

MODELING THE FOURIER TRANSFORM NEAR INFRARED SPECTRA
OF UNREACTED STARCH IN CORN FOR MONITORING APPLICATIONS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Agricultural and Biological Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

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ABSTRACT

The dry grind ethanol process is an important part of the renewable biofuels industry. In this process corn must be stored and used throughout the year. Corn quality changes, in terms of unreacted starch content, in the dry grind process have not been extensively studied, although they impact the efficiency of bioconversion to ethanol and its coproducts. The goal of this research was to monitor changes in unreacted starch content in corn stored for 5 to 12 months under the following conditions: (1) ambient outdoor temperatures of -10°C to 35°C; (2) refrigerated temperature of 1°C; and (3) room temperature of 20°C. In addition, a high throughput assay based on the Fourier transform near infrared spectra of starch blends and corn flour was developed to predict unreacted starch contents in corn.

Results of the study showed unreacted starch content in corn increased during the first four weeks after harvest, then decreased until 10 to 15 weeks, and finally increased for the remainder of the 5 to 12 months in storage. Unreacted starch content was found to be strongly correlated ($r = 0.744$) with storage time and mildly correlated ($r = 0.555$) with storage temperature at the temperatures used in this study. Partial least squares regression models for blends of native and resistant starches and ground corn, dried and un-dried, were created with RPD values of 7.28, 2.07, and 2.16, respectively, which may be used for rough screening of blends and flours.

Acknowledgments

A special thanks to the Jonathon Baldwin Turner Fellowship and to Novozymes for providing funding to support my graduate thesis research. Special thanks to the following individuals, including my thesis committee, for their technical assistance and advice: Shih-Fang Chen, Mary-Grace Danao, Haibo Huang, Wei Liu, Kent Rausch, and Vijay Singh. Thanks also to my family and friends for their continuous support.

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CHAPTER 1. INTRODUCTION

Production of ethanol from corn has become an essential fuel industry in the United States of America. With the goal of an ethanol output of 15 billion gallons by 2022 (EPA, 2009), the ethanol industry is clearly of great economic importance. Greater process efficiency could lead to increased production so it is important to understand and be able to monitor corn quality and how it changes over time.

The most common method used for converting corn into ethanol is the dry grind process, which is responsible for 86% of domestic production (Mueller, 2010). The dry grind ethanol process involves grinding the corn, producing a slurry, then breaking down starch molecules with alpha amylase in the presence of heat and water. Glucoamylase is added to further break down the starch into glucose so that it can be consumed by yeast and converted into ethanol. The conversion of starch into ethanol is not complete, however, as distillers dried grains with solubles (DDGS), a coproduct of the process, contains more than 5% starch (Belyea et al., 2004; Kim et al., 2008). Since 3.78 liters (1 U.S. gallon) of ethanol produces 2.7 kg of DDGS (Szulchyk, et al., 2010), this means that for the 43 billion liters (11.4 billion gallons) of ethanol produced by the dry grind ethanol process in the United States in 2011, 1.5 billion kg starch was unreacted or unused. This represents inefficiencies in the dry grind ethanol process and reducing the amount of unreacted starch is particularly important to improving the profitability of the ethanol industry. Also, a decrease in unreacted starch content DDGS increases the protein content, which translates into a higher value DDGS.

In order to minimize the amount of unreacted starch in the dry grind ethanol process, it is important to understand the effects that storage temperature and time have

on the enzymatic digestibility of the corn starch and to develop a high throughput procedure for monitoring unreacted starch levels. Correlations between unreacted starch content in corn and storage temperature and time are not well understood, but starch yield in the wet milling process have been shown to vary by both storage time and temperature (Singh et al., 1998).

The objectives of this study were (1) to determine the changes in unreacted starch content during storage and (2) to develop a high throughput assay based on Fourier transform near infrared spectroscopy to provide a measure of unreacted starch content in corn.

CHAPTER 2. LITERATURE REVIEW

2.1 Corn grain for fuel ethanol production

Corn is the primary crop used for fuel ethanol production in the United States. While endosperm and germ are the major components of the corn kernel, starch makes up 70-73% of the kernel dry weight (Srichuwong et al., 2010). It is known that enzymatic digestibility of raw starch granules varies among corn varieties (Perera et al., 2001) due to the differences in amylose content and branch-chain-length of the amylopectin (Ji et al., 2003; Jane, 2007). The physical damage in starch granules that result from dry grinding corn can also lead to greater digestibility of the starch (Karkalas et al., 1992; Tester et al., 1994).

It is expected, however, that even before dry grinding corn, changes in the chemical structures and functional properties of the corn occur during storage. These changes can impact corn processing characteristics and quality of the final products. For example, some grain crops require storage for a period of time before they can be used for processing. In the case of wheat, dough rheology and properties improve with storage time (Zeleny, 1948). For wet milling of corn, Singh et al. (1998) reported that starch yields vary by both storage time and temperature. Corn wet millers have observed that ethanol yields from freshly harvested corn are typically lower than yields from corn that has been stored for two to three months and yield efficiency continue to decrease with storage time (Singh, 2012). Long term storage reduces starch yield from the crops during wet milling (Abera and Sudip, 2004). Long-term storage of grain also results in increases in lipid oxidation and free fatty acid content (Pomeranz, 1992); decreases in grain protein solubility and digestibility (Onigbinde and Akinyele et al., 1998); and alters

the physical and nutritional properties of the grain and its final products (Hasjim et al., 2010).

2.2 Resistant starch content in corn and corn products

Since yeast cannot utilize starch directly the production of ethanol from corn requires hydrolysis of starch to glucose, and glucose is then fermented by yeast to produce ethanol. During enzymatic starch hydrolysis, not all starch is converted to sugars. Unconverted (sometimes called unreacted, residual, or resistant) starch is recovered in the distillers dried grain with solubles (DDGS). The amount of unconverted starch in DDGS depends on the type of raw starch in the corn and process parameters, such as temperature, pH, enzyme activity, and duration of hydrolysis (Berry et al. 1986; Sharma et al., 2010).

While the terms unreacted, residual, and resistant starch are used interchangeably in the literature, in this study, the term *unreacted starch* is used to refer to starch that is not broken down by enzymes, such as those in the dry grind corn ethanol process while *resistant starch* will be used to describe starch that is resistant to acid or digestive systems. There are four main sources of resistant starches: RS1, starches that are resistant because they are sterically inaccessible; RS2, ungelatinized starch or starches that are still a part of intact starch granules; RS3, retrograded starch; and RS4, chemically modified or cross linked starch (Fuentes-Zaragoza et al., 2010; Hodsagi, 2011).

Much of the literature relating to resistant starch has focused on changes in the starch due to food processing techniques, often in relation to digestive resistance. These studies reported an increase in starch resistance or a decrease in starch quality with an increase in storage time and higher temperatures, both for storage and for food processes.

Turning corn into tortillas increased the content of digestive resistant starch, as did increased storage time of the tortilla (Garcia-Rosas et al., 2009). High temperature processing techniques such as baking, boiling, roasting, or storage from 12 to 24 hours increased resistant starch content (Rendon-Villalobos et al., 2002; Vaidya and Sheth, 2011). In addition warm holding at 65-70°C for 1 hr. increased digestive resistant starch from 2.2 to 4.8% and slow cooling from 3.6 to 6.3% (Gormley and Walshe, 1999). Starch extracted through the corn wet milling process typically decreased when higher heat drying processes were used (Paulsen et al., 2003).

Similar trends were reported from enzyme based studies. Enzymatic starch hydrolysis percentage generally decreased with an increase in storage time (Setiawan et al., 2010), although these results were not as consistent as the results from acid hydrolysis or digestibility studies. A 5% decrease in starch yield, the wet basis percentage of starch that was recovered from wet milling, from dried cassava was reported after 8 months of storage (Abera and Rakshit, 2004). The fiber content of the cassava was greater in the samples stored at 30°C than in the samples stored at 5°C, although no reliable trend was seen over storage time. Both peak viscosity and light transmittance decreased over time, while syneresis increased for both storage temperatures and varieties.

Processing temperature of starch was shown to have an effect on alpha amylase/pullulanase resistant starch content of wheat starch, with higher autoclaving temperatures producing more resistant starch (Berry et al., 1986). Although higher processing temperatures cause an increase in enzymatic resistant starch, higher temperatures in the liquefaction stage of the dry grind ethanol process can have the opposite effect. Processes that had higher temperature in the liquefaction stage were

shown to have lower final amounts of residual starch (Sharma et al., 2010). It has also been theorized that high temperature processing with amylase may cause lipid-amylose complexes to form, serving as competitive inhibition to resistant starch formation (Gruchala and Pomeranz, 1992).

The amylose to amylopectin content of the starch is an important factor in the development of enzymatically resistant starch, as waxy maize was shown to have around 1% enzymatic resistant starch across after autoclave treatments were applied, whereas amylomaize starches ranged from 24% to 48% (Berry et al., 1986). This conclusion was supported by the Singh et al. (1998) study, where the difference in the starch yield were measured and may have been due to differences in amylose content. Alpha and pullulanase resistant starches increased with starch gelatinization, implying enzymatic resistance increased in the presence of water (Berry et al., 1986).

The size, shape, and frequency of pores in starch granules all affected the ability of hydrolyzing enzymes to degrade the starch (Valetudie et al., 1993). These results may explain differences in enzymatic digestibility across different starch sources. X-ray diffraction measurements revealed starch structure has an effect on the enzymatic digestibility of the starch molecule (Jane, 2009; Perez et al., 2009). Type A starch, which typifies the starch found in cereal grains, structures consist of amylopectin molecules that are 23 to 29 glucose units and characterized by a double helical outer structure with hydrogen bonds between the hydroxyl groups, along with amylose chains packed in between (Jane, 2009; Perez et al., 2009). Type B structures have longer amylopectin chains with 30 to 44 glucose units infused with water instead of amylose, whereas type C structures are a cross between A and B structures. Type V structures are amylose chains

that have crystallized with nonstarch compounds, such as alcohols or fatty acids (Jane, 2009). Studies have indicated that type B starch structures are less susceptible to hydrolysis by alpha amylase and, therefore, more enzymatically resistant than type A structures (Jane et al., 1997; Planchot et al., 1997). Type A starch molecules provide more branch linkages and short chain double helices, which form weak points that allow enzymatic pitting to occur (Jane et al., 1997).

2.3 Unreacted starch determination

Currently, resistant starch contents are determined by assays such as AOAC Method 991.43 (Hollmann et al., 2013). This method requires incubation with alpha amylase and protease, followed by chromatography. Vidal et al. (2009) developed a procedure which involved carrying out a full fermentation and then using a GOPOD assay to estimate the glucose content in the corn mash. Ethanol plants can determine the unreacted starch content after fermentation by analyzing the DDGS or comparing predicted yields to actual yields. All of these methods take several days to complete and usually involve operating a wet chemistry lab and complex equipment. As such, there are no reliable yet quick methods of predicting unreacted starch content of corn that can be accomplished before the dry grind ethanol process is complete. In this study, the development of a high throughput assay based on Fourier transform near infrared spectroscopy (FT-NIR) to predict values of unreacted starch content in corn was explored as a means to monitor corn quality during storage, blending, and processing operations in ethanol facilities.

2.4 Use of infrared spectroscopy in grain analyses

Infrared spectroscopy is most commonly used in biological applications relating to food and agricultural industries. Its popularity as an analytical technique expanded rapidly in the 1960s since it can be conducted without with minimal sample preparation (i.e., not requiring a pretreatment of the sample); can provide information on both physical and chemical characteristics; does not require destroying or damaging the test sample; and is relatively easy to use (Blanco and Bano, 1998; Tikuisis et al., 1993). These factors made near infrared spectroscopy a common choice for agricultural, food, pharmaceutical, and biological systems. Quantitative analyses of near infrared spectral data require a calibration step with a set of standard samples (Blanco and Bano, 1998).

Several studies have been reported in the literature with regards to grain quality and processing analyses. Qualitative changes in starch resistance to enzymes in processed corn tortillas were analyzed using FT-NIR, where retrograded resistant starch had broader peaks with fewer spectral details than the native starch (Garcia-Rosas et al., 2009). Ruminant digestive resistant starches from a variety of plants, such as corn, wheat, and potato, were modeled using mid IR spectroscopy with an $R^2 = 0.995$ on a model involving 98 samples (Uden, 2009). This study also included a calibration test against chemical methods with a mean squared prediction error of 0.0076. Hydrolyzates from the dry grind ethanol process were measured for sugar concentrations by FT-NIR as well with under 5% error (Blanco and Bano, 2003). DDGS samples from operating corn ethanol plants were analyzed by FT-NIR to predict the quantity of components. While a prediction model was constructed for a variety of components, residual starch quantities were among the least accurately predicted with $R^2 = 0.81$ as compared to other

components whose $R^2 = 0.88$ for protein and 0.87 for moisture (Paulsen et al., 2010). In a separate study, FT-NIR was used on whole corn to predict protein, starch, and oil, but again, starch values showed the lowest correlation values, with $R^2 = 0.86$ for calibration, and 0.89 for validation (Jiang et al., 2007). Calibration for the percentage of corn starch that can be utilized in the corn wet milling process was conducted with $R^2 = 0.8$ (Paulsen et al., 2004). Although FT-NIR has been used to quantify starch content, the differentiation among types of resistant starch has not been thoroughly explored in literature.

2.5 Preprocessing of spectral data

When analyzing macromolecules such as starch with NIR spectroscopy, many chemical bonds in the samples are stretched or vibrated. Each of these stretches can emit multiple signals, called overtones, which overlap with each other, making the signal more difficult to interpret. While overtones are necessary for analysis, specific overtones are found over multiple ranges of wavelengths. The exact wavelength of the overtone may change depending on environmental factors, potentially complicating analysis. Fundamental bands correspond to the emitted energy from vibrational excitement from the lowest energy position to the next lowest. Those bands tend to have a stronger signal than either overtone bands, which are excitations from lowest energy level to an energy level higher than the fundamental, or combination bands, which are bands that exist from multiple simultaneous fundamental excitations (Stark et al., 1986).

FT-NIR spectra of ground corn typically show water has strong absorption bands which overlap with the absorption bands of sugars, proteins, oils, and starches (Table 2.1). Glucose, maltose and dextrans overlap between 2100 nm and 2350 nm, or

4762 cm^{-1} and 4255 cm^{-1} (Blanco and Bano, 2003). Other important spectral regions in starch are absorption bands at 2276 nm (4394 cm^{-1}) to 2100 nm (4762 cm^{-1}), and 1400 nm (7143 cm^{-1}) to 1600 nm (6250 cm^{-1}), the last of which corresponds to the first overtone of hydroxyl groups (Noah et al., 1997). The region around 2100 nm is associated with C–O and O–H deformation vibrations, and the region at 2276 nm corresponds to a combination band of O–H and C–C stretch vibrations (Noah et al., 1997). Water absorption values are typically around 760, 970, 1190, 1450 and 1940 nm (Paulsen and Singh, 2004). Strong water absorption can obscure starch spectra in similar wavelengths, causing error in the model. For this reason, dry samples are preferred in FT–NIR spectroscopy.

Table 2.1. NIR wavebands of different constituents in corn 9

Samples	Wavenumbers (cm ⁻¹)	Assignment	Reference
Yellow dent corn	4301-4415	Starch yields for wet milling	Wehling et al. (1993)
Starch blends	4283-4329	C-H bending and C-H stretching, carbohydrate IV	Hodsagi et al. (2012) Hao et al. (2012)
Starch blends Yellow dent corn	4367-4396	O-H stretching and C-C stretching, carbohydrate III	Hodsagi et al. (2012) Wehling et al. (1993)
Maize	4587-4673	NH band indicating protein	Paulsen et al. (2003)
Maize	4864-4873	Protein	Hao et al. (2012) Paulsen et al. (2003)
Maize	5250	Amylose-amylopectin	Lindberg and Kaila (1980)
Maize	5225-5319	Starch	Hao et al. (2012)
Maize	4274 4333 5814 5682 8562	CH ₂ bands that indicate fat or oil	Paulsen et al. (2003)
Maize	5800	C-H stretching, first overtone	Hao et al. (2012)
Starch blends	6289-6349	O-H stretching, first overtone, carbohydrate I	Hodsagi et al. (2012)
Maize	6684	NH band indicating protein	Paulsen et al. (2003)
Yellow dent corn	8889-9524	Starch yields for wet milling C-H stretching and C-C stretching associated with lipids and other hydrocarbons	Wehling et al. (1993)
Soybeans	5155 6897 8403 10309	Water absorption bands	Ben-Gera and Norris (1968) Paulsen and Singh (2004)
Maize	10823	Extractable starch	Paulsen and Singh (2004)

A variety of methods for statistical analysis of FT–NIR data are available and used in literature. To develop a proper model, it is necessary to divide the data points into two groups, one of which is used to calibrate the model, and the other validate the

model once it is derived (Esbensen, 2010). The percentage of data devoted to the validation set varies, but is typically between 20% and 33% of the data in the study. It is important, however, that the statistical distributions of the calibration and validation sets are representative of the entire data set.

In many cases involving multiple variables, it is necessary to normalize data with different magnitudes; however, since the only data analyzed are spectra and resistant starch values, normalization is likely not required in this instance. Other forms of data pretreatment are applicable for spectral data, such as multiplicative scatter correction (MSC), or a derivative-based smoothing technique such the Savitzky–Golay (SG) (Delwiche and Reeves, 2010). These pretreatment methods are used to minimize error, such as baseline shifts, or overlapping peaks (Szlyk et al., 2005). The process of derivation removes constant terms like baseline shift, but the second derivative is more commonly used than the first derivative for NIR spectral analysis because it preserves peak location (Naes et al., 2002). MSC attempts to correct for drift with a transformation equation that removes additive effects so only multiplicative effects are seen (Naes et al., 2002). The MSC pretreatment is most useful in readings that are not expected to have much interference and, therefore, are not thought to need significant adjustment. MSC is calculated by creating a mean centered data set by subtracting the mean from each of the points. The mean centered data set is then divided by a correction factor that is calculated using a reference mean centered spectrum, and then adding in the mean centered reference value. The SG method, a numerical technique that estimates the derivative of a curve is used commonly, it suffers from distortions if severe smoothing is required (Stark et al., 1986). SG acts as a filter that combines derivation with a moving

point smoothing, where each point is the average of the points surrounding it, the number of points being independent. MSC has an advantage over SG derivations in that it simplifies the calibration model; however, it is heavily affected if the sum of all the light absorbance constituents does not equal a constant amount (Naes et al., 2002).

Although MSC and SG derivations are the most common methods for analyzing FT-NIR data, other methods do exist, such as independent modeling of class analogy (SIMCA). Soft modeling is a technique that analyzes significance by separating data into classes and is commonly used in the pharmaceutical industry because of its close attention to error limits (Candolfi et al., 1999; De Maesschalck et al., 1999).

To understand the quality of models obtained from the data, a number of factors can be analyzed, such as the number of iterations performed, percentage of variance explained by the number of factors in the model, coefficient of determination (R^2), root mean square error of prediction (RMSEP), and ratio of performance to deviation (RPD). The coefficient of determination is calculated using linear regression, the mean square error is the sum of squares of differences between predicted y values and actual y values, RPD is calculated as the standard deviation of the reference data set divided by the root mean square error of prediction. The number of iterations is important to understand how much transformation of the data was required to achieve the model. Having fewer iterations is better as the data had to undergo less manipulation. The number of factors in a partial least squares (PLS) regression model is typically minimized without compromising the percentage of the variance in the spectral data that is explained by model. The R^2 value of the calibration and the validation shows how well the line of prediction fits the data, with higher values signifying a more effective fit. RMSEP is

another commonly used measurement for how far off the models predictions are from the data. The RPD used to sort the model into different categories of usefulness. An RPD value of 2.5 to 2.9 is needed for rough screening, and good quality screening is possible with models having an RPD values greater than 3.0.

In this study, changes in the unreacted starch content of corn stored under different temperatures over a period of 5 to 12 mo. were corded and analyzed. Predictive models of unreacted starch content based on the FT-NIR spectra of dry blends of native and digestive resistant starches and ground corn (wet and dry) were developed using partial least squares regression. The dry blends were used to determine which wavebands of the FT-NIR spectra were most responsive to changes in unreacted starch content. The FT-NIR spectra of dry ground corn were compared to the FT-NIR wavebands of dry starch blends to see if the identified wavebands remained prominent, i.e., not masked by other constituents in the corn flour, and useful in developing a PLS regression model for dry corn flour. However, for in line monitoring of ground corn in storage, blending, and ethanol processing, it will be most advantageous to have predictive models that can be used with un-dried ground corn. Therefore, FT-NIR spectra of un-dried ground corn were also collected, compared to the spectra of starch blends and dry ground corn samples, and modeled using PLS. All regression models were compared and recommendations for the use of FT-NIR spectroscopy as a high throughput assay for monitoring unreacted starch content in corn were made.

CHAPTER 3. EFFECTS OF STORAGE TEMPERATURE AND TIME ON UNREACTED STARCH LEVELS IN CORN

3.1 Introduction

Since the dry grind ethanol process is an important component of the renewable fuels industry, which relies upon corn stored for up to a year, it is necessary to understand the aging process of stored corn and its effects the efficiency of the dry grind ethanol industry. For wet-milling of corn, Singh et al. (1998) reported that starch yields vary by both storage time and temperature. Dry millers have observed that ethanol yields from freshly harvested corn are typically lower than yields from corn that has been stored for two to three months and yield efficiency continue to decrease with storage time (Singh, 2012). In order to understand this aging process, the first goal of this research was to monitor changes in unreacted starch content in corn stored for 5 to 12 mo. under differing storage conditions, in order to determine unreacted starch trends over the course of the storage year, and how they are affected by storage time and by storage temperature.

3.2 Materials and Methods

3.2.1 Ground corn preparation and moisture content determination

Pioneer P1395R yellow dent corn was harvested from the Agricultural and Biological Engineering Farm in Urbana, IL in October 2011 and the same hybrid was grown in Dupont Pioneer, Johnston, IA and provided by Pioneer in October 2012. The corn was stored in sealed plastic pails (5 gallon capacity) either in a refrigerated room (set at 1°C), under a sheltered awning outdoors (-10 to 37°C), or in the laboratory (14 to 24°C) for a period of 5 to 12 mo. Both relative humidity and temperature inside the pails were monitored every 6 hr using wireless dataloggers (TrackIt®, MicroDAQ,

Contoocook, NH). Storage time was measured in weeks after harvest, and the first reading is begun within a day of harvest.

Every two weeks during the first 3 mo. of storage and every 4 weeks thereafter, 200-300 g samples from each storage condition were removed and a 25 g subsample was used to determine the moisture content of the whole corn using ASABE Standard S358.2 (2003). The rest of the corn samples were ground with a hammer mill using a 0.5 mm sieve. A 10 g subsample from each storage condition was used to determine the ground corn moisture content by gravimetric method and drying at 135°C for 2 hr (AACCI, 2000).

For corn harvested in 2011, ground corn was first liquefied for 1 hr with Liquozyme SC alpha amylase (Novozymes, Franklinton, NC) at pH 5.7 and 85°C followed by saccharification with Spirizyme Ultraglucoamylase (Novozymes, Franklinton, NC) for 72 hr at pH 5.0 and 65°C (Figure 3.1). The samples were then washed with 40 ml deionized water three times in a centrifuge set at 500 rpm for 20 min. to remove glucose from the samples. The samples were dried at 49°C overnight and treated with either deionized water (blank) or 0.4 M HCl (test) to break apart the remaining starch molecules. All blank and test samples were diluted 10X using deionized water and the unreacted starch content was determined using a glucose oxidase-peroxidase (GOPOD) colorimetric assay (Megazyme International, Wicklow, Ireland). Estimates of the glucose content obtained were used to calculate the original unreacted starch content remaining after saccharification based on a procedure developed by Vidal et al. (2009). The GOPOD procedure relied on dilute HCl acid to measure the enzymatic unreacted starch; acid-resistant starch was not measured in this study.

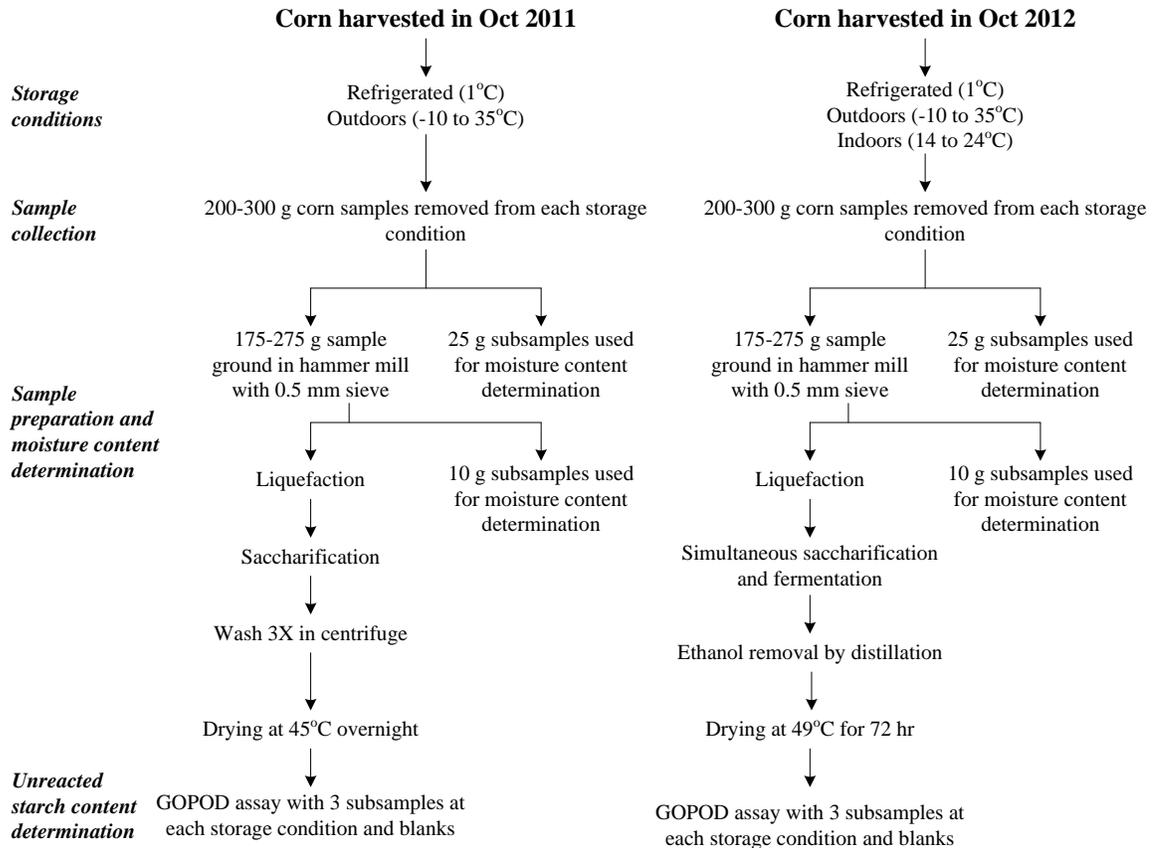


Figure 3.1. Test procedure for monitoring unreacted starch content in corn harvested in 2011 and 2012.

For corn harvested in 2012, the procedure was modified by conducting a simultaneous saccharification and fermentation with Spirizyme Ultraglucoamylase at pH 4.5 and 32°C for 72 hr using dry red yeast, which is more representative of procedures in modern dry grind ethanol facilities and complete removal of fermentable sugars in corn mash samples. Ethanol was removed by distillation at 90°C for 3 hr and dried at 49°C for 72 hr. The GOPOD colorimetric assay was used to determine the amount of glucose remaining in the sample and converted to unreacted starch content. Three replicates from each storage condition were used, and in the GOPOD assay, each replicate was measured with three repeated measures.

3.2.2 Statistical analyses

Pearson correlation coefficients between storage temperatures, storage time, and unreacted starch levels in corn from the 2011 harvest were computed in R environment (Version 2.15.5, 2012) and analyses were repeated for the 2012 harvest. Comparisons of means were performed in R using Tukey's test and evaluated at a p -level of 0.05, between values from different storage conditions within the same harvest year. Least squares regressions were conducted to determine the rates of change, μ , in unreacted starch content at different time periods (Figure 3.2), which were compared and evaluated at a p -level of 0.05. The program code is available in Appendix A.

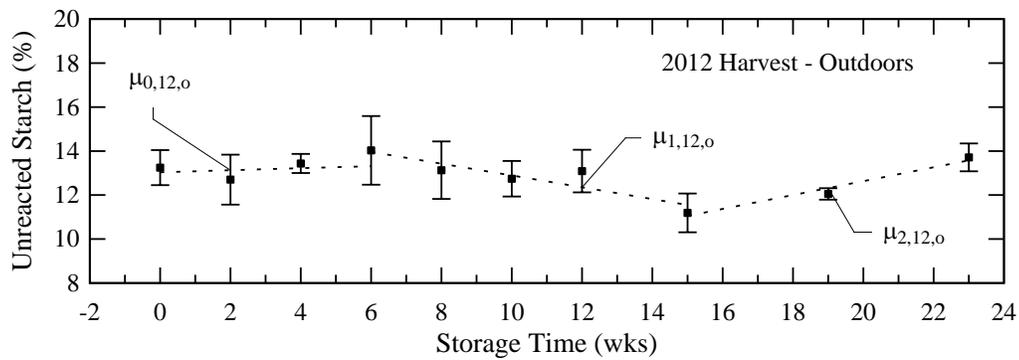


Figure 3.2. Different rates of change ($\mu_{\text{segment,year,conditions}}$) exhibited by unreacted starch in corn. Storage segment = 0 (0 to 6 weeks), 1 (6 to 16 weeks), and 2 (>16 weeks); Year = 11 or 12; and conditions = o (outdoors).

3.3 Results and Discussion

The values for unreacted starch in the 2011 harvest year were within values reported for enzymatically unreacted starch content (Berry, 1986; Gormley and Walshe, 1999), although the values for unreacted starch in the 2012 harvest year were higher than the 2011 results and fell outside the ranges typically reported for unreacted starch. Unreacted starch content of the during the first 5 mo. of storage of corn from the 2011 harvest year decreased from 5.5% at four weeks of storage to minimum values of 1.5 and

2.5% for corn stored at 1°C and outdoors, respectively, for the corn harvested in 2011 (Figure 3.3).

The minima were obtained after 10 weeks of storage, on 19 January 2012, when the midday outdoor temperature averaged -4°C. The unreacted starch content increased to 8% after 20 weeks of storage, when outdoor temperatures averaged 16°C. Unreacted starch contents in both outdoor and refrigerated storage conditions remained around 8% until weeks 28-32 of storage. After the 32nd week, unreacted starch contents increased steadily until month 12.

For the 2012 harvest year, corn stored in all three storage conditions had 13.5% unreacted starch content at the time of harvest. This value was higher than that of the 2011 harvest year which might be due to the drought conditions during kernel development during the summer months of 2012 (Below, 2013). In each of the storage conditions, unreacted starch content increased for the first weeks of storage and then decreased. By the 15th week of storage, all corn samples reached a minimum value of 11% unreacted starch content. This occurred at the same calendar date (19 January 2013), as the minimum readings from the 2011 harvest year, despite the fact the corn in 2012 was harvested earlier. Unreacted starch values then increased from the 15th to 23rd week of storage.

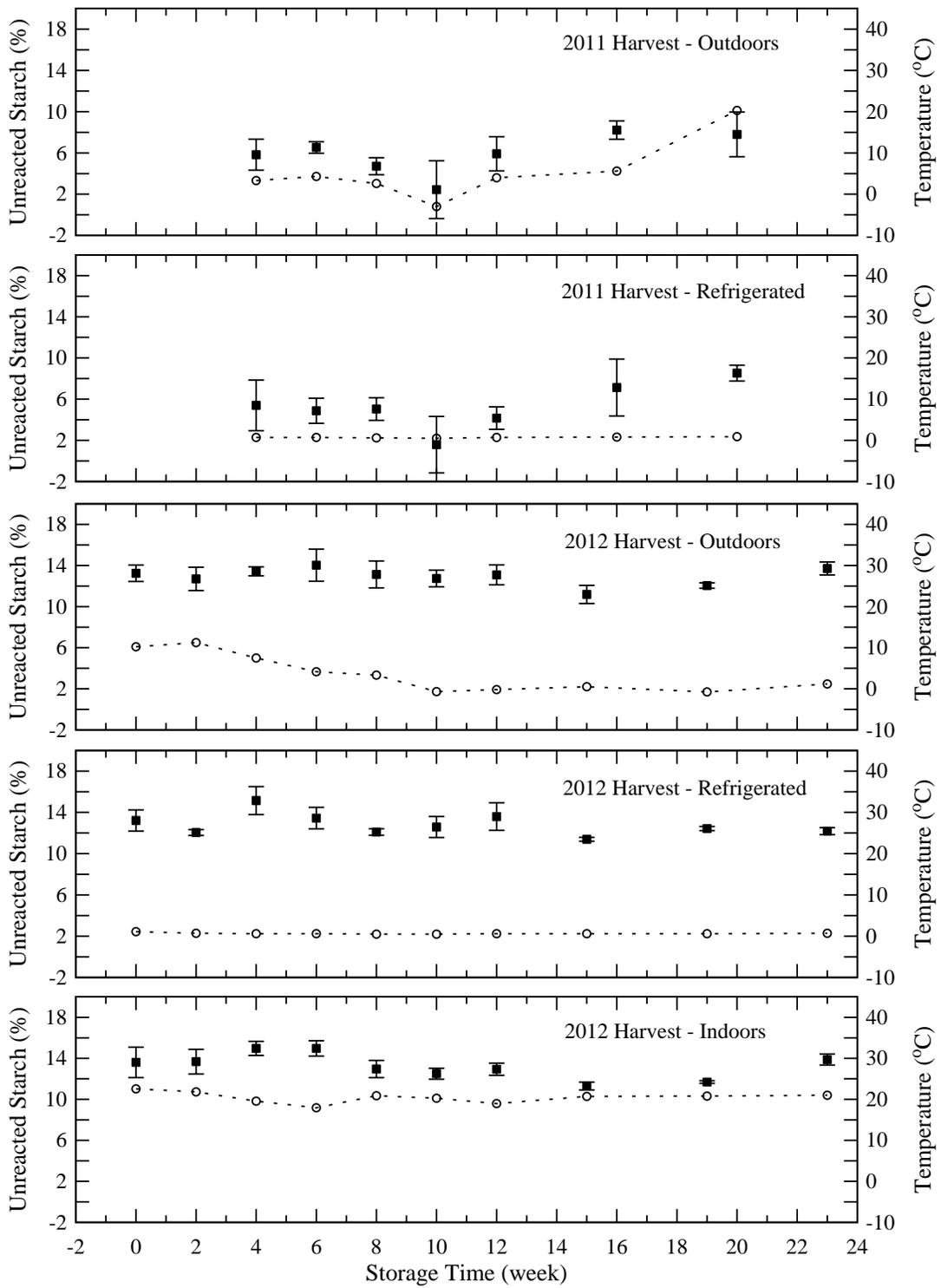


Figure 3.3. Unreacted starch content (■) in corn harvested in 2011 and 2012 and temperature conditions (○) during the first 5 mo. in storage. Storage time was equal to the number of weeks after harvest.

For corn harvested in 2011, the Pearson correlation coefficients between unreacted starch content and time after harvest was $r = 0.74$, suggesting a strong correlation between unreacted starch and storage time (Table 3.2). There was a moderate correlation between unreacted starch content and temperature and between storage time and temperature $r = 0.55$. For corn harvested in 2012, however, there were weaker correlations among the three variables, r values ranging from -0.135 to -0.396.

Table 3.1. Pearson correlation coefficients for storage time, temperature, and unreacted starch for corn harvested in 2011 and 2012.

	2011 Corn		2012 Corn	
	Temperature	Unreacted starch	Temperature	Unreacted starch
Storage time	0.449	0.744	-0.135	-0.396
Temperature	1	0.555	1	0.193

The correlations between unreacted starch and storage time have different signs given the different lengths of storage times in the study. Corn harvested in 2012 was only stored for five months, where the general trend was decreasing unreacted starch contents; whereas data for 12 months of storage, as in 2011, where the general trend was increasing unreacted starch contents. The correlations between unreacted starch and temperature were also less significant than the correlations between unreacted starch and storage time. From the literature, both temperature and time should be factors that are generally influencing unreacted starch contents (Garcia-Rosas et al., 2009; Gormley and Walshe, 1999; Onigbinde and Akinyele et al., 1998; Paulsen et al., 2003). In this study, however, the differences between outdoor and refrigerated conditions were too small to see the temperature effect.

Since the trends of unreacted starch content in corn stored in refrigerated and outdoor conditions were similar for the first 5 mo. suggested that storage time had a larger effect on unreacted starch content in corn than storage temperature (Table 3.2).

However, there was a late harvest time of the 2011 corn crop, due to the wet weather in Illinois, causing the temperatures in the different storage locations to be similar for the first few weeks of the study. Pronyk et al. (2004) reported that the “sweating” process and postharvest ripening can be influenced by temperatures of the grain during the first 6 weeks of harvesting, which might explain why there would be similar trends in late fall for corn stored outdoors and corn stored under refrigeration.

Variation in the first few months of the 2011 study could be caused by changes within the corn due to variability in moisture contents (Table 3.2). Differences in moisture content between corn stored outdoors and corn stored under refrigerated conditions were larger, possibly due to moisture due to temperature gradients (Stewart, 1975). Therefore, in subsequent sampling, corn was thoroughly mixed to reduce variation in moisture content.

In 2011, the standard deviations of the unreacted starch contents before the removal of excess glucose ranged from 1.00 to 3.00% with the coefficient of variation ranging from 3.00 to 8.5% of the measurement. In the published note on the acid GOPOD method used for these measurements, Vidal et al. (2009) recorded standard deviations of a six replicate sample at 3.4% coefficient of variation which was within the range of standard deviations in this study. For the corn harvested in 2012, unreacted starch was initially around 13%, higher than those observed at the beginning of the 2011 experiment. With drought conditions during the corn development period in 2012 and the unreacted starch assay was modified to include a simultaneous saccharification and fermentation, direct comparisons between years was difficult. For the 2012 harvest year study, standard deviations ranged from 0.13 to 1.56%, which resulted in coefficients of

Table 3.2. Unreacted starch content in corn, moisture content, and temperature conditions during the first five months of storage.

Storage time (weeks)	Mean unreacted starch content ¹ ± S.D. ² (% d.b.)											
	0	2	4	6	8	10	12	16	20	24	28	
2011 Corn												
Outdoors	---	---	5.83a ± 1.50	6.53a ± 0.56	4.71a ± 0.82	2.44a ± 2.80	5.92a ± 1.66	8.22a ± 0.89	7.80a ± 2.17		8.01a ± 2.17	
Refrigerated	---	---	5.40a ± 2.46	4.87b ± 1.22	5.04a ± 1.10	1.58a ± 2.74	4.16b ± 1.09	7.13a ± 2.77	8.53a ± 0.77		8.20a ± 0.85	
2012 Corn												
Outdoors	13.25a ± 0.80	12.70ab ± 1.14	13.44a ± 0.43	14.03a ± 1.56	13.13a ± 1.31	12.74a ± 0.81	13.09a ± 0.97	11.19a ± 0.88	12.05a ± 0.26	13.71a ± 0.63		
Refrigerated	13.21a ± 1.03	12.05a ± 0.29	15.15b ± 1.35	13.44a ± 1.04	12.09a ± 0.31	12.58a ± 1.02	13.59a ± 1.33	11.39a ± 0.19	12.43a ± 0.21	12.18a ± 0.34		
Indoors	13.61a ± 1.48	13.68b ± 1.20	14.97ab ± 0.69	14.97a ± 0.75	12.96a ± 0.84	12.50a ± 0.54	12.94a ± 0.59	11.29a ± 0.39	11.69a ± 0.13	13.88a ± 0.54		
	Moisture content of corn flour (% d.b.)											
	0	2	4	6	8	10	12	16	20	24	28	
2011 Corn												
Outdoors	---	---	12.1	25.3	15.4	15.7	7.44	12.4	12.1		12.9	
Refrigerated	---	---	11.8	24.8	12.9	20.5	6.44	13.1	13.3		12.9	
2012 Corn												
Outdoors	9.70	10.54	10.58	9.46	10.78	10.44	10.10	10.40	10.60	10.17		
Refrigerated	10.20	9.25	9.58	10.39	10.33	10.42	9.49	9.97	9.97	10.78		
Indoors	9.64	9.94	9.67	9.69	10.14	9.96	9.36	9.73	10.03	9.51		
	Mean temperature during one week prior to sampling date (°C)											
	0	2	4	6	8	10	12	16	20	24	28	
2011 Corn												
Outdoors	---	---	3.3	4.3	2.6	-3	4	5.6	20.3		20.7	
Refrigerated	---	---	0.7	0.7	0.6	0.5	0.7	0.8	0.9		1.90	
2012 Corn												
Outdoors	10.24	11.25	7.50	4.16	3.33	-0.68	-0.19	0.51	-0.76	1.19		
Refrigerated	1.10	0.70	0.60	0.6	0.5	0.5	0.6	0.60	0.60	0.70		
Indoors	22.6	21.8	19.6	17.9	20.9	20.3	19.0	20.7	20.8	21.0		

¹Per year, mean values followed the same lowercase letter in the same column are not significantly different ($p > 0.05$). ²S.D. = one standard deviation. ³Data from the first four weeks of storage in 2011 were lost.

variation from 1.00 to 11.00%, though most were under 10%, with most of the variation coming from differences in fermentation runs instead of the GOPOD assay, as the repeated measures on each replicate generally within 0.5% of each other.

The rate of changes in unreacted starch content from the 4th to 10th week for outdoor and refrigerated samples were lower than -0.5 % per week. Comparison of slopes showed that there was not a significant difference between these slopes (Table 3.3). This decrease was then followed by a general increase in unreacted starch content in both outdoor and refrigerated conditions. The rates of increase in unreacted starch content from 10 to 20 weeks of storage were 0.5 % per week for outdoor corn and 0.68% per week for refrigerated corn and, again, not statistically different. For the first four weeks after harvest, values from the 2012 harvest year tended to increase, except for the outdoor corn, which had a small increase; however, they were not different from each other. The downward slope in outdoor stored corn was statistically different between harvest years, but the upward slope was not. In refrigerated corn, the decrease was not statistically different between harvest years whereas the following increase was.

Table 3.3. Rates of change in unreacted starch content at different storage temperatures.

Segment ²	Rates of change in unreacted starch content ¹ (% per week)								
	0			1			2		
Storage Conditions ³	OUT	REF	IN	OUT	REF	IN	OUT	REF	IN
2011 Corn ⁴	---	---	---	-0.55aC	-0.54aC	---	0.50aC	0.68aC	---
2012 Corn	0.05a	0.44a	0.23a	-0.20aC	-0.24aC	-0.28b	0.32aC	0.10aD	0.32a

¹On a per storage segment basis, values followed by the same lowercase letter (a or b) in the same row and by the same uppercase letter (C or D) in the same column are not significantly different (p > 0.05).

²Segment 0 = 0 to 6 weeks; 1 = 6 to 16 weeks; and 2 = greater than 16 weeks of storage.

³Storage conditions OUT = outdoors, REF = refrigerated, IN = indoors.

⁴Data from the first segment, i.e., first 4 weeks of storage, in 2011 were lost. Also, corn was not stored indoors in 2011.

From the 4th to 15th week after harvest, unreacted starch tended to decrease across all three storage locations, with corn stored indoors exhibiting the greatest rate of

decrease and different from the other stored corn. This behavior was likely related to the decrease seen for corn harvested in 2011 during the 4th to 10th week, although the decrease in unreacted starch content was not as steep. When comparing between years, the rates of changes in corn harvested in 2012 were, in general, different from the rates of changes seen with corn harvested in 2011 except for the corn stored under refrigerated conditions.

The 2012 harvest year corn showed a general increase in unreacted starch content from the 15th to the 23rd week of storage, which was also observed starting at the 10th week of storage of the 2011 harvest year corn. Statistically, none of these slopes from different storage conditions in the 2012 storage year were different from each other. When this trend in 2012 corn was compared to storage conditions counterparts from 2011, the outdoor corn slopes were not statistically significant, but the refrigerated corn slopes were considered statistically significant.

Long term changes in unreacted starch content for the 2011 harvest year showed a moderate leveling off between 20 and 32 weeks of storage (Figure 3.4). After this period, the unreacted starch content of the corn stored outdoors increased through the 44th week and began to drop again. The increasing trend for the corn stored outdoors, in general, coincided with the increase in ambient temperature during the summer months, although the highest unreacted starch contents lagged behind the highest temperatures. The refrigerated corn also exhibited an increase in unreacted starch content during the 40th to 44th week in storage.

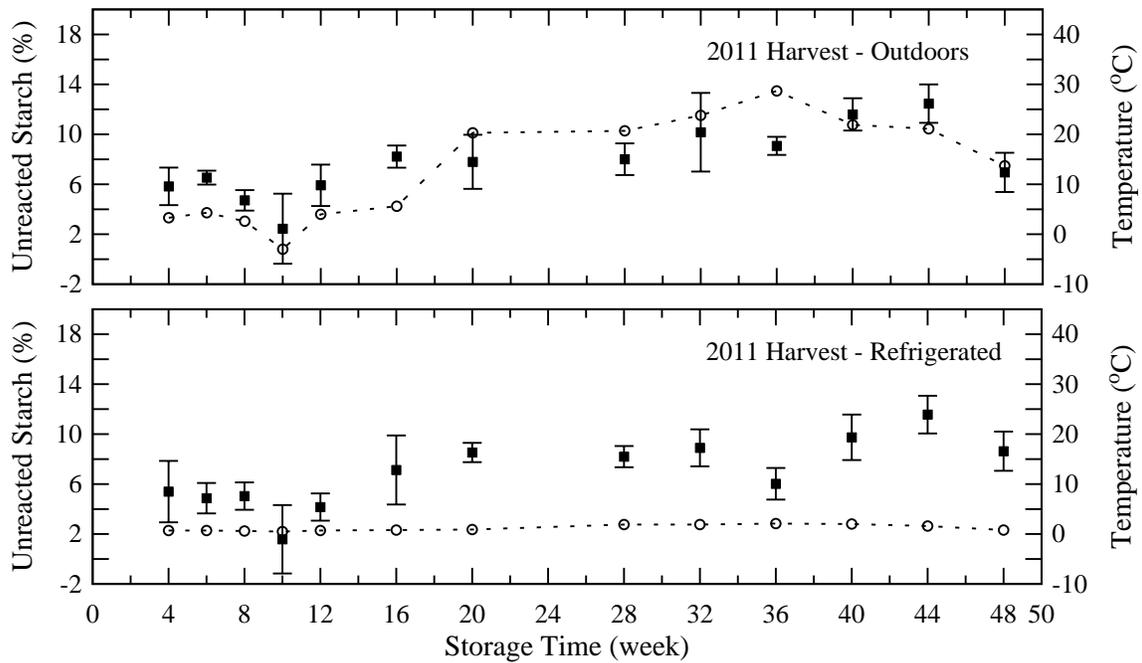


Figure 3.4. Unreacted starch content (■) in corn harvested in 2011 and temperature storage conditions (○) during the 48 weeks in storage.

3.4 Conclusion

This study showed that unreacted starch content in corn increased during the first four weeks after harvest, then decreased until 10 to 15 weeks, and finally increased for the remainder of the 5 to 12 months in storage. Unreacted starch content was strongly correlated with storage time and mildly correlated with storage temperature. For the 4th to 15th week of storage, the corn stored indoors had a different rate of decrease in unreacted starch content than the corn stored outdoors and under refrigerated conditions. In each year of the study, at all other time periods of storage, there were no differences amongst the rates of change in unreacted starch content. However, there was a difference between rates of increase or decrease in unreacted starch content across years.

CHAPTER 4. PREDICTING UNREACTED STARCH LEVELS IN CORN BY FOURIER TRANSFORM NEAR INFRARED SPECTROSCOPY

4.1 Introduction

In order for ethanol companies to minimize the amount of unreacted starch in the dry grind ethanol process and in the DDGS, it would be beneficial to develop a high throughput procedure for monitoring unreacted starch levels in incoming corn. Preferably the method would provide a rapid assessment of unreacted starch levels and instrumentation be installed online at an ethanol facility. This method would allow dry grind ethanol plants to have greater control of managing their corn inventory, provide a better understanding of possible sources of error in the process, and increase processing efficiency by adapting conditions and enzymes used to fit the corn. Given the fast speed of near infrared spectroscopy and its usefulness in quantifying similar organic compound, the second goal of this research was to develop a model for unreacted starch content in ground corn flour using near infrared spectroscopy.

4.2 Materials and Methods

4.2.1 Sample preparation and unreacted starch determination

Starch blends (0 to 33% unreacted starch content) were prepared by mixing weight proportions of Hi-maize resistant starch (Honeyville, Brigham City, UT) with starch (Product No. S516-500, Fisher Scientific). Honeyville's product contains Hi-maize[®]260 (Ingredient, Bridgewater, NJ) resistant starch that has been isolated from high amylose corn hybrids produced through traditional plant breeding and contains 33% digestible, or glycemic, starch. The unreacted starch contents of the blends were verified using the same liquefaction and saccharification procedure used with the ground corn

samples described in Section 3.2. Results of the verification tests are available Figure B.1 in Appendix B.

Pioneer P1395R yellow dent corn was harvested from the Agricultural and Biological Engineering Farm in Urbana, IL in October 2011 and the same hybrid was provided from Pioneer directly in October 2012. Ground corn samples, dried and undried (or “wet”), were prepared (Figure 4.1) and the procedures for determining their unreacted starch contents were determined according to the procedures described in Section 3.2. Note that the FT-NIR scans were conducted prior to the samples undergoing the dry grind process, and those scans matched to the results of the GOPOD assay. Any change in starch resistance that was a result of the dry grind process is not represented in the NIR spectral scans.

4.2.2 Scanning, processing and analyses of FT-NIR spectra

A spectrophotometer (Perkin Elmer Spectrum One NTS Waltham, MA) was used to scan 100 dry ground corn samples and 50 starch blend samples. Approximately a 5 g subsample was poured in a smaller NIRA cup, leveled, and scanned 16 times with a manual rotation between each scan. This procedure was repeated five times with separate subsamples and the resulting spectral scans were averaged.

Because it will be advantageous to develop a high throughput procedure to estimate unreacted starch content in wet corn samples, 49 “wet” ground corn samples were scanned prior to moisture content determination. Approximately 25 g of sample was poured in a NIRA sample cup and leveled. The cup was placed on a spinner attachment and the spectrophotometer was set to take an average spectral scan from 16 readings.

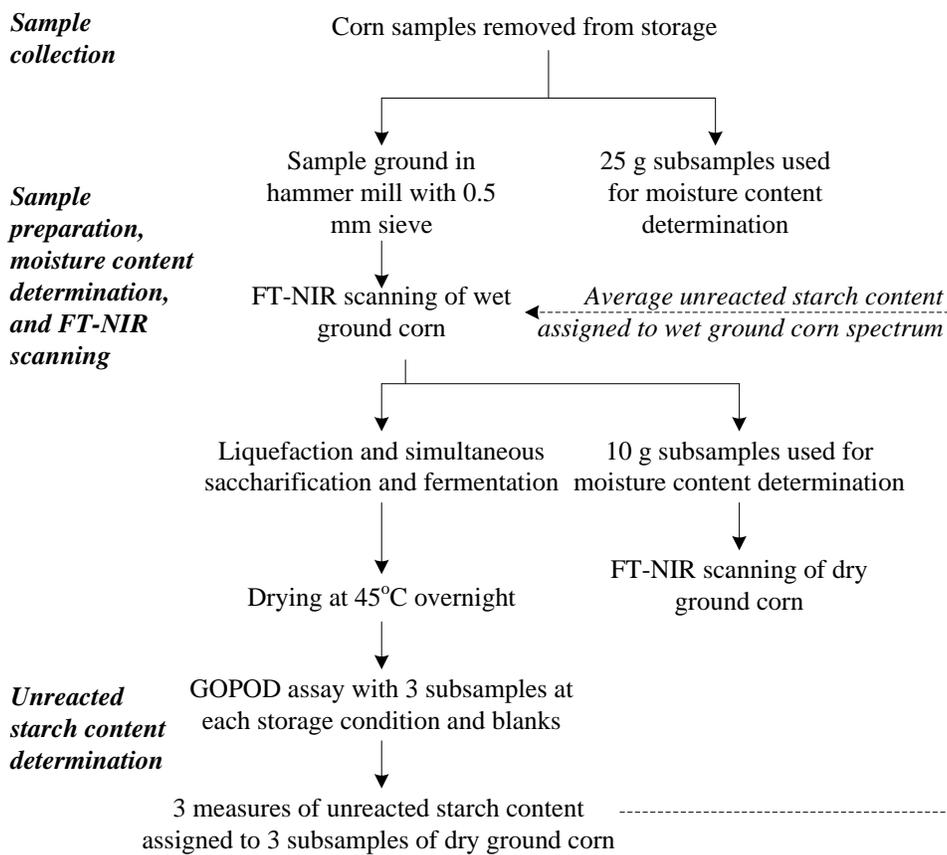


Figure 4.1. Preparation of dry and wet ground corn used in developing predictive models of unreacted starch content based on FT-NIR spectroscopy.

Unscrambler® (Version 10.2., Camo Software Inc., Woodbridge, NJ) was used to process and analyze the spectral data, build and validate the calibration, and test the regression models.

4.3 Results and Discussion

4.3.1 Unreacted starch content in starch blends, dry and wet ground corn

A total of 56 samples of unreacted starch blends were used to create unreacted starch models. The histograms for the starch blends are shown in Figure 4.2. The samples measured ranged from 0 to 33.3% resistant starch, with a mean of 16.69%. There were 36 samples were used in the calibration set, 14 samples were used in the validation set, and six samples were used in the test set.

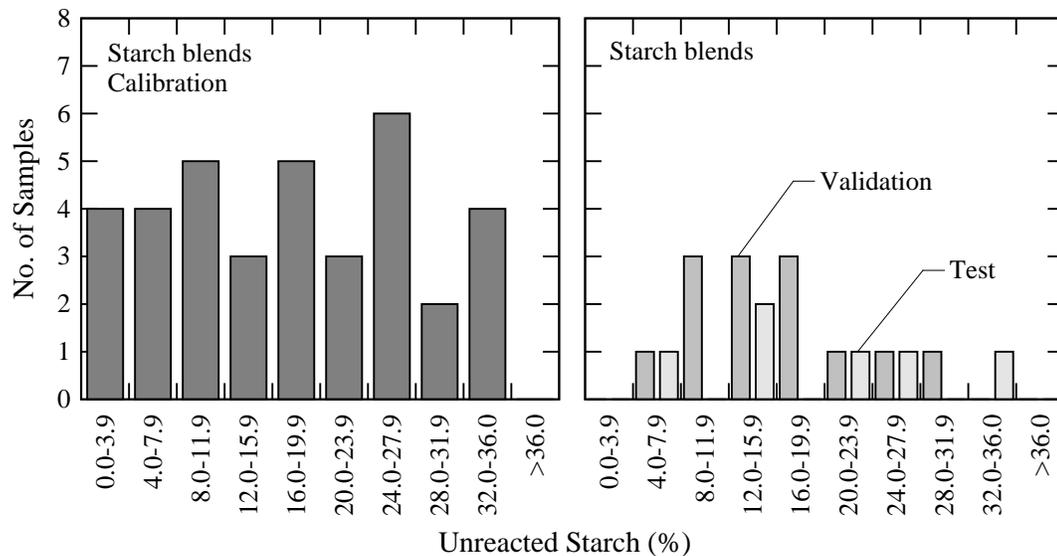


Figure 4.2. Histograms of the calibration, validation, and test sets used in developing predictive models for the starch blends.

The dry ground corn models were made using 100 samples of corn from the 2011 and 2012 harvest years. For calibration, validation, and testing, 72, 20, and 8 samples were used, respectively (Figure 4.3). The values of resistant starch ranged from 5.1% to 16.6%, with the mean value being 11.9%. The wet ground corn models were made using 49 samples of corn from the 2011 and 2012 harvest years. There were 36 samples were

used for calibration, 10 samples for validation, and three samples for the test set. The values of resistant starch ranged from 1.6% to 15.1%, with the mean value being 10.1%.

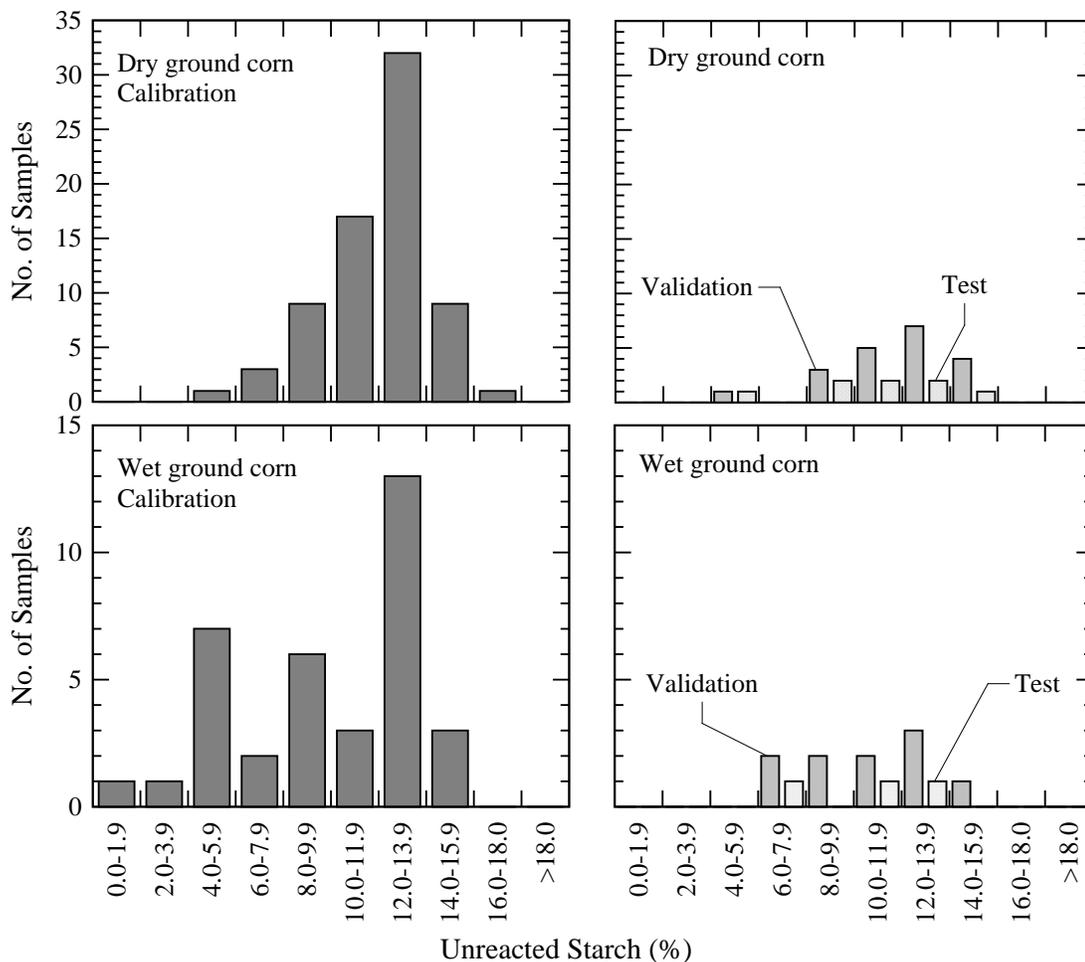


Figure 4.3. Histograms of the calibration, validation, and test sets used in developing predictive models for the dry and wet ground corn.

4.3.2 FT-NIR Spectra

The FT-NIR spectra of the starch blends, dry corn flour, and wet corn flour showed prominent peaks, both crests and troughs, at around 5250 cm^{-1} and 7000 cm^{-1} (Figures 4.4). The region around 5250 cm^{-1} has been noted for O-H stretching and O-H deformation and was discussed as a possible measure of the stability amylose and amylopectin gel structures (Lindberg and Kiala, 1980), as both are components of

unreacted resistant starch. While all of the samples tend to show the same trend, a sharp crest, then a sharp trough in that region, the heights and slopes of these vary across all the samples we studied, particularly for dry samples. The peak at 7000 cm^{-1} has been reported as an important region for starch and protein (Noah et al., 1997) but is also near an overtone of water absorption (Paulsen and Singh, 2004). This peak tended to be higher in the wet corn flour samples than in the dry corn flour samples so predictive models of unreacted starch content should not have high regression coefficients in this region since high moisture contents would influence the degree of absorption.

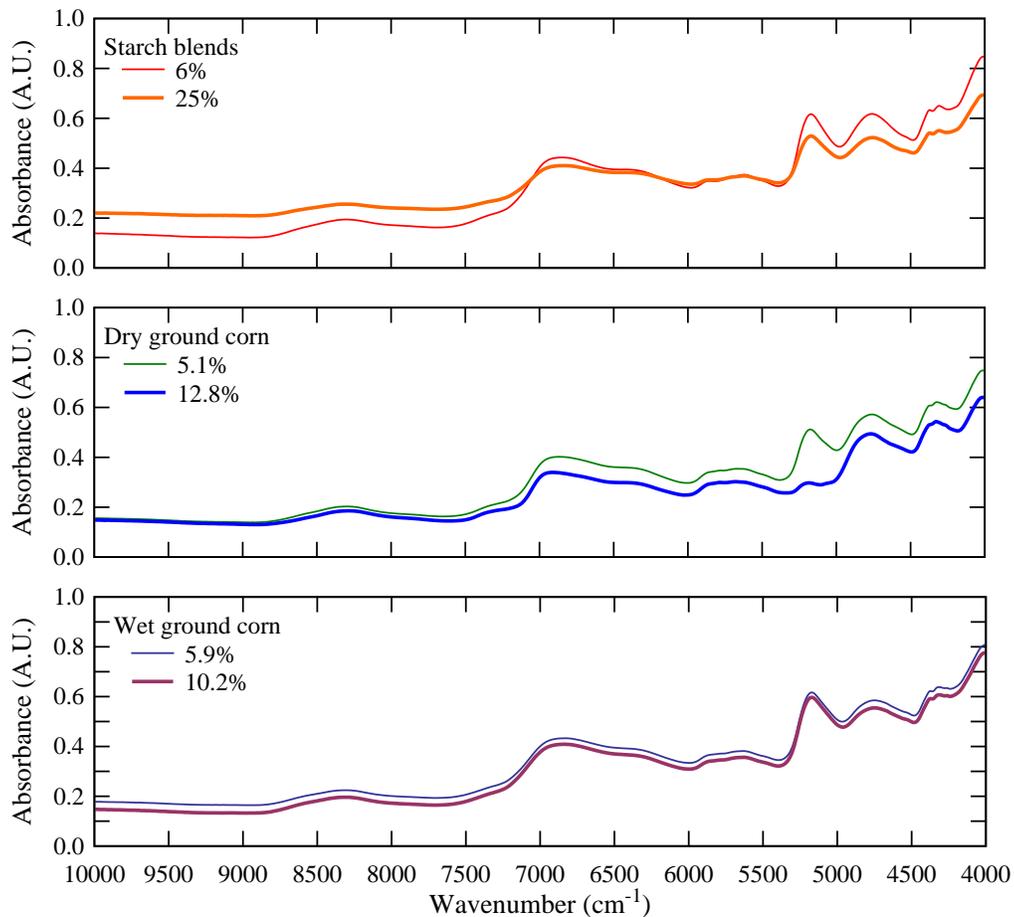


Figure 4.4. FT-NIR spectra of select starch blends and ground corn (dry and wet) samples.

NIR scans of waxy corn and high amylose corn were also conducted for reference (Figure 4.5), as the difference in amylose and amylopectin ratios would mean that those samples likely represent the opposite ends of theoretical unreacted starch content in corn. From $4,000\text{ cm}^{-1}$ to $6,000\text{ cm}^{-1}$, the peak and trough for waxy corn were steeper than that of high amylose corn, and the absorbance values were higher for waxy corn in both the peak and the trough. Since higher amylose contents are known to correlate with an increase in unreacted starch, one would expect higher unreacted starch contents to correlate with higher values in this region. This trend held true for most samples as samples with lower unreacted starch content had higher absorbance values in this region and, beyond this region, samples with lower amounts of unreacted starch had higher absorbances. In the $4,000\text{ cm}^{-1}$ to $4,500\text{ cm}^{-1}$ region, absorbance values increased with decreasing unreacted starch content.

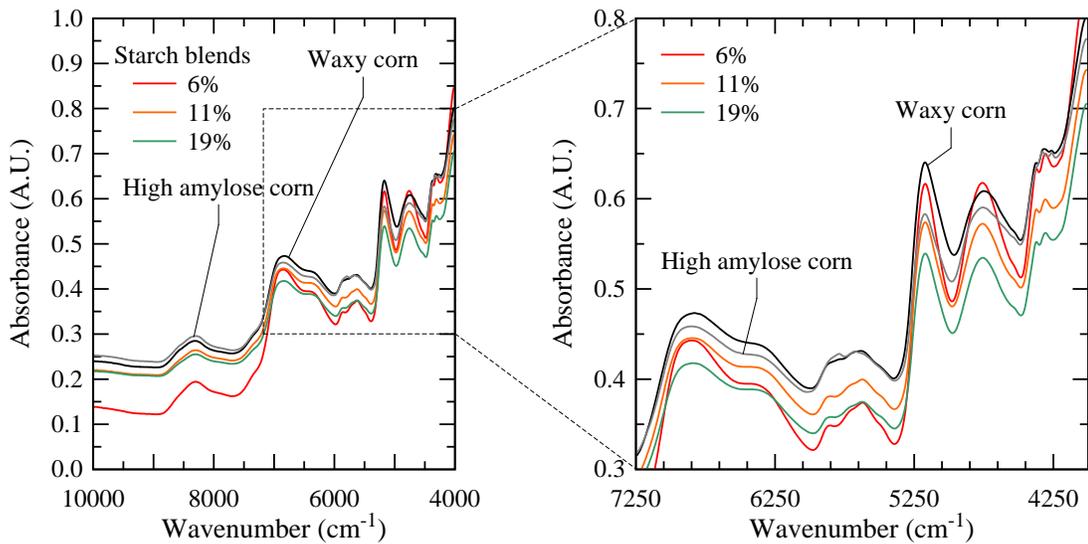


Figure 4.5. FT-NIR spectra of select starch blends with different unreacted starch content (%) and ground samples of waxy and high amylose corn.

4.3.3 Processing of FT-NIR data

The baseline shifts in the raw spectral data (Figures 4.4 and 4.5) often do not represent compositional information but likely common sources of error in sample preparation or spectral data collection. To remove the baseline shifts and other errors in the spectra, pretreatment techniques such as MSC and SG were used (Figure 4.6). MSC corrected simple baseline shifts without overprocessing, or changing, the appearance or location of peaks as much as derivative based pretreatments.

The SG first and second derivative calculations were useful in showing changes in the spectra, but being derivatives, they shifted the peaks and troughs that mark the functional group wavebands. The first derivative curves were easier to interpret as the location of peaks and troughs of the spectra did not change.

During the SG derivative pretreatment, the number of smoothing points used changed how severely the spectra were processed (Figure 4.7). Too few points resulted in “noisy” or undersmoothed curves whereas too many points resulted in oversmoothed curves. While undersmoothed curves still presented small, yet important, absorption bands, spectral noise was not eliminated. Oversmoothed curves eliminated noise effectively, but also tended to eliminate small absorption bands or shoulders that were important indicators of unreacted starch.

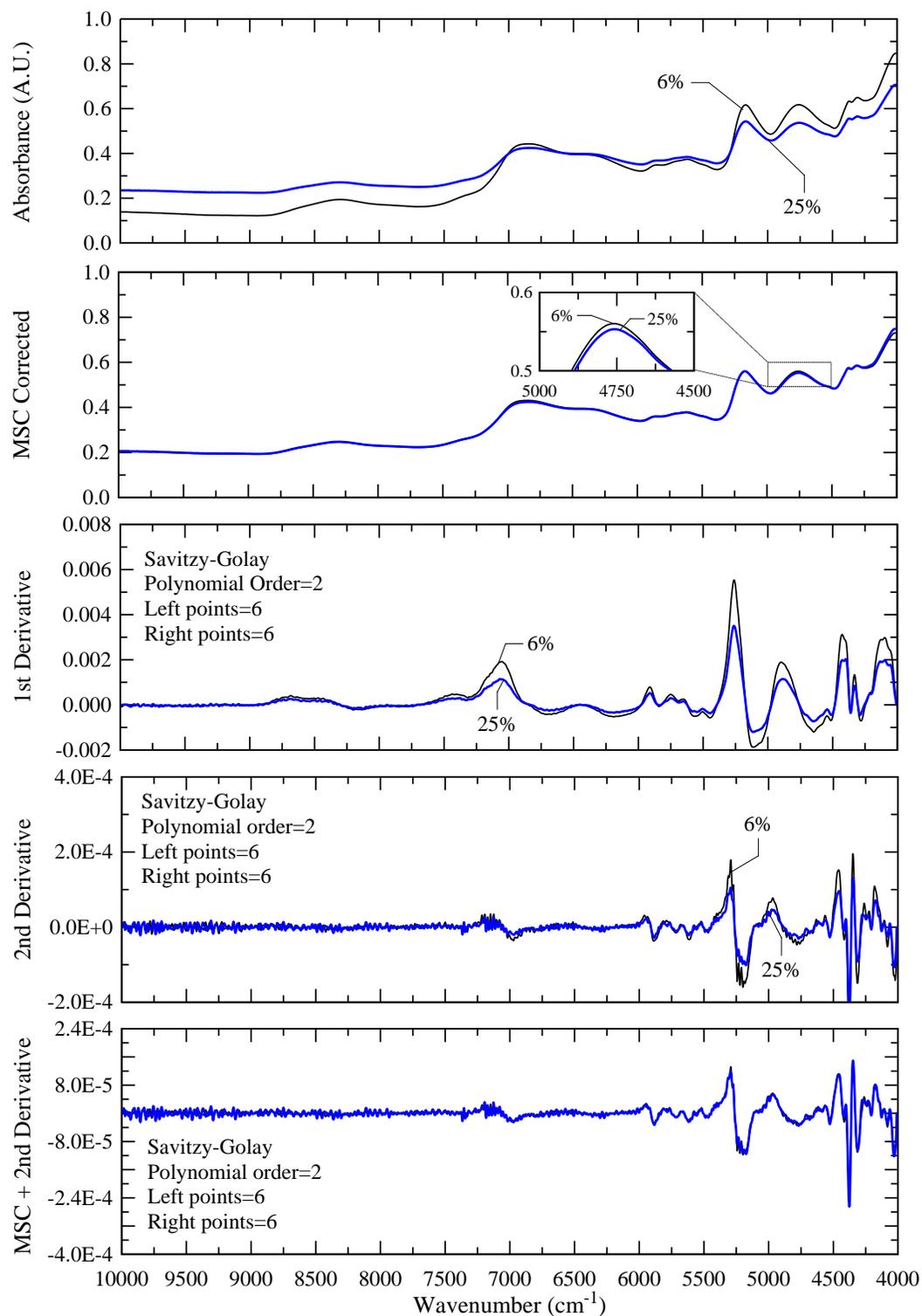


Figure 4.6. Processing of the FT-NIR spectra of starch blends involved conducting a multiplicative scatter correction (MSC), derivative calculations based on the Savitzky-Golay (SG) algorithm, and combined smoothing and derivative calculation.

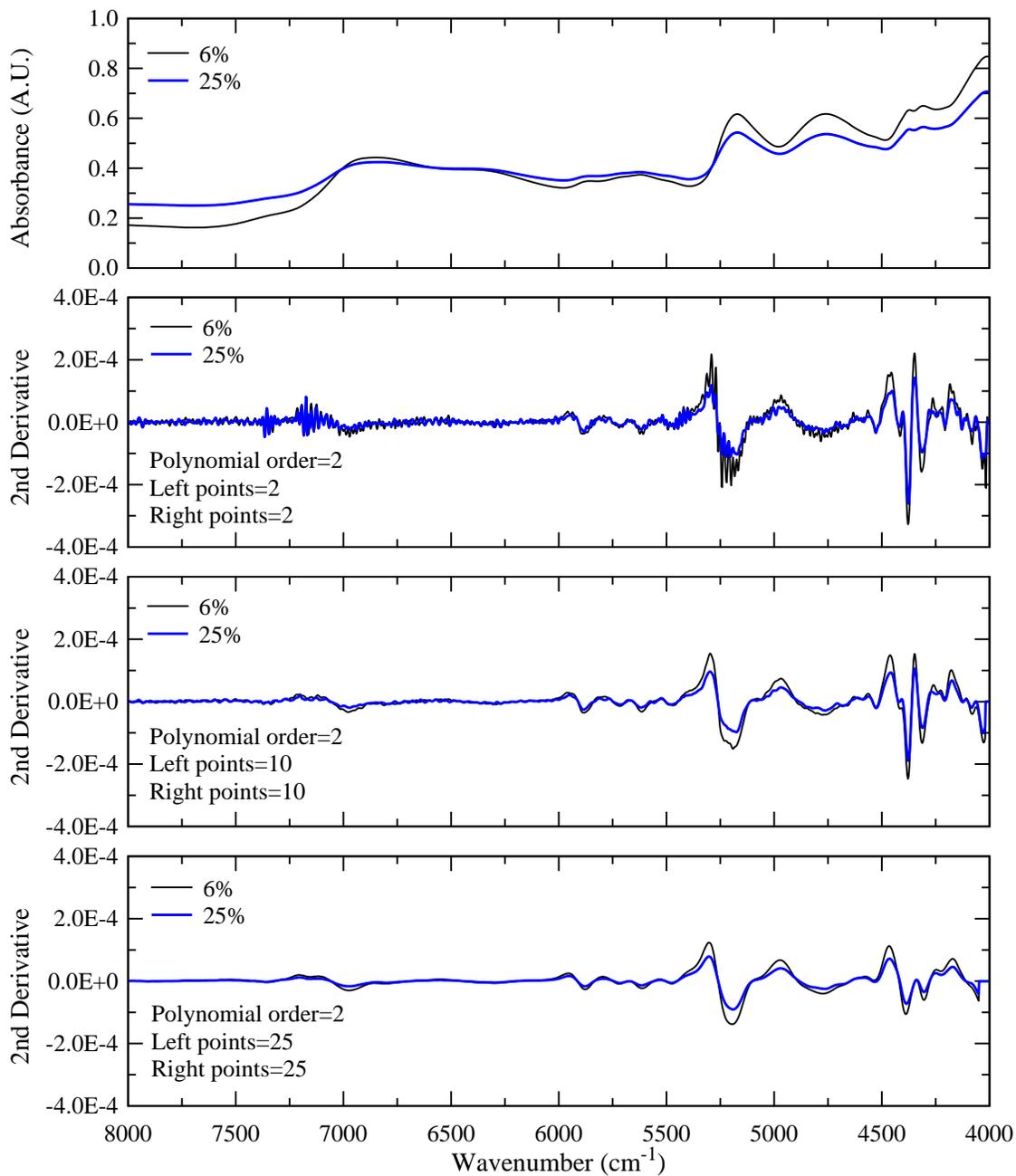


Figure 4.7. The size of the bandwidth used in the Savitzky-Golay (SG) algorithm determines the smoothness of the resulting derivative curves. Undersmoothed curves were noisy while certain features in the spectra are lost in oversmoothed curves.

4.3.4 PLS Modeling

Partial least squares models using MSC, SG first and second derivatives, and a combination of MSC with SG second derivative pretreated spectral data were developed for starch blends and ground corn. Results of calibration, validation, and prediction are presented in Table 4.1, Figure 4.8, and Appendix C.

Models for the unreacted starch blends outperformed the other models with RPD values up to 7.28, $R^2 = 0.97$ and 95% explained variance. The best model for the unreacted starch blends was the model made with combined MSC and SG second derivative pretreatments, although results of the MSC model were similar. The unreacted starch blend models were expected to yield better results than the models developed for corn, as these samples likely did not have large or varying amounts of other constituents in corn, such as proteins, lipids, etc. The calibration and validation sets of the starch blends also had a wider range compared to the corn flour sets.

The best model for dry ground corn was also based on the spectra pretreated with a combined MSC and SG second derivative. It had 85% explained variance and an RPD of 2.07. This model was based on the highest number of calibration and validation data points, although the range of unreacted starch values was narrow, from 5.1% to 16.6% with a standard deviation of 2.34%. This model could be improved with the addition of more samples with a wider range of unreacted starch content and a more accurate procedure for measuring glucose than the colorimetric GOPOD assay, such as high performance liquid chromatography.

Table 4.1. PLS regression models of unreacted starch content in starch blends and ground corn.

Data Pretreatment	Starch blends				Dry corn flour				Wet corn flour			
	MSC ¹	SG-1 ²	SG-2 ³	MSC +SG-2 ⁴	MSC	SG-1	SG-2	MSC +SG-2	MSC	SG-1	SG-2	MSC +SG-2
No. of factors	3	2	2	2	4	4	2	2	3	4	3	3
Explained variance (%)	95	90	95	95	37	66	80	85	70	90	85	85
No. of points in the derivative calculation	---	13	31	31	---	13	21	21	---	11	51	51
R^2												
Calibration	0.97	0.97	0.98	0.98	0.41	0.83	0.83	0.86	0.70	0.93	0.83	0.94
Validation	0.96	0.93	0.93	0.97	0.49	0.64	0.75	0.80	0.19	0.38	0.54	0.47
Prediction	0.97	0.98	0.98	0.97	0.30	0.33	0.42	0.38	0.99	0.97	0.99	0.75
RMSE ⁵ [%]												
Calibration	1.81	1.63	1.44	1.23	1.77	0.89	0.89	0.82	2.14	1.01	1.59	0.96
Validation	1.51	1.94	1.95	1.29	1.29	1.51	1.26	1.13	2.23	1.95	1.67	1.81
Prediction	4.02	4.5	4.38	2.67	2.67	2.82	2.83	2.49	1.81	1.28	0.80	1.43
RPD ⁶	6.22	4.84	4.82	7.28	1.81	1.55	1.86	2.07	1.61	1.85	2.16	1.99

¹MSC = multiplicative scatter correction

²SG-1 = Savitzky-Golay 1st derivative calculation using a 2nd order polynomial

³SG-2 = Savitzky-Golay 2nd derivative calculation using a 2nd order polynomial

⁴MSC+SG-2 = multiplicative scatter correction with a Savitzky-Golay 2nd derivative calculation using a 2nd order polynomial

⁵RMSE = root mean square error

⁶RPD = ratio of performance to deviation

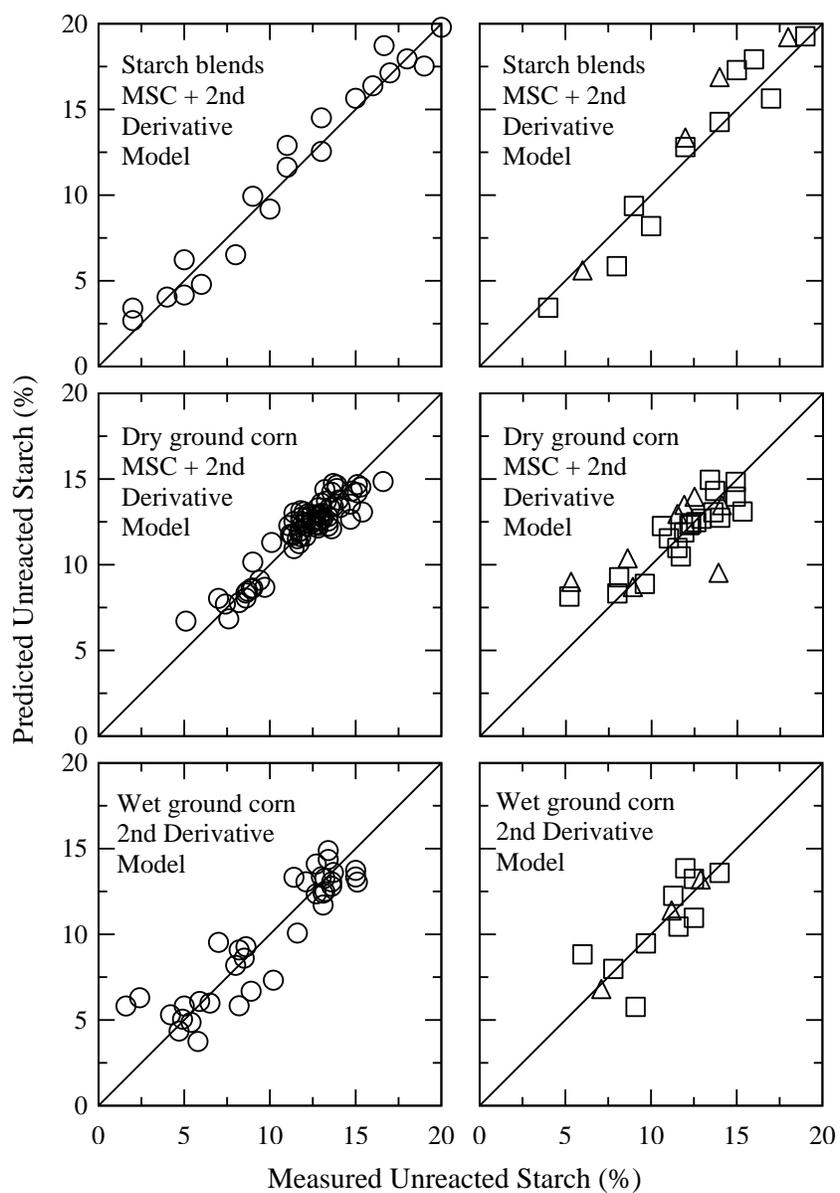


Figure 4.8. Comparison of predicted to measured unreacted starch content using the calibration (○), validation (□), and test (△) sets of starch blends and ground corn.

The best model for wet ground corn used the SG second derivative pretreated spectra data. It had 85% explained variance and an RPD of 2.16. This was better than the model for dry ground corn despite the fact that water would have interfered and masked important absorption bands in the spectra. One possible explanation for this was that the unreacted starch values for wet corn flour samples were based on was the average

of three replicates, unlike for dry ground corn samples, measures were based on one replicate. The calibration set for wet ground corn also had a wider range than for dry ground corn, from 1.6% to 15.1% with a standard deviation of 3.6%.

All of the models had high correlation coefficients in the important regions of 4000 to 5000 cm^{-1} , 5250 cm^{-1} , and 7000 cm^{-1} for starch (Figure 4.9). However, the 7000 cm^{-1} waveband likely was attributable to protein and water content in corn. The wet ground corn model also relied on a peak around 5,100 cm^{-1} , which is a known water absorption band (Ben-Gera and Norris, 1968; Paulsen and Singh, 1994). Since the peaks and valleys of regression coefficients coincided with the important starch regions reported in the literature and in the starch blends, with high values at 5,250 cm^{-1} , the resulting regression models were accurate predicting unreacted starch content instead of other constituents in the ground corn.

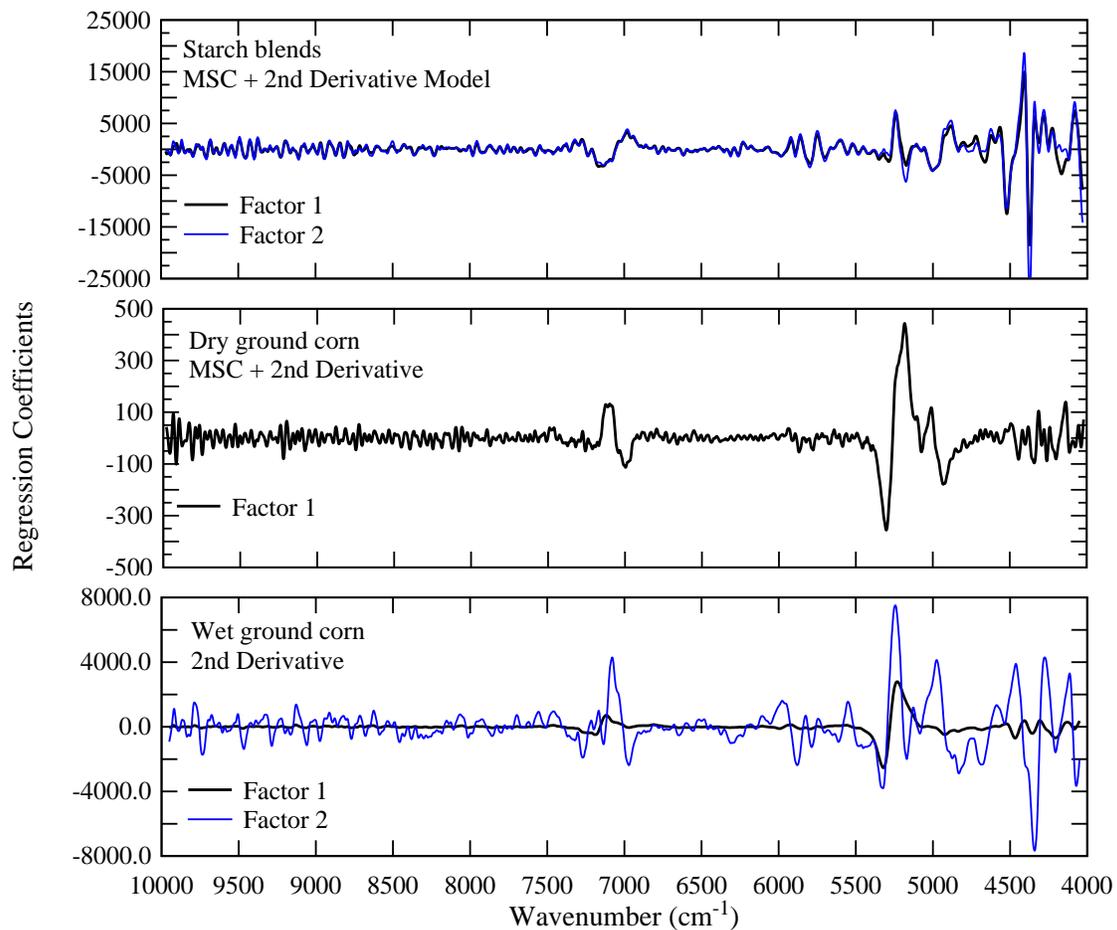


Figure 4.9. Regression coefficients of the best models developed for predicting unreacted starch content in starch blends and ground corn.

4.4 Conclusion

Using FT-NIR spectroscopy to predict unreacted starch content in corn as an in line monitoring tool in ethanol facilities is promising, as several models approached rough screening quality and were based on the important regions known to correlate with starch. The starch blend models had an RPD of 7.28. The models for dry ground corn and wet ground corn had RPD values of 2.07 and 2.16, respectively, which is almost at a rough screening level.

CHAPTER 5. CONCLUSIONS AND FUTURE WORK

This study showed that unreacted starch content in corn increased during the first four weeks after harvest, then decreased until 10 to 15 weeks, and finally increased for the remainder of the 5 to 12 months in storage. Unreacted starch content was strongly correlated with storage time and mildly correlated with storage temperature. From the 4th to 15th week of storage, the corn stored indoors had a different rate of decrease in unreacted starch content than the corn stored outdoors and under refrigerated conditions. In each year of the study, at all other time periods of storage, there were no differences amongst the rates of change in unreacted starch content. However, there was a difference between rates of increase or decrease in unreacted starch content across years.

Using FT-NIR spectroscopy to predict unreacted starch content in corn as an in line monitoring tool in ethanol facilities is promising, as several models approached rough screening quality and were based on the important regions known to correlate with starch. Models for starch blends, dry ground corn, and wet ground corn had RPD values of 7.28, 2.07, and 2.16, respectively.

In the future, experiments could be conducted to determine the effect other factors, such as corn variety, types of mixes of enzymes used, or processing conditions, on unreacted starch content. The storage experiments could be expanded to include a wider range of temperature differences between storage conditions, to see if larger differences in temperature cause would have a larger impact on the unreacted starch content. The predictive models developed in this study could be further improved by increasing sample sizes and including different corn varieties, especially waxy and high amylose corn to get a wider range of unreacted starch content.

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APPENDIX A. STATISTICAL CODE IN R ENVIRONMENT

Code for Statistical Significance and Tukey's test

```
mean18 <- read.table("U:/DanaoLab/Ben Plumier/mean18.csv", sep="," ,
header=T)
attach(mean18)
summary(aov(glm(US ~ factor(Cond))))
mean20 <- read.table("U:/DanaoLab/Ben Plumier/mean20.csv", sep="," ,
header=T)
attach(mean20)
amod <- aov(US ~ Cond)
library(agricolae)
HSD.test(amod, "Cond", group=TRUE)
```

Code to determine Pearson correlation coefficients

```
pearson <- read.table("C:/2011pearson.csv", sep="," , header=T)
attach(pearson)
cor(pearson[,c("Weeks", "Temperature", "US")], use="complete.obs")
```

Code for least squares regression and comparison of slopes

```
week <- c(4,4,4,6,6,8,8,10,10)
us <- c(5.94, 5.85, 5.71, 6.86, 6.21, 4.66, 4.76, 2.19, 2.70 )
fit <- lm(us~week)
summary(fit)
```

P values for comparisons of slopes were then calculated using slopes and standard error of the slopes by the equation

$$T = \frac{b_1 - b_2}{\sqrt{(Sb_1)^2 + (Sb_2)^2}}$$

where b_1 and b_2 are the slopes the lines and Sb_1 and Sb_2 are the standard errors of the slopes.

APPENDIX B. VERIFICATION OF THE UNREACTED STARCH CONTENT IN STARCH BLENDS

In a small experiment to test the possibility of creating resistant starch standards, commercial starch from Honeyville, advertised as one third digestibly resistant starch was tested with the unreacted starch assay outlined in Section 2.2. Three samples were prepared with unreacted starch content ranging from 16% to 34%. Results, however, were misleading as nearly all of the digestible starch were converted to glucose and removed, leaving nothing but the unreacted starch. The calculations lead to unreacted starch contents of 80% and above. Based on dry weights of recovered hydrolyzate, the relations could be graphed between expected unreacted starch and actual unreacted starch (Figure B.1).

A small amount of glucose, ranging from 12 % to 17%, was found to remain after centrifuge washing. The percentage of nonstarch in the hydrolyzate ranged from 4% to 9%. However, the amount of solid hydrolyzate apparent in the samples varied greatly after centrifuge washing, and a strong correlation was found between the theoretical resistant starch contents and the weights of the hydrolyzate, with an R^2 value of 0.9982.

This test demonstrated that our unreacted starch procedure is effective at differentiating between enzyme resistant and nonresistant starch, and that commercially available digestive resistant starch is a reasonably adequate analogue for enzymatically resistant starch.

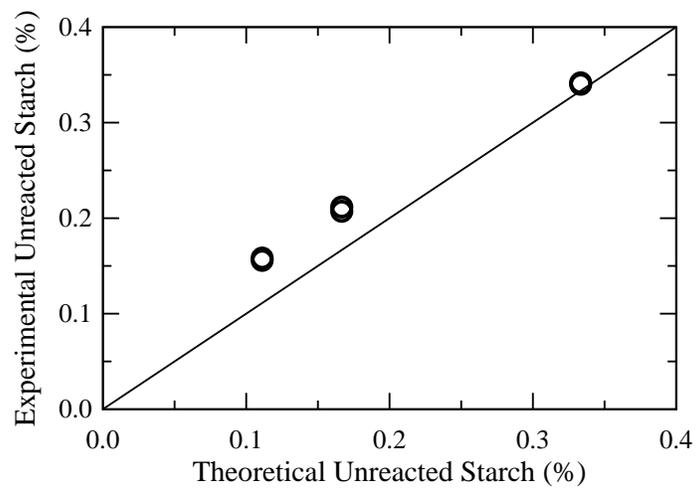


Figure B.1. A comparison between theoretical unreacted starch content and resulting unreacted starch yields measured by weight after centrifuge washing.

APPENDIX C. FT-NIR SPECTRAL DATA PRETREATMENT AND REGRESSION MODELS

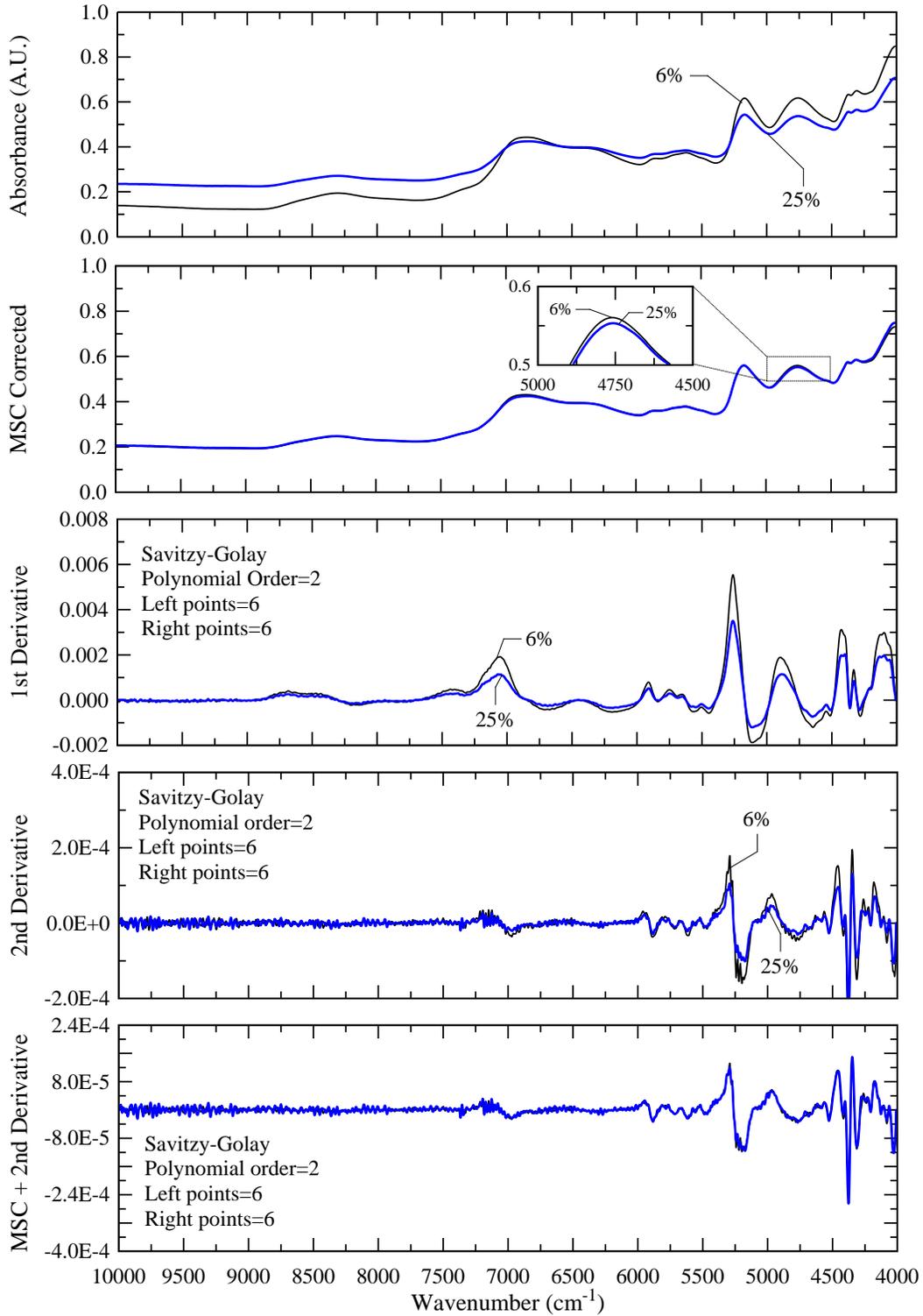


Figure C.1. Raw and pretreated spectra of starch blends used in PLS regressions and analyses.

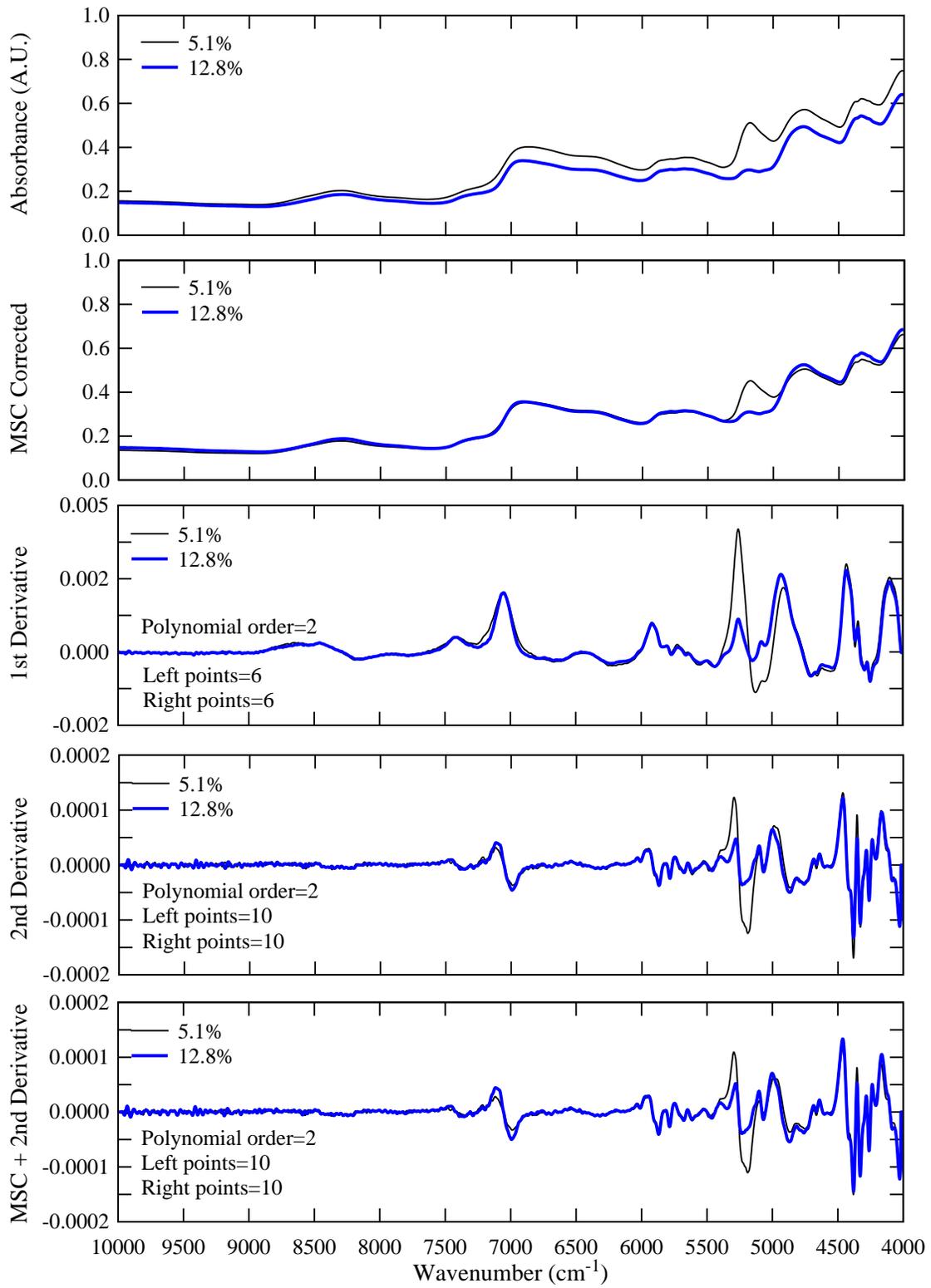


Figure C.2. Raw and pretreated spectra of dry ground corn used in PLS regressions and analyses.

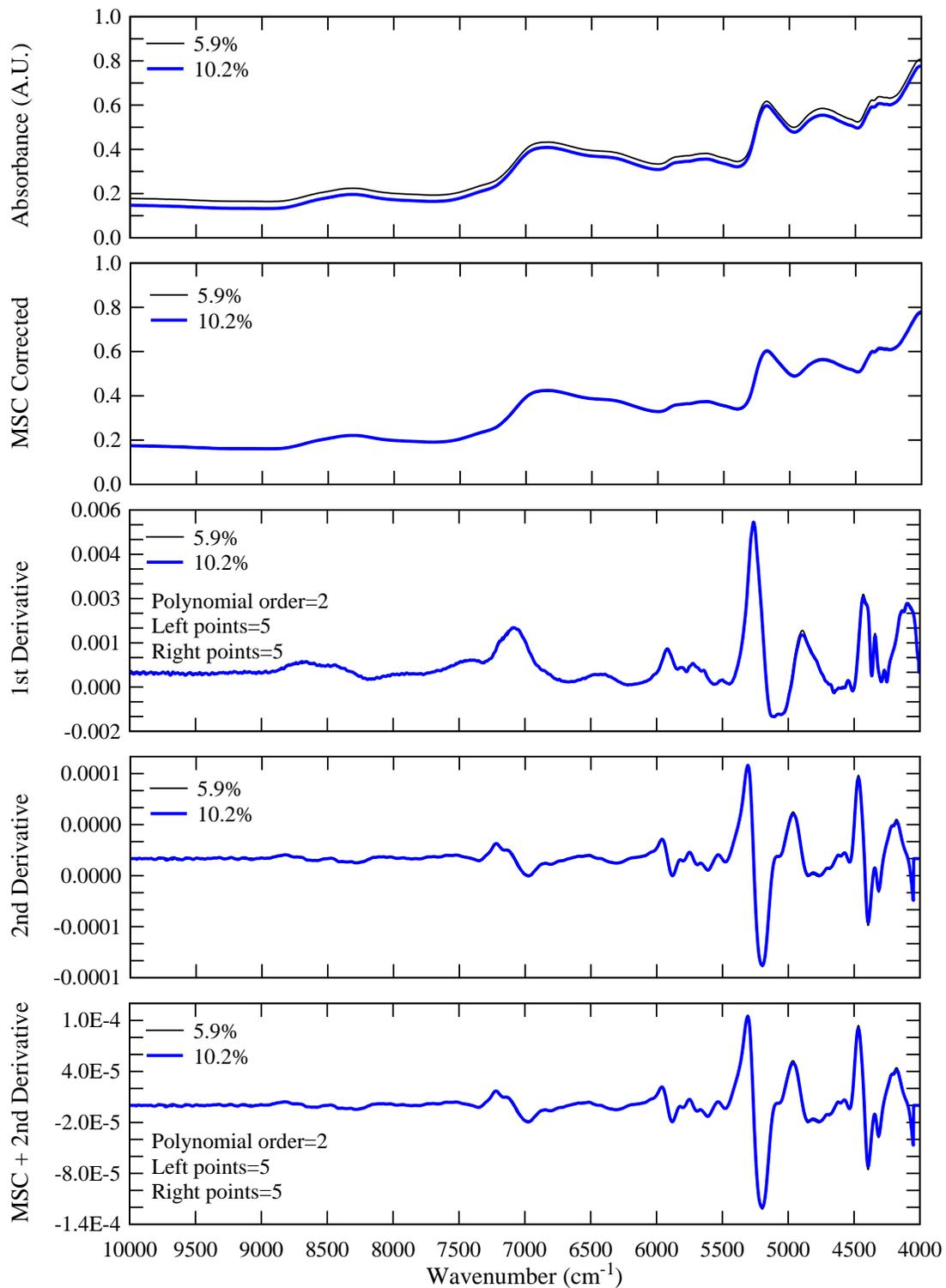


Figure C.3. Raw and pretreated spectra of wet ground corn used in PLS regressions and analyses.

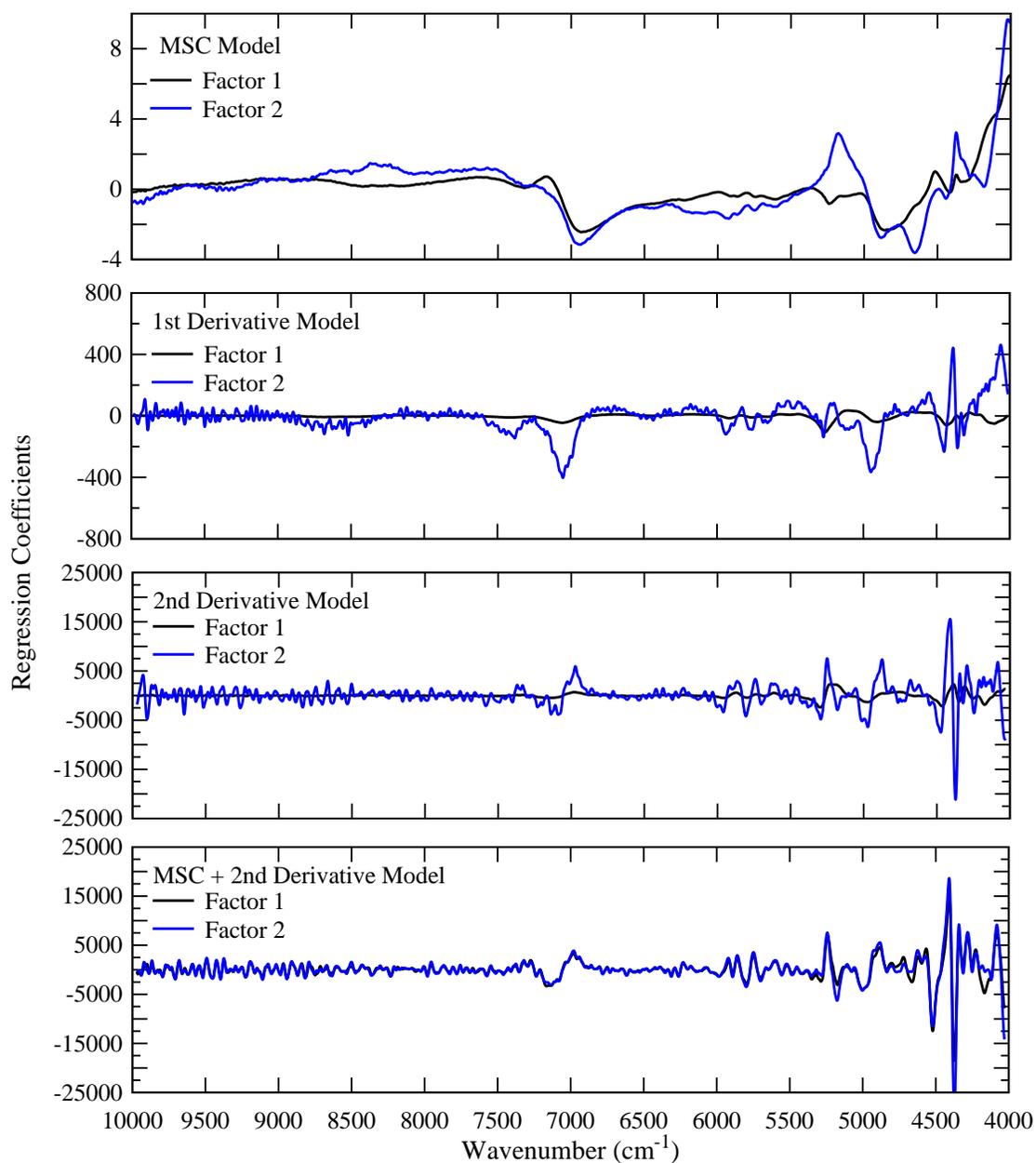


Figure C.4. Regression coefficients of the first two factors of the resulting PLS models for unreacted starch content in starch blends.

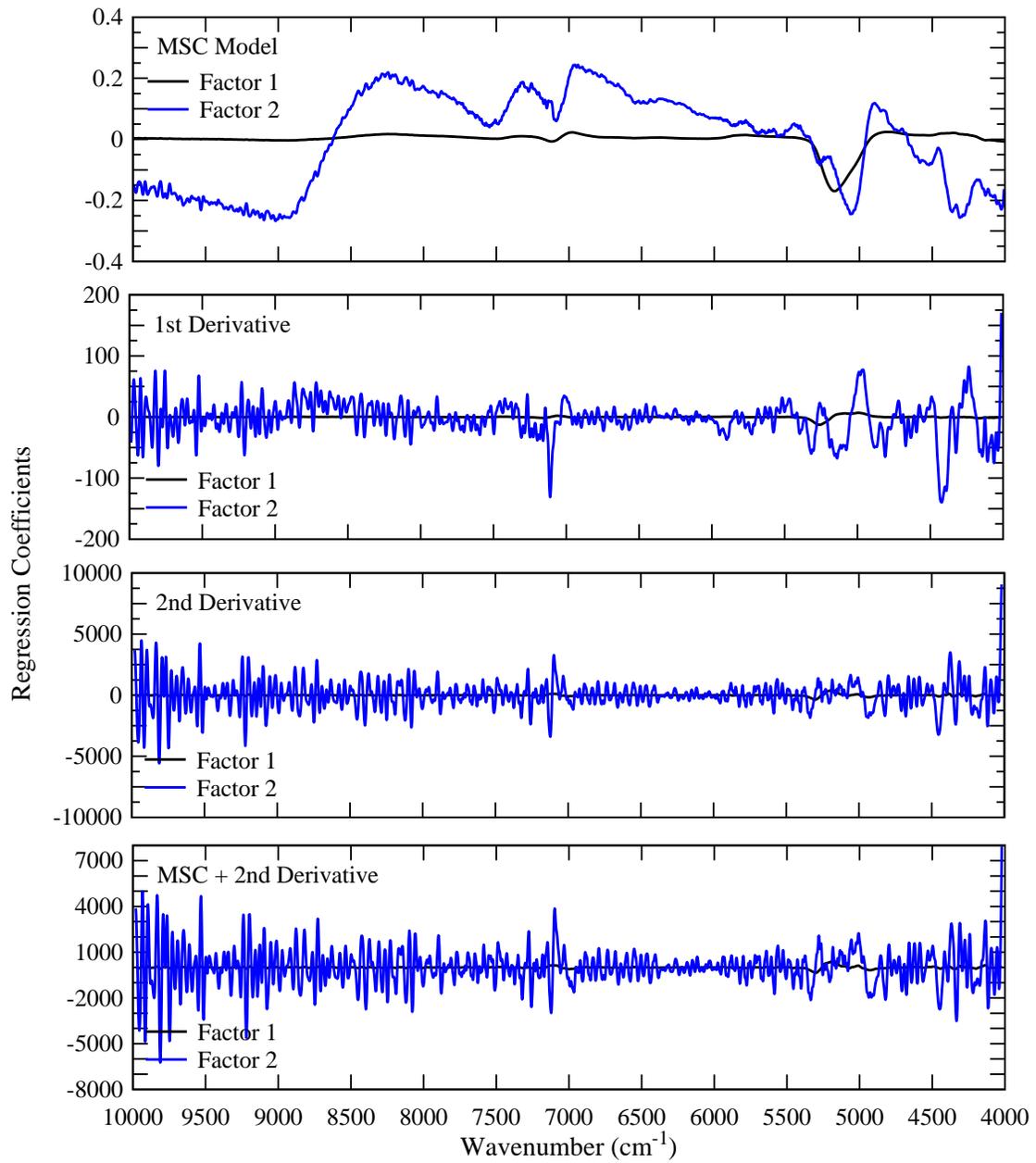


Figure C.5. Regression coefficients of the first two factors of the resulting PLS models for unreacted starch content in dry ground corn.

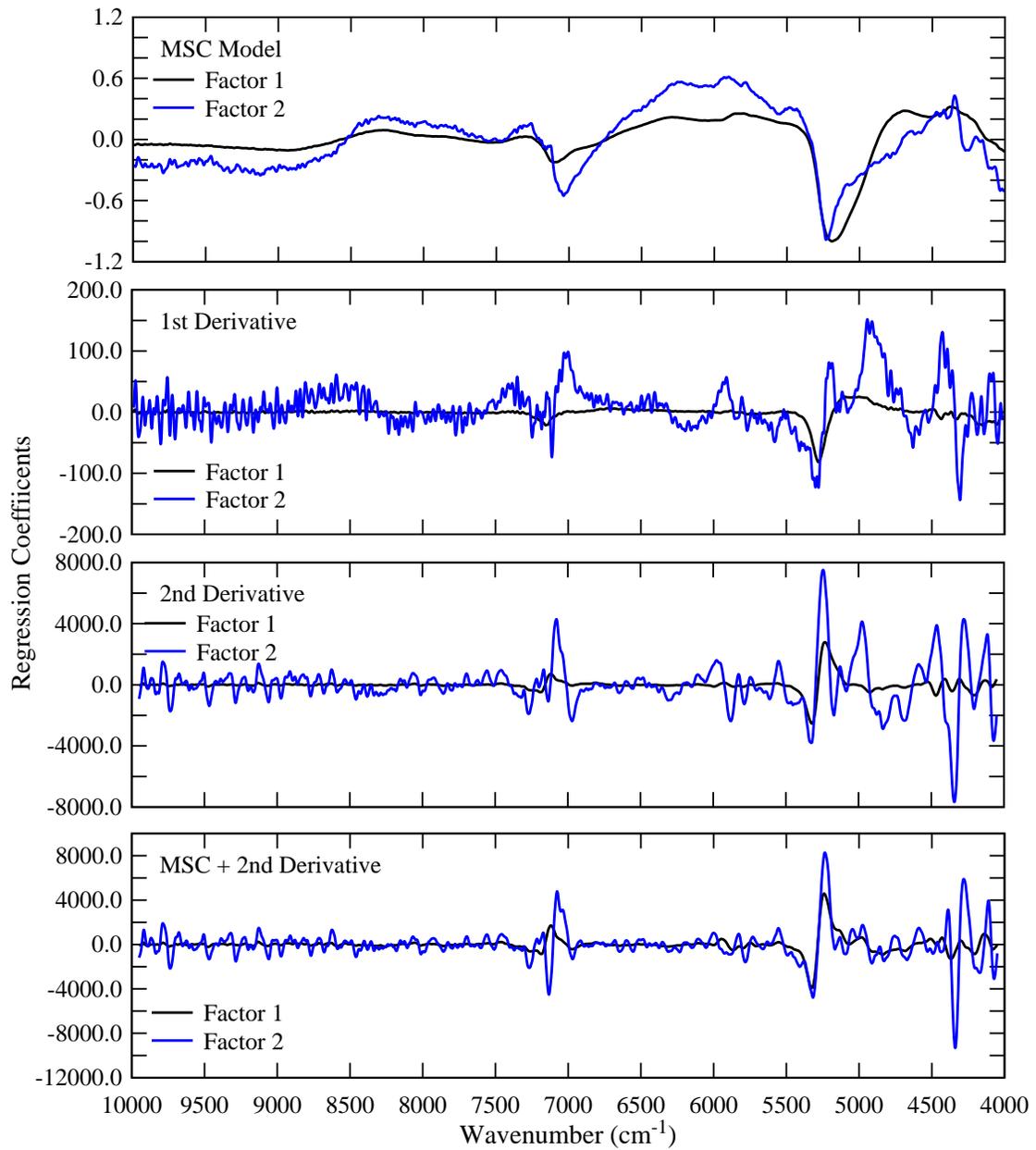


Figure C.6. Regression coefficients of the first two factors of the resulting PLS models for unreacted starch content in wet ground corn.

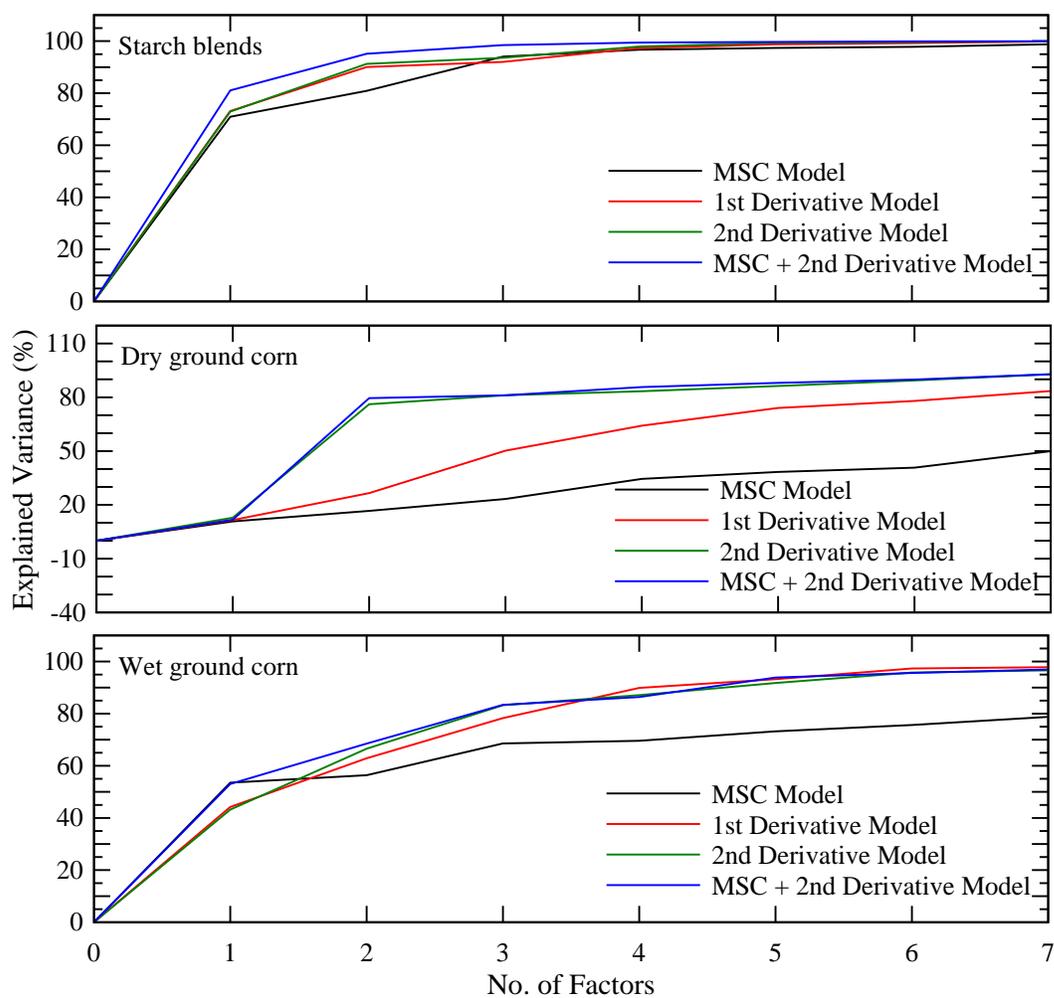


Figure C.7. Cumulative variance explained by the different factors of the resulting PLS models (MSC, black; SG 1st derivative, red; SG 2nd derivative, green; combined MSC and SG 2nd derivative, blue).

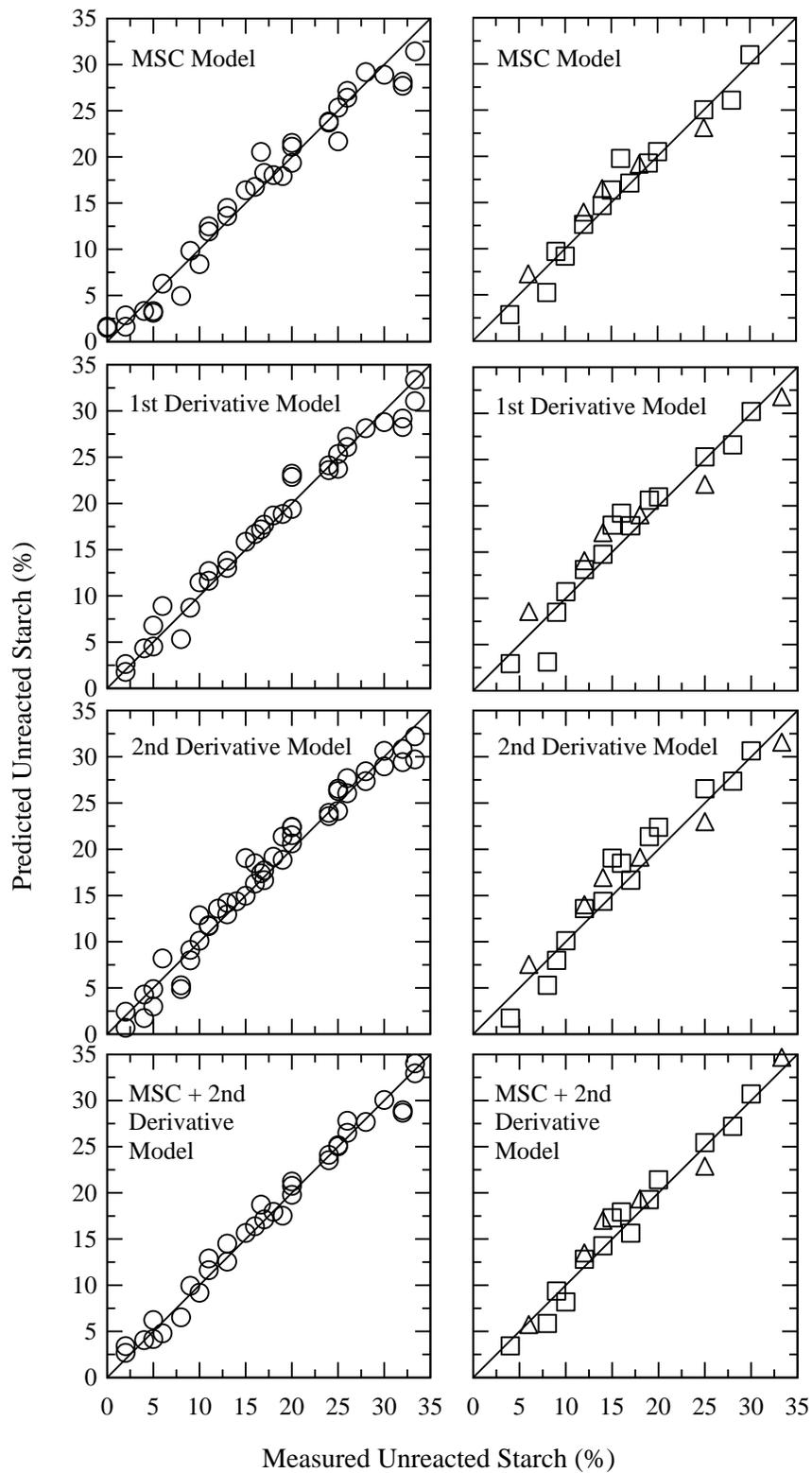


Figure C.8. Comparison of predicted to measured unreacted starch content using calibration (○), validation (□), and test (△) sets of starch blend samples.

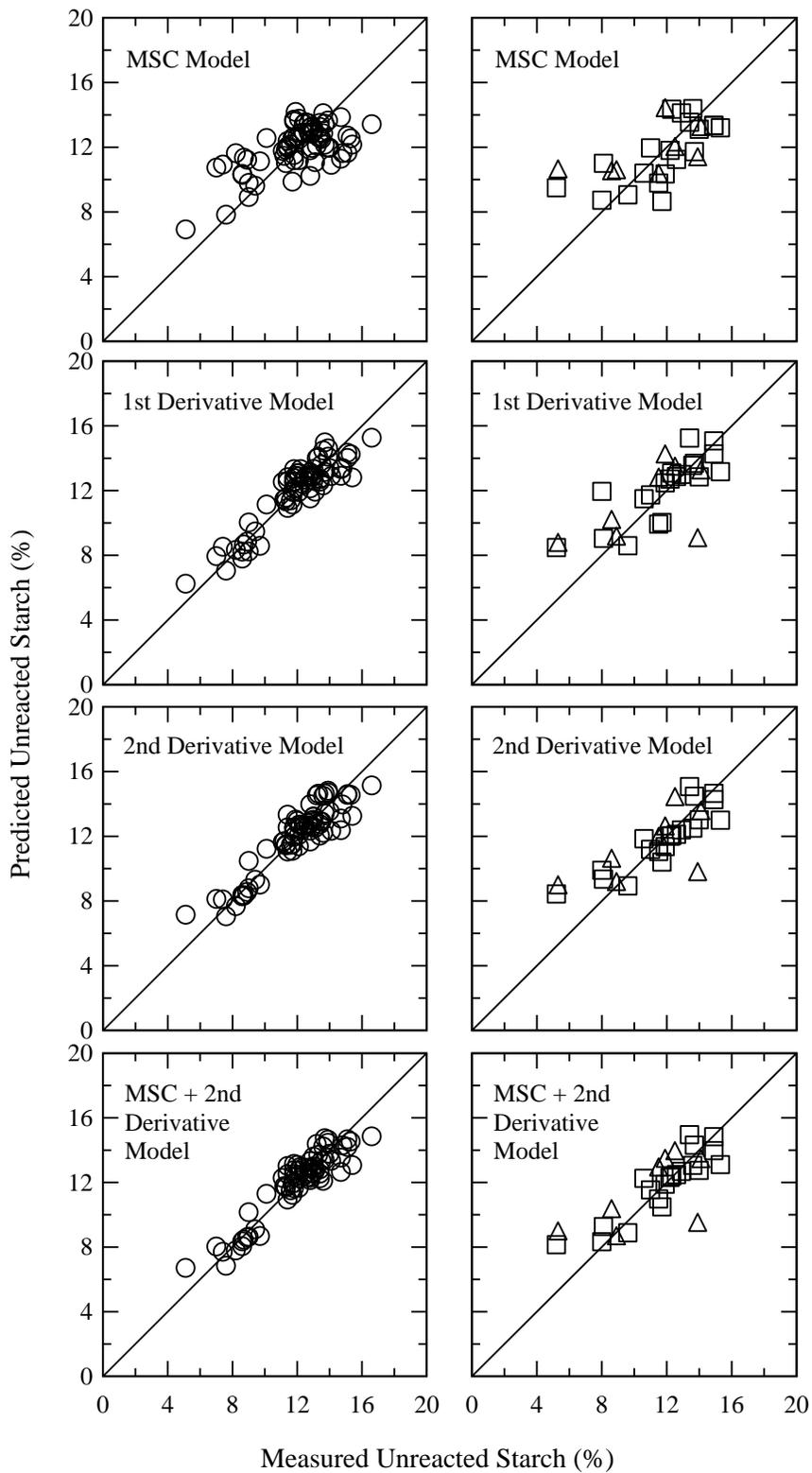


Figure C.9. Comparison of predicted to measured unreacted starch content using calibration (○), validation (□), and test (△) sets of dry ground corn samples.

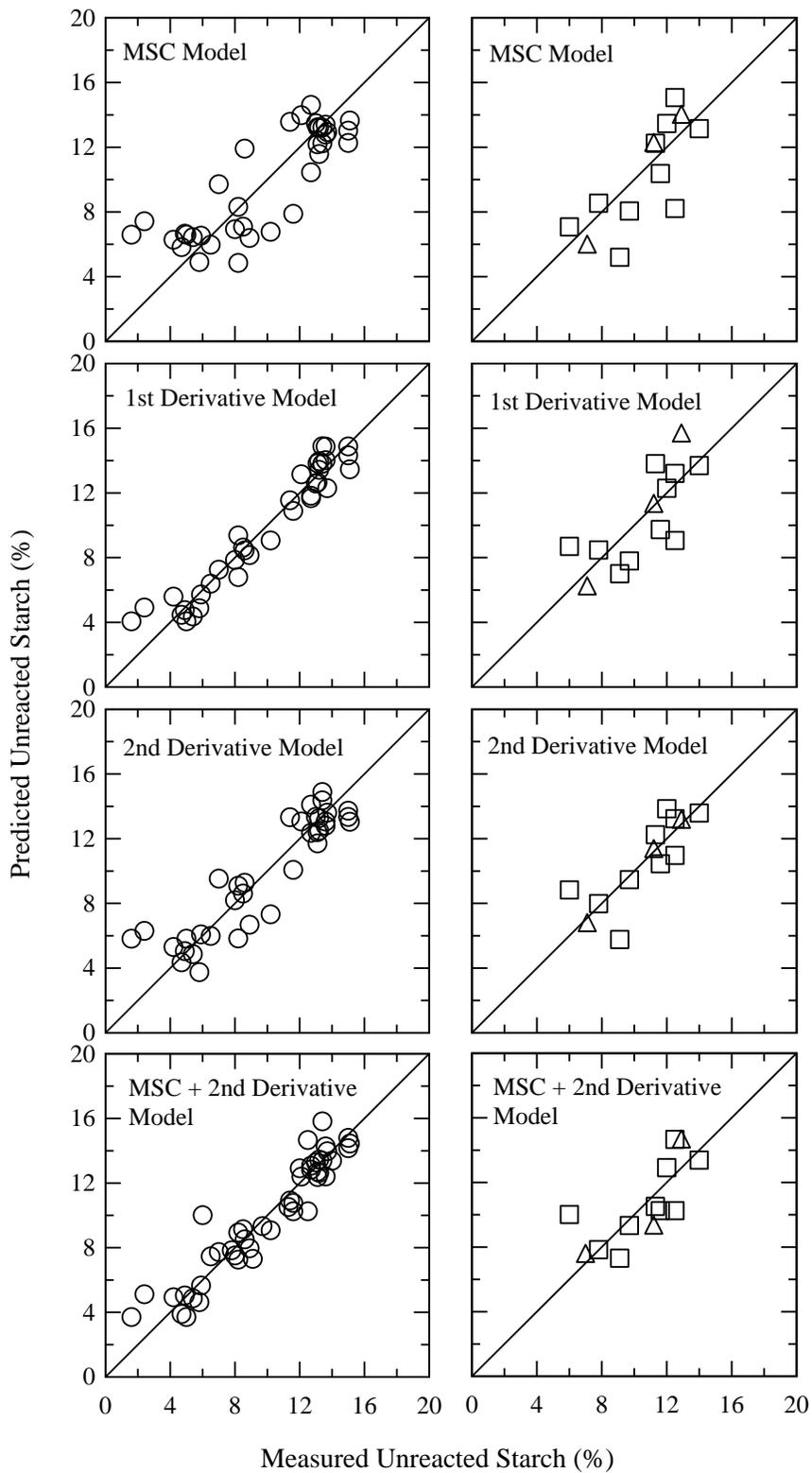


Figure C.10. Comparison of predicted to measured unreacted starch content using calibration (○), validation (□), and test (△) sets of wet ground corn samples.