity. In general, the RSQ values of the calibrations for the farinograph parameters were higher than those of the mixograph. The predictive calibrations that were created from these hard red spring wheat samples is similar in nature to what others have reported. Indirect wheat quality parameters such as color values, loaf volume, baking water absorption, gluten content, farinograph measures, and mixograph measures have been found suitable for screening purposes with NIRS

calibrations (Dowell et. al, 2006). The NIRS predictive calibrations trended downward in their predictive powers as the parameters become further from flour (flour protein, flour moisture, flour ash) and closer to baking potential. For example, NIRS predictive calibrations are much better for flour protein than loaf volume, which is expected but not to be discredited. NIRS scanning of a flour to predict end quality parameters with relative accuracy (RSQ>0.7) has the potential to save valuable time and sample quantity that a breeder has available. NIRS is especially useful in that it can predict multiple factors with a single scan, and the data can be rapidly compared and analyzed. Protein level of wheat is often relied on for quality indication on wheat, but NIRS has been found to perform more reliably (higher RSQ) in predicting baking qualities such as loaf volume (Gabriel et. al, 2017). This demonstrates the importance and benefit in establishing these models.

Table 11. NIRS calibration statistics of selected wheat constituents and functionality parameters from combined 2018/2019 growing years

	Calibration				
Constituent	**N**	**Mean**	**RSQ**	**SEC**	**SECV**	**1-VR**
Moisture(%) (Farinogarph)	155	13.92	0.77	0.42	0.587	0.546
Measuring Time (sec)(Farinograph)	155	1641.2	0.42	325.9	334.5	0.388
Dough development time(sec)(Farinograph)	153	1347.5	0.95	100.96	146.2	0.899
Consistency (FU)(Farinograph)	154	500.97	0.0089	9.93	10.399	-0.095
Water absorption(%)(Farinograph)	155	67.3	0.9	1.016	1.36	0.812
WAC(%) (Farinograph)	154	67.34	0.88	1.094	1.35	0.815
WAM(%) (Farinograph)	154	67.3	0.87	1.32	1.78	0.762
Mixing tolerance index(FU) (Farinograph)	153	23.69	0.6	6.14	8.18	0.279
Time to breakdown (sec)(Farinograph)	154	1183.8	0.62	227.4	260.8	0.502
NIR Moisture(%)	155	12.87	0.92	0.164	0.224	0.85
NIR Protein(%)	155	14.56	0.99	0.0754	0.105	0.99
NIRAsh(%)	155	0.471	0.9	0.012	0.0128	0.882
Wet bad gluten	152	0.462	0.054	0.327	0.33	0.0298
Total gluten	155	4.24	0.58	0.205	0.212	0.545
Dry gluten	153	1.5	0.66	0.071	0.073	0.6333
Good Gluten	152	3.77	0.54	0.236	0.24	0.522
Gluten index	152	89.3	0.069	7.22	7.29	0.043
Wet gluten	155	42.4	0.58	2.05	2.12	0.545
Water binding (Glutomatic)	154	27.39	0.4	1.64	1.74	0.329

N: number of samples; RSQ: coefficient of determination; SEC: standard error of calibration; SECV: standard error of cross validation; 1-VR: 1 minus variance ratio

Table 12. Comparison of reference means and NIRS predicted means for parameters >0.6 RSQ of validation set 2018/2019 combination

Parameter	**Reference Mean (ANKOM)**	**Reference SD**	**Predicted Mean (NIRS)**	**Predicted SD**
*Dry gluten	1.51	0.13	1.50	0.10
*Moisture % (Farinograph)	13.93	0.87	13.93	0.77
*Water absorption (Farinograph)	67.34	3.16	67.34	3.00
*WAC (Farinograph)	67.43	3.33	67.36	2.97
*WAM (Farinograph)	67.22	3.79	67.30	3.41
*NIR Moisture	12.87	0.58	12.87	0.56
*NIR Ash	0.471	0.037	0.471	0.035
*NIR Protein	14.56	1.05	14.56	1.05
*Time to breakdown (Farinograph)	1195.88	398.89	1187.30	298.32
*Dough development time (Farinograph)	1350.76	458.23	1350.51	454.41
*Mixing tolerance index (Farinograph)	24.10	10.24	23.69	7.57

*Reference mean and NIR predicted mean are statistically the same (paired t-test 95% confidence interval includes 0).

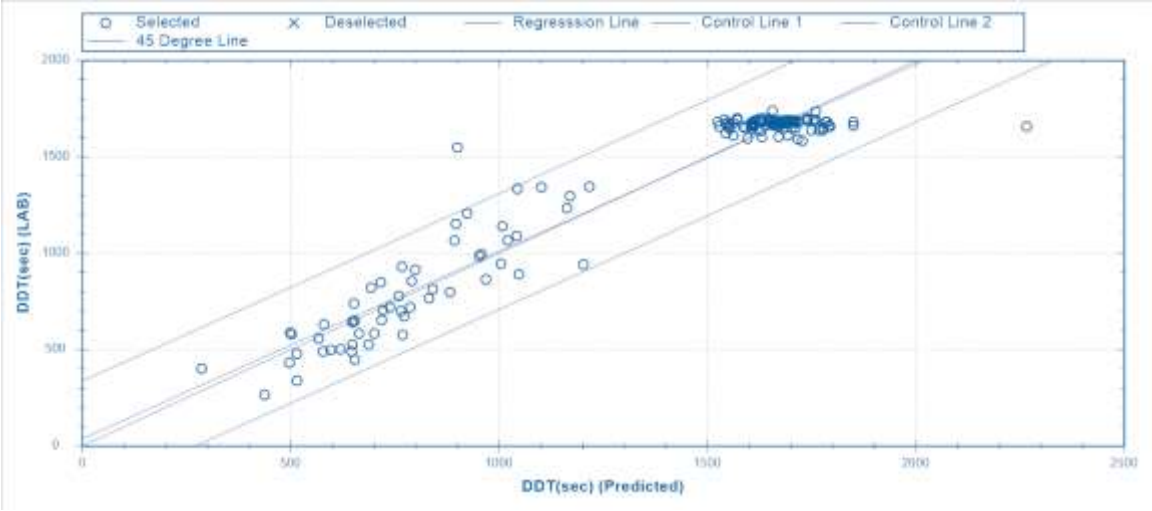

Figure 8. Correlation plot between NIRS method and reference analysis method of dough development time (DDT in sec) with combined 2018/2019 calibration dataset (N=153)

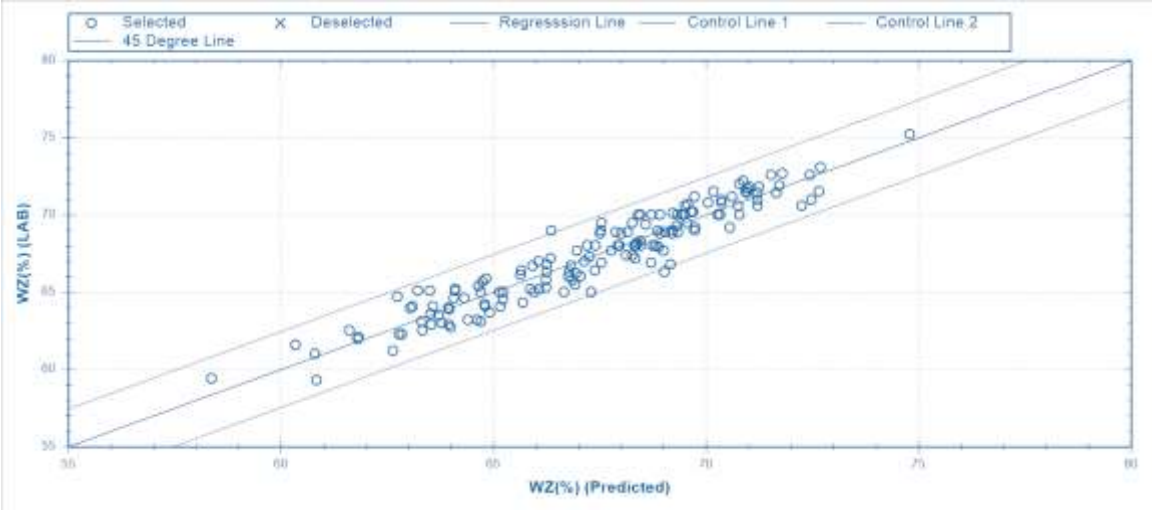

Figure 9. Correlation plot between NIRS method and reference analysis method of water absorption % (farinograph) with combined 2018/2019 calibration dataset (N=155)

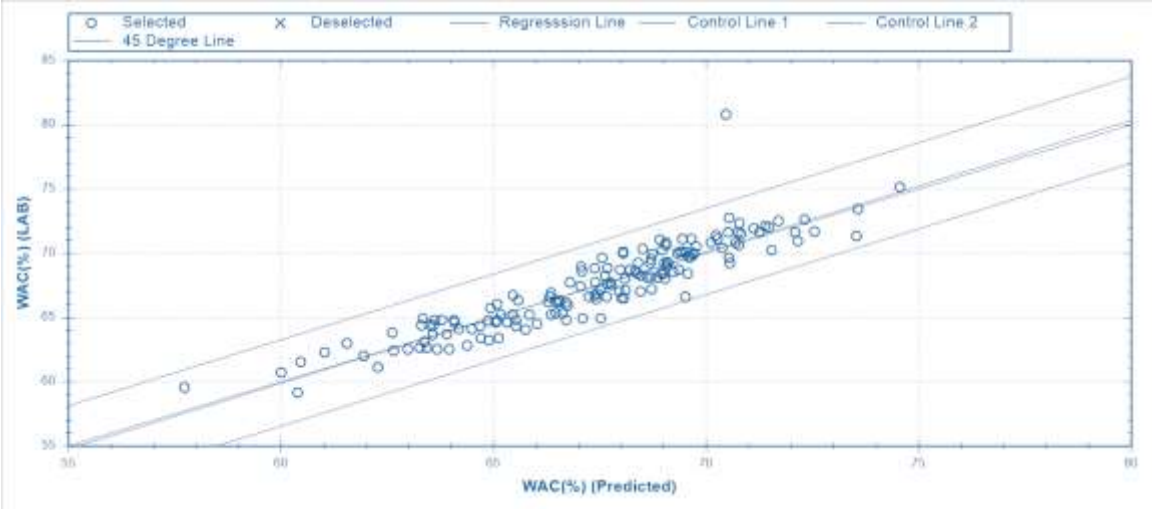

Figure 10. Correlation plot between NIRS method and reference analysis method of WAC% (farinograph water absorption correction factor) with combined 2018/2019 calibration dataset(N=154)

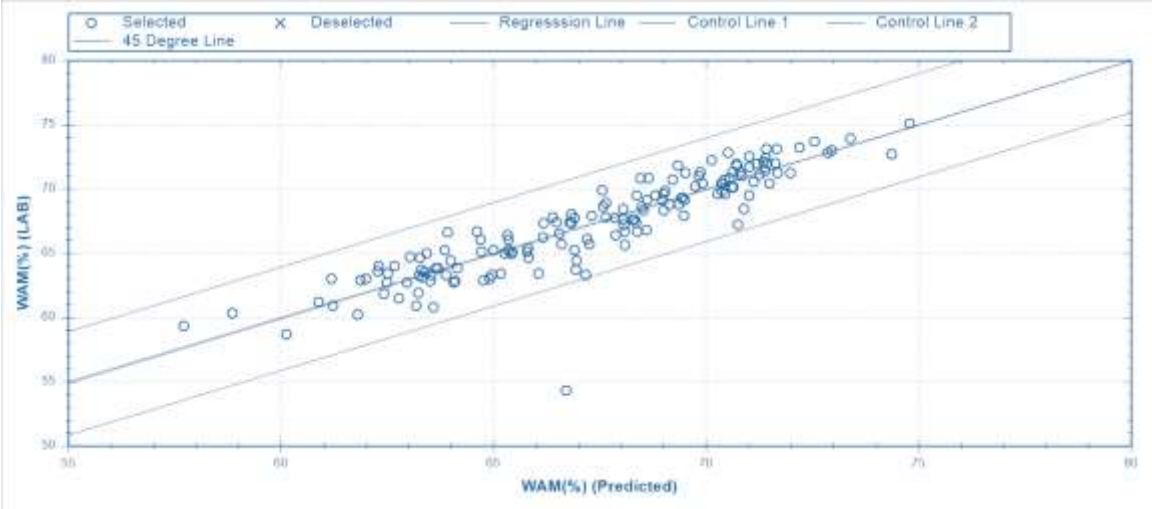

Figure 11. Correlation plot between NIRS method and reference analysis method of WAM% (farinograph water absorption correction factor) with combined 2018/2019 calibration dataset(N=154)

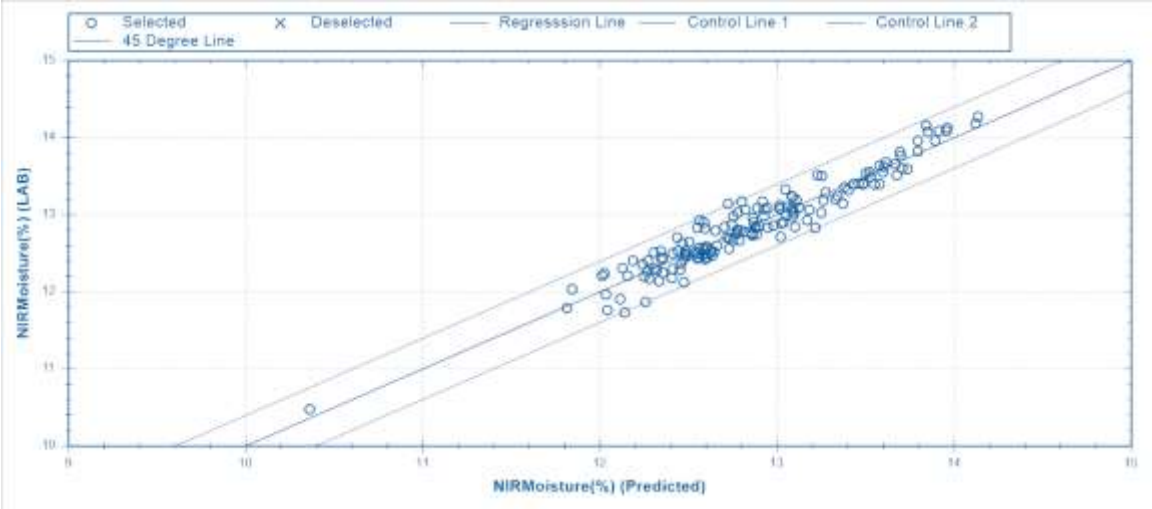

Figure 12. Correlation plot between NIRS method and reference analysis method of NIR moisture% with combined 2018/2019 calibration dataset(N=155)

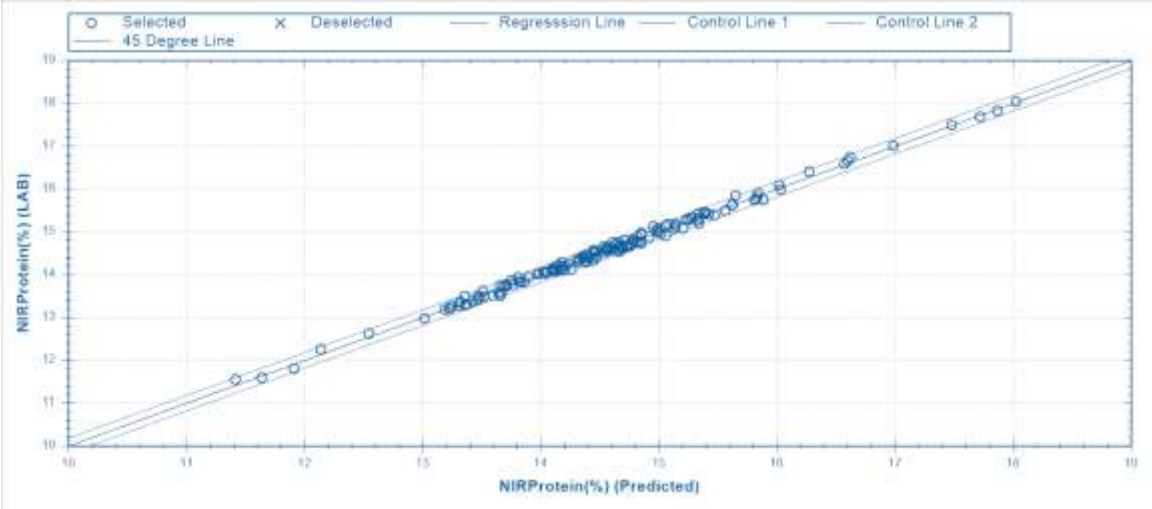

Figure 13. Correlation plot between NIRS method and reference analysis method of NIR protein% with combined 2018/2019 calibration dataset(N=155)

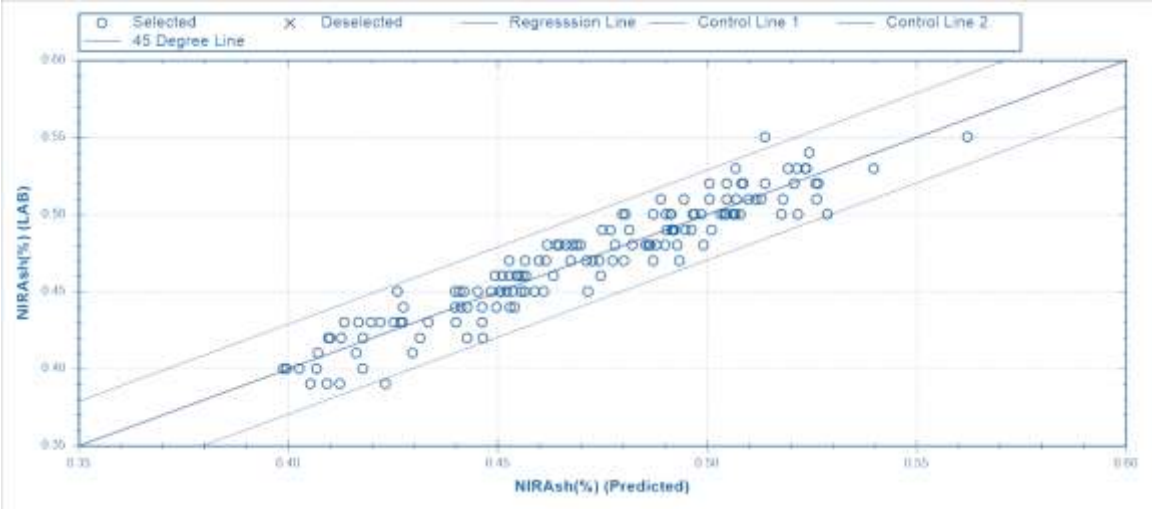

Figure 14. Correlation plot between NIRS method and reference analysis method of NIR ash % with combined 2018/2019 calibration dataset(N=155)

Interrelation of wheat constituents, mixing parameters, and baking potential

The interrelationships of wheat constituents, mixing parameters, and baking parameters are important for predictive potential of desirable traits of a wheat variety. Pearson's correlations coefficients were conducted for wheat constituents (protein, ash, gluten, TDF, etc.), mixing parameters (mixing time), and baking parameters (loaf volume, baking absorption, etc.). A value from -1.0 to 1.0 are outputted, with 1.0 indicating a perfect positive correlation of the variables. A value of -1.0 is a perfect negative correlation of the variables, and a value of 0 indicates no statistical correlation. A correlation coefficient larger than 0.70 is indicative of a statistically significant relationship, with larger than 0.80 indicating a very significant relationship. Table 13 shows the interrelationships between the variables from the 2018 data that was found to have a correlation coefficient greater than 0.70. For example, moisture content analytically measured is related to the NIR moisture output with a 0.81 correlation coefficient, indicating that the same entity can be accurately measured in multiple ways. Similarly, NIR protein, kernel protein, and flour protein are all highly correlated. Table 14a and 14b show the interrelationships between the variables from the 2018 data that were found to have a correlation coefficient between 0.50 and 0.69, lower than the statistically significant value of 0.70, but the connections are worth noting. For example, NIR protein is correlated to baking absorption with a correlation coefficient of 0.52. The mixogram pattern is related to baking absorption with a correlation coefficient of 0.58. NIR protein is related to loaf volume with a correlation coefficient of 0.58.

For the 2019 data that was available, gluten factors were highly correlated with one another (Table 15). Total gluten was related to wet gluten at almost a perfect correlation

rounded to 1.0, and to dry gluten with a correlation coefficient of 0.74. Dry and wet gluten were correlated with a correlation coefficient of 0.74. Total gluten was correlated to water binding with a correlation coefficient of 0.94. The relation of gluten content and water binding can be explained, as gliadin and glutenin proteins combine to make gluten, which swells with the addition of water into a network that traps water.

Using the combined data from both 2018 and 2019, correlation coefficients were calculated to determine the relationships among the parameters using all available datapoints (Table 16). Again, the gluten factors were highly correlated with one another as seen in the 2019 data. Many of the mixograph parameters were related to the farinograph parameters. The farinograph's water absorption was correlated to the mixograph's mid-line peak value with a correlation coefficient of 0.70. The dough development time measured by a farinograph was related to the time to breakdown by a correlation coefficient of 0.78. These correlations have the potential to be used as predictive factors. The strength of the relationships can be useful to establish trends, and as a starting point for the explanation of the interrelations of variables. Knowing how much of a variable is statistically tied to another variable is beneficial to understanding the science of bread baking from kernel to dough to bread.

Table 13. Correlation coefficients for paired comparisons of 2018 wheat constituents and parameters data with correlation coefficients >0.70

Variable 1	**Variable 2**	**Correlation Coefficient**
Measuring time (Farinograph)	Dough development time (Farinograph)	0.75
Water absorption(Farinograph)	WAC (%) (Farinograph)	0.99
Water absorption(Farinograph)	WAM%(Farinograph)	0.93
WAC (%)	WAM%(Farinograph)	0.94
Measuring time (Farinograph)	Time to breakdown (Farinograph)	0.82
Time to breakdown (Farinograph)	Time to breakdown (Farinograph)	0.78
Flour Moisture (%)	NIR Moisture (%)	0.81
NIR Protein (%)	Kernel protein	0.80
NIR Protein (%)	Flour protein	0.86
Kernel protein	Flour protein	0.92
Bake mix time	Mid-line peak time (mixograph)	0.80
Water absorption(Farinograph)	Mid-line peak value (mixograph)	0.72
WAC (%) (Farinograph)	Mid-line peak value (mixograph)	0.71
Baking water absorption	Mid-line peak value (mixograph)	0.71
Mid-line peak value (mixograph)	Mid-line peak width (mixograph)	0.82
Water absorption (farinograph)	Mid-line peak value (mixograph)	0.70
Baking water absorption	Mid-line peak value (mixograph)	0.75
Mid-line peak value (mixograph)	Mid-line peak value (mixograph)	0.96
Mid-line peak width (mixograph)	Mid-line peak value (mixograph)	0.84
Bake mix time	INTEG (mixograph)	0.85
Mid-line peak time (mixograph)	INTEG (mixograph)	0.94
Single kernel weight	Single kernel diameter	0.80
Total gluten	Dry gluten	0.91
Wet Bad gluten	Gluten index	-0.99
Total gluten	Wet gluten	1.00
Total gluten	Dry gluten	0.91
Wet gluten	Dry gluten	0.91
Total gluten	Water binding (glutomatic)	0.96
Dry Gluten	Water binding (glutomatic)	0.76
Wet gluten	Water binding (glutomatic)	0.96

Table 14a. Correlation coefficients for paired comparisons of 2018 wheat constituents and parameters data with correlation coefficients 0.50-0.69

Variable 1	Variable 2	Correlation coefficient
Dough development time (Farinograph)	WAM%(Farinograph)	0.50
Mixing tolerance index (Farinograph)	Time to breakdown (Farinograph)	-0.59
Water absorption (farinograph)	NIR Protein (%)	0.60
WAC (%) (farinograph)	NIR Protein (%)	0.59
WAM (%) (farinograph)	NIR Protein (%)	0.52
NIR Protein (%)	Loaf volume	0.58
Loaf volume	Kernel protein	0.60
Test weight	Kernel ash	-0.61
Water absorption(Farinograph)	Flour protein	0.55
WAC (%) (farinograph)	Flour protein	0.55
WAM (%) (farinograph)	Flour protein	0.54
Loaf volume	Flour protein	0.64
NIR Ash (%)	Flour ash	0.67
Kernel ash	Flour ash	0.68
Time to breakdown (farinograph)	Mixigram pattern	0.55
Time to breakdown (farinograph)	Bake mix time	0.52
Water absorption(Farinograph)	Bake water absorption	0.59
WAC (%)(farinograph)	Bake water absorption	0.60
NIR Protein (%)	Bake water absorption	0.52
Loaf volume	Bake water absorption	0.56
Flour protein	Bake water absorption	0.55
Mixigram pattern	Bake water absorption	0.58
WAM (%)(farinograph)	Mid-line peak value (mixograph)	0.67
NIR Protein (%)	Mid-line peak value (mixograph)	0.65
Loaf volume	Mid-line peak value (mixograph)	0.64
Kernel protein	Mid-line peak value (mixograph)	0.51
Flour protein	Mid-line peak value (mixograph)	0.66
Water absorption(Farinograph)	Mid-line peak width(mixograph)	0.58
WAC (%)(farinograph)	Mid-line peak width(mixograph)	0.57
WAM (%)(farinograph)	Mid-line peak width(mixograph)	0.59
NIR Protein (%)	Mid-line peak width(mixograph)	0.57
Loaf volume	Mid-line peak width(mixograph)	0.56
Kernel protein	Mid-line peak width(mixograph)	0.52
Flour protein	Mid-line peak width(mixograph)	0.64
Baking water absorption	Mid-line peak width(mixograph)	0.60
WAC (%)(farinograph)	Mid-line right value(mixograph)	0.70
WAM (%)(farinograph)	Mid-line right value(mixograph)	0.67
NIR Protein (%)	Mid-line right value(mixograph)	0.62
Loaf volume	Mid-line right value(mixograph)	0.66
Kernel protein	Mid-line right value(mixograph)	0.52
Flour protein	Mid-line right value(mixograph)	0.65
Mixgram pattern	Mid-line right value(mixograph)	0.51
Measuring time (farinograph)	INTEG (mixograph)	0.54

Table 14b. Correlation coefficients for paired comparisons of 2018 wheat constituents and parameters data with correlation coefficients 0.50-0.69

Variable 1	Variable 2	Correlation coefficient
Time to breakdown (farinograph)	INTEG (mixograph)	0.60
Mixogram pattern	INTEG (mixograph)	0.63
WAC (%) (farinograph)	Single kernel harness index	0.50
Water absorption(Farinograph)	Single kernel diameter	0.50
WAC (%) (farinograph)	Single kernel diameter	0.52
WAM (%) (farinograph)	Single kernel diameter	0.53
Measuring time (farinograph)	Wet Bad gluten	-0.52
Mixing tolerance index (farinograph)	Wet Bad gluten	0.51
Time to breakdown (farinograph)	Wet Bad gluten	-0.58
Mixigram pattern	Wet Bad gluten	-0.56
Bake mix time	Wet Bad gluten	-0.60
Mid-line peak time (mixograph)	Wet Bad gluten	-0.58
INTEG (mixograph)	Wet Bad gluten	-0.65
NIR Protein (%)	Total gluten	0.58
Wet Bad gluten	Total gluten	0.59
NIR Protein (%)	Dry gluten	0.61
Flour protein	Dry gluten	0.51
Dough development time (farinograph)	Good gluten	0.53
Time to breakdown (farinograph)	Good gluten	0.56
NIR Protein (%)	Good gluten	0.60
Loaf volume	Good gluten	0.56
Kernel protein	Good gluten	0.61
Flour protein	Good gluten	0.61
Mixogram pattern	Good gluten	0.61
Mid-line peak width (Mixograph)	Good gluten	0.55
Mid-line peak value (Mixograph)	Good gluten	0.60
Wet Bad gluten	Good gluten	-0.56
Dry gluten	Good gluten	0.51
Measuring time (Farinograph)	Gluten index	0.54
Mixing tolerance index (Farinograph)	Gluten index	-0.53
Time to breakdown (Farinograph)	Gluten index	0.60
Mixogram pattern	Gluten index	0.59
Bake Mix Time	Gluten index	0.60
Mid-line peak time (Mixograph)	Gluten index	0.58
INTEG (Mixograph)	Gluten index	0.67
Total gluten	Gluten index	-0.51
Good gluten	Gluten index	0.62
NIR Protein (%)	Wet gluten	0.58
Wet Bad gluten	Wet gluten	0.59
Gluten index	Wet gluten	-0.51
NIR Protein (%)	Water binding (glutomatic)	0.50
Bake mix time	Water binding (glutomatic)	-0.50
Wet Bad gluten	Water binding (glutomatic)	0.68
Gluten index	Water binding (glutomatic)	-0.63

Table 15. Correlation coefficients for paired comparisons of 2019 wheat constituents and parameters data with correlation coefficients >0.70

Variable 1	**Variable 2**	**Correlation Coefficient**
Water absorption(%) (farinograph)	WAC (%) (farinograph)	0.922
Water absorption(%) (farinograph)	WAM (%) (farinograph)	0.898
WAC (%) (farinograph)	WAM (%) (farinograph)	0.840
Wet Bad gluten	Good gluten	-0.761
Wet Bad gluten	Gluten index	-0.996
Good gluten	Gluten index	0.805
Total gluten	Wet gluten	1.000
Dry gluten	Wet gluten	0.739
Total gluten	Dry gluten	0.739
Total gluten	Water binding (glutomatic)	0.939
Wet gluten	Water binding (glutomatic)	0.939

Table 16. Correlation coefficients for paired comparisons of combined 2018/2019 wheat constituents and parameters data with correlation coefficients >0.70

Variable 1	**Variable 2**	**Correlation Coefficient**
Measuring time (farinograph)	Dough development time (farinograph)	0.752
Water absorption (farinograph)	WAC (farinograph)	0.995
Water absorption (farinograph)	WAM (farinograph)	0.930
WAC (farinograph)	WAM(farinograph)	0.936
Measuring time (farinograph)	Time to breakdown (farinograph)	0.824
Dough development time (farinograph)	Time to breakdown (farinograph)	0.779
Moisture (farinograph)	NIR Moisture (%)	0.812
NIR Protein (%)	Kernel protein	0.799
NIR Protein (%)	Flour protein	0.861
Kernel protein	Flour protein	0.918
Baking mix time	Mid-line peak time (mixograph)	0.796
Water absorption (farinograph)	Mid-line peak value (mixograph)	0.720
WAC (farinograph)	Mid-line peak value (mixograph)	0.715
Bake water absorption	Mid-line peak value (mixograph)	0.713
Mid-line peak value (mixograph)	Mid-line peak width (mixograph)	0.816
Water absorption (farinograph)	Mid-line right value (mixograph)	0.700
Bake water absorption	Mid-line right value (mixograph)	0.748
Mid-line peak value (mixograph)	Mid-line right value (mixograph)	0.960
Mid-line peak width (mixograph)	Mid-line right value (mixograph)	0.837
Baking mix time	INTEG (mixograph)	0.852
Mid-line peak time (mixograph)	INTEG (mixograph)	0.938
Single kernel weight	Single kernel diameter	0.801
Wet Bad gluten	Gluten index	-0.989
Total gluten	Wet gluten	1.000
Dry gluten	Wet gluten	0.907
Total gluten	Dry gluten	0.907
Total gluten	Water binding	0.962
Wet gluten	Water binding	0.962
Dry gluten	Water binding	0.757

Chapter 5:

Summary and Conclusion

Wheat is a valuable cereal grain in terms of its growability, versatility, and multifunctional nutritional components. Research into the genetic characteristics and growing conditions of the grain is advantageous to everyone, especially wheat breeders, farmers, food processors, and end consumers. NIRS technology is rapid, cost-effective, and a powerful analytical tool that can be harnessed to create predictive calibrations for estimations of wheat parameters.

The first objective of this study was to develop and validate a predictive NIRS calibration equation for the estimation of dietary fiber content in South Dakota wheat. It was hypothesized that the NIRS prediction values for TDF of wheat samples are statistically the same as reference TDF analysis values for those samples. This hypothesis was rejected, as the predictive calibration equation had an RSQ of 0.07, indicative of a poor calibration model. Dietary fiber is unique in that it is comprised of many constituents and is not a singular variable, thus rendering the measurement and prediction of the parameter difficult.

The second objective of this study was to statistically analyze the variability of South Dakota wheat based on growing location and wheat variety. It was hypothesized that both the growing location and variety would have statistically significant effects on the dietary fiber content of wheat grown in South Dakota. This hypothesis was accepted. Ninety-nine hard red spring wheat samples, including 33 varieties grown in three locations

(Brookings, Miller, Groton) in 2018 were analyzed. Two replicates of TDF residue values were obtained for each of the 99 samples. Both variety and growing location were found to be statistically significant at the 0.001 level.

The third objective of this study was to create and evaluate predictive NIRS calibrations for selected wheat constituents, dough mixing parameters, and baking output parameters. It was hypothesized that these calibrations would be accurate and precise with high RSQ, low SEP, and low SEP values. This hypothesis was accepted, for some parameters. Predictive NIRS calibration estimations for TDF and other selected wheat constituents, and mixing and baking parameters were created for 2018, 2019, and combined 2018/2019 data. For 2018, the parameters with an RSQ>0.6 included the single kernel hardness index, dry gluten, water absorption%, WAC%, NIR moisture, NIR protein, NIR ash, flour protein, flour ash, mixograph's mid-line peak value, total gluten, good wet gluten, wet gluten, WAM%, kernel ash, kernel protein, and flour extraction. For 2019, the parameters with an RSQ>0.6 included the Farinograph moisture%, water absorption%, WAC%, WAM%, NIR protein, NIR ash, and NIR moisture. For the combination 2018/2019 calibration model, the model showed to have greater predictability than the singular years calibrations. The parameters with RSQ> 0.60 included the Farinograph's dough development time, water absorption, WAC%, WAM%, NIR moisture, NIR protein, NIR ash, Farinograph moisture, mixing tolerance index, time to breakdown, and dry gluten. The accuracy of these calibrations was validated with a validation subset of data. The validation dataset included data that were independent of those used in the calibration development, but their reference values were known. The NIRS predictions were compared to the reference data, and a paired t-test showed that the NIRS predictions

were not statistically different than the actual values at a 95% confidence level for all tested parameters except for 2018 NIR ash, flour ash, and kernel ash.

End quality parameters that can be predicted via NIRS calibrations have the potential to save time and money, as well as reducing the sample quantity that is needed for analytical quality testing. These base calibration models can be expanded upon with data from future years to enhance the robustness of the models. As additional outliers and sample numbers get added to the calibration estimation equations, via additional years of sample data, the predictive power will likely increase.

In addition to the calibration models, correlations between wheat constituents, mixing parameters, and baking parameters were generated for the predictive potential of desirable traits of a wheat variety. Pearson's correlations coefficients indicated strong correlations among gluten parameters, water binding of flour, and mixograph/farinograph measurement values.

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