RAPID ANALYSIS OF CHEDDAR CHEESE AND NONFAT DRY MILK USING MID INFRARED SPECTROSCOPY

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by

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ABSTRACT

The first objective of this work was to develop methods for the creation of liquid Cheddar cheese calibration standards and evaluate the calibration set using a combination MIR transmittance analysis and an in-line conductivity sensor within the MIR milk analyzer for measuring fat, protein, moisture, and salt content. Annatto colored cheese samples were collected from one cheese factory. Each cheese sample was analyzed using reference chemistry and then blended into a particle free dispersion to run through the FT-MIR. The remaining cheese was combined and used to create an 8-sample calibration set with decreasing amounts of fat, protein, total solids, and salt. Validation samples were also run using the new calibration model. Standard error of prediction (SEP) values for fat, moisture, and salt of the validation samples on the MIR produced SEP values for fat A+B, moisture, and salt of 0.177, 0.176, and 0.022, respectively. These low SEP values indicate that MIR could be used as an effective alternative to cheese producers for the rapid analysis of cheese using equipment most dairy processors already have in their facility.

Our second objective was to develop and evaluate the performance of the rapid determination of fat, protein, lactose, and moisture of nonfat dry milk powders using mid-infrared transmittance analysis. Nonfat dry milk powders were reconstituted and analyzed using reference chemistry and the FT-MIR to create a calibration model. Validation was done at a separate time using the same nonfat dry milk powders. The SEP values for the validation samples were low. Moisture, fat A, protein, and lactose had SEP values of 0.292, 0.023, 0.110, and 0.334, respectively. This indicates that MIR could be used as an easy and effective way for NFDM producers to measure the components of their product.

BIOGRAPHICAL SKETCH

Emmalee Peterson was born and raised on a family farm in Lowell, MI where she grew up raising and showing livestock at the county fair. Upon graduation from Lowell High School in 2014, she began her undergraduate studies at Michigan State University while serving as a Michigan FFA State Officer. Emma studied Food Science and worked in a variety of different positions while at Michigan State University. She helped her peers as an undergraduate learning assistant for the dairy processing course and the food chemistry course, and she conducted an independent research project on protein functionality of a2 milk. While at Michigan State, Emma also continued to serve the Michigan FFA after her time as a state officer by working in the FFA office on campus and serving as the Audio and Video Director for the Michigan FFA State Convention.

Emma's passion for dairy foods led her to pursue internships at two dairy processing companies during her tenure at MSU. In 2016 she interned in Quality Assurance with Glanbia Nutritionals at their plant in Gooding, ID. In 2017, she interned in Research & Development with Schreiber Foods at their headquarters in Green Bay, WI.

With the skills and knowledge gained during her graduate studies, Emma began working as an Academic Specialist at Michigan State University, specifically acting as the Food Processing, Technology, and Safety Coordinator for a new program in the Institute of Agricultural Technology. While in this role, she has increased student enrollment in the program and developed many new courses related to food science, processing, and safety.

For my husband, Ben, who never lets me give up, and my parents, Gary and Laurie, who provide endless encouragement to pursue my goals and dreams, no matter how crazy.

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LIST OF ABBREVIATIONS

AOAC…….....Association of Official Analytical Chemists

FDB.....Fat dry basis

FTIR.....Fourier transform infrared spectroscopy

MD.....Mean difference

MIR.....Mid-infrared

NIR.....Near-infrared

PLS.....Partial least squares

SDD.....Standard deviation of the difference

SEP.....Standard error of prediction

SMEDP.....Standard methods for the evaluation of dairy products

SNF.....Solids not fat

TP.....True protein

CHAPTER ONE

ANALYSIS OF THE COMPOSITION OF CHEDDAR CHEESE AND DAIRY POWDERS

THE USE OF MID INFRARED SPECTROSCOPY IN THE DAIRY INDUSTRY

Milk Analysis and Payment Testing

Milk analysis in the United States has changed drastically through the years. Producers are compensated for their milk based on composition and as a result, accurate milk analysis results are important. Traditionally, chemical methods for analysis, like the Mojonnier ether extraction, Babcock, and Gerber methods, were the main options for determining the fat content of milk, while the Kjeldahl method was used to determine protein content of milk. While accurate, these methods are slow and inefficient for use in testing large quantities of milk samples. By the 1960s, Milko-testers (Shipe 1972) had replaced most chemical methods for determining fat due to their increased efficiency (Barbano and Clark 1989). At that time dye binding (Ashworth 1966; Sherbon 1978) was the only choice for rapid measurement of protein content of milk.

When the industry wanted more milk composition information, like protein, lactose, and solids-not-fat, quantitative mid-infrared (**MIR**) milk analysis was developed to meet this need (Barbano and Clark 1989). Along with being able to measure more components, infrared analysis offers a rapid and more cost-effective

method for milk analysis. When infrared first entered the field, industry and regulatory agencies did not have a firm understanding of it. To ensure high quality milk testing in the United States and to develop testing procedures, the federal milk marketing orders established a committee in 1984 (Barbano and Clark 1989). The increasing scale of production in the dairy industry led to the development and improvement of faster methods of analysis. As MIR analysis methods improved, milk payment shifted from being based on weight and fat content to being based on the weight of each of the components including fat, protein, and solids (Barbano and Lynch 2006).

The large scale of milk production in the United States increases the importance of milk payment testing. These results dictate the flow of large quantities of cash between processors and producers. Even slightly inaccurate compositional results could mean a difference of millions of dollars in milk payment transactions across the country. Today, almost all milk payment testing and herd improvement testing is done by infrared making accurate and consistent results important in economic and business decision making.

Mechanics of Mid Infrared (MIR) Analysis and Calibration

Mid infrared is a rapid secondary testing method. Accurate reference chemistry is the basis for calibration of instruments that are secondary testing methods. MIR instruments measure infrared light absorbance at frequencies within the mid infrared spectra that represent chemical bonds (Barbano and Lynch 2006) in structure of major milk components. In the first generation of infrared milk analyzers, optical filters were used to select bands of MIR light wavelengths that were useful for measurement of fat, protein and lactose content of milk. The filters were called Fat B, Fat A, Lactose, and

Protein in which the Fat A filter corresponds to the carbonyl group stretch, the Fat B filter corresponds to the carbon hydrogen stretch, the protein filter corresponds to the amide group stretch, and the lactose filter corresponds to the hydroxyl group stretch (Lynch et al. 2006). Filter based MIR instruments use pairs of optical filters, a sample and a reference at each of the filters listed above, to select a band of wavelengths for measurement of fat, protein, and lactose (Barbano and Lynch 2006). There were a total of 4 pairs of filters in the optical filter based MIR milk analyzers. Once Fourier transform MIR optical systems using an interferometer became available and were cost effective, the Fourier transform (FT) systems that produce a full spectra rapidly replaced the optical filter based instruments. The FT instruments that produced a full spectra on every sample created the opportunity to make "virtual" digital filters in the instrument software. These virtual filters provided an opportunity to fine tune center wavelength and band pass of the traditional MIR milk analysis wavelengths that were limited by the process of making the optical filters.

The optimized basic filter model wavelengths for fat A, fat B, protein, and lactose as reported by Kaylegian et al. (2009) are shown in Table 1.1. These reference filters account for the impact of water absorption and light scattering (Lynch et al. 2006). Because water is in high concentration in milk and absorbs light strongly in the MIR spectra, it has a large impact on the infrared absorption by a milk sample. Total solids measurements are estimated using a calculation based on the values for fat, protein, and lactose along with a slope and intercept adjustment made based on the reference values for total solids (Barbano and Lynch 2006).

Table 1.1 Optimized Virtual Filter Sample and Reference Wavelengths for MIR Basic Filter Models from Kaylegian et al. 2009

Component	*Sample Wavelength (cm^{-1})*	*Sample Wavelength Bandwidth (cm^{-1})*	*Reference Wavelength (cm^{-1})*	*Reference Wavelength Bandwidth (cm^{-1})*
Fat B	2,851	26	2,812	24
Lactose	1,048	20	1,293	14
Protein	1,541	20	1,491	12
Fat A	1,748	16	1,791	16

These filters form the foundation that the calibration of the MIR instrument is built on for measurement of fat, protein, and lactose. Calibration is done using a set of samples with known concentrations of each of the components being measured. These known concentrations of the main components must be established using official reference methods. Each component is calibrated using a linear regression analysis in which a slope and intercept adjustment is made to establish a corrected instrument signal (Lynch et al. 2006).

Methods for the Chemical Analysis of Milk

The current reference method for the determination of fat in milk is the Mojonnier ether extraction method (AOAC International 2012 method 989.05, SMEDP method 15.086). While specific changes have been implemented to improve the accuracy of the traditional method for fat determination of milk, the Babcock method, the Mojonnier ether extraction method has been found to be more precise than the Babcock method and can achieve better within lab repeatability and between lab reproducibility (Hooi et al. 2004). The Mojonnier method uses a combination of diethyl and petroleum ethers is used to extract the fat from a known weight of milk in

3 extractions. The 3 ether extracts of the milk are decanted into dried, pre-weighed pans and the ether is evaporated in a hood after which the pans are dried to a constant weight in an oven, cooled, and then weighed (AOAC International 2012 method 989.05, Hooi et al. 2004). Wojciechowski et al. (2016), analyzed the proficiency of this method of analysis. They analyzed the uncertainty of reference values determined using Mojonnier ether extraction. The ether extraction method is also applicable to other dairy products, but the method performance on other dairy product matrices have not been evaluated using collaborative studies (Hooi et al. 2004).

Currently, Kjeldahl true protein analysis is used as the reference method for measuring protein content of milk in the US (AOAC International 2012 method 991.22, SMEDP method 15.133). True protein was adopted as the reference method for milk protein analysis in the USDA Federal Milk Markets in 2000 (USDA 1999). Prior to this, protein content of raw milk was measured using the Kjeldahl total nitrogen method. In 1991 Barbano et. al determined that the Kjeldahl total nitrogen method overestimates true protein in milk by about 6%. This overestimation is due to the non-protein nitrogen present in milk which is not accounted for when using the Kjeldahl total nitrogen method. Therefore, Kjeldahl true protein provides a more accurate result. Kjeldahl true protein analysis is based on the precipitation of protein from a milk sample using 12 % trichloroacetic acid (TCA) followed by filtration to separate the protein precipitate (Hooi et al. 2004). Then the nitrogen content of the precipitate, which contains the protein nitrogen contents of the sample, is determined through digestion to convert organic nitrogen into ammonium sulfate (Hooi et al. 2004). Sodium hydroxide is added to free the ammonia followed by distillation of the

ammonia into boric acid solution and then titration with hydrochloric acid (Hooi et al. 2004). The amount of ammonia present is used to determine the nitrogen content (Hooi et al. 2004).

To measure total solids, the forced air oven drying method is the accepted reference method for milk (AOAC International 2012 method 990.20, SMEDP method 15.114). In this method, a known amount of sample is dried at 100°C for 4 hours (Hooi et al. 2004). The sample should be warmed in a water bath to 38°C ± 1C and inverted to ensure sample is mixed thoroughly (Hooi et al. 2004). While this seems like a straightforward method, there are important factors that can impact the accuracy of the results. Temperature variability of the oven plays an important role in the final result, so an oven used for determination of total solids of milk should not vary in temperature more than ± 2°C (Hooi et al. 2004).

There are two accepted chemical methods for the determination of lactose. The classical method is polarimetry, but spectrophotometric based enzymatic and HPLC methods are more commonly used (Barbano and Lynch 2006). When using the enzymatic method, the lactose in milk is hydrolyzed into glucose and galactose using B-galactosidase and water after which the B-galactose present is oxidized into galactonic acid by NAD while in the presence of B-galactose dehydrogenase (Hooi et al. 2004). This forms NADH which is proportional to the amount of lactose present. The amount of NADH is measured by absorbance at 340 nm (Hooi et al 2004). To get the final lactose results, the difference of a lactose plus galactose assay and a galactose assay must be taken (Hooi et al. 2004).

Factors that Impact Mid Infrared Instrument Accuracy

MIR instrument accuracy is dependent on many factors because it is an indirect method of analysis. Results from MIR instruments can be impacted by the instrument itself and by issues with the information and samples used during calibration. There are many mechanical and electronic aspects of the instrument that impact accuracy. These instrument factors include signal to noise ratio, repeatability, linearity, gain, homogenization efficiency, purging efficiency, and intercorrection response (Kaylegian et al. 2006b). Specific information on these elements can be found in the next section on precalibration and calibration of the MIR.

The other elements that impact accuracy are analytical factors, milk sample composition factors, and calibration set characteristics (Kaylegian et al. 2006a). The main analytical factor that can decrease accuracy is the analytical uncertainty in the chemical reference values (Kaylegian et al. 2006b). As a secondary testing method, MIR instruments are dependent on calibration using accurate chemical reference values. Any analytical uncertainty in the reference chemistry will translate into analytical uncertainty in the final MIR result for unknown samples. Milk sample composition also impacts MIR accuracy. Variation in fatty acid chain length and degree of unsaturation, variation in non-protein nitrogen as a percentage of total nitrogen, and the citrate and free fatty acid content from sample-to-sample are all elements of milk composition that can influence instrument accuracy (Kaylegian et al. 2006b).

The final element that plays a role in MIR accuracy is the composition of the milk set used to calibrate the instrument. The number of samples, the range of

concentration of the components, the distribution of the samples within the range, and the correlation between fat and protein content are characteristics that influence calibration performance (Kaylegian et al. 2006a). It is important to calibrate the instrument with a large enough number of samples and to ensure that the sample composition range is representative of the samples that will be tested. While these calibration sets can be created by individual facilities, the use of modified milk calibration sample sets, created in a central location and shipped out to multiple facilities, is common for calibration of MIR instruments.

Kaylegian et al. (2006b) studied the difference in calibration performance between producer (i.e., individual farm) calibration sets versus modified calibration sets made in a central location. Producer calibration sets are made from individual farm raw milks selected to get a range of fat, protein, and lactose. Modified calibration sets are made in a central location in which one bulk tank farm milk is separated into cream, skim milk, and ultrafiltration retentate and permeate and then combined with some water and pure lactose to produce a set of 14 samples designed to form an oorthogonal matrix of fat, protein, and lactose concentrations..Kaylegian et al. (2006b) found that the modified calibration sets had a larger range of the components, a more even distribution of component concentration, and lower correlation of fat and protein which created smaller 95% confidence interval around the linear regression calibration line. Even spaced distribution of the concentration of components decreases the influence that single samples have on the calibration, i.e. avoiding high leverage samples (Kaylegian et al. 2006b). High leverage samples can increase variation of the slope and intercept values in a way that does not represent the desired component

range. Kaylegian et al. (2006b) also found that producer calibration sets had a positive correlation between fat and protein. This is not a good characteristic because when creating the regression, there is an assumption of independence of all terms in the regression equation. Many other factors influence MIR accuracy including instrument factors, analytical factors, and the composition of the calibration set.

Precalibration and Calibration of MIR Instruments

Precalibration. Before a MIR instrument can be used to accurately provide rapid results, the instrument itself needs to be checked to ensure mechanical and electrical soundness. Precalibration assures that an instrument is in good working order and that the instrument readings prior to being calibrated are stable and optimized (Lynch et al. 2006). When performing a precalibration, the readings used from each of the component channels must be from uncorrected signals (i.e., slope 1, intercept 0, and all intercorrection factors set at zero), and after precalibration, the intercorrection factors can be established (Lynch et al. 2006). Intercorrection factors are an important component in ensuring instrument accuracy. Each component not only absorbs infrared energy at their primary wavelength, but also absorb infrared energy at the primary wavelengths of the other components to a lesser extent (Lynch et al. 2006). Intercorrection factors account for this absorbance at the other wavelengths when determining the final measurement of the primary component. After intercorrection factors are determined, the uncorrected signal used during precalibration is changed to the intercorrected signal (Lynch et al. 2006).

Precalibration serves a valuable purpose in the maintenance and upkeep of MIR instruments. Routine performance of precalibration procedures provides

continuous documentation of the performance of the instrument along with a record of any adjustments made to the instrument. It also helps in determining the cause of issues with the machine. There are a few factors that play a key role in the effectiveness of the precalibration. First, all samples and zeroing solutions need to be at 41°C +- 1°C because the homogenizer efficiency is influenced by sample temp (Lynch et al. 2006). It is also important that the milk used is high quality and well mixed before being measured (Lynch et al. 2006). Samples must be mixed thoroughly in order to get a representative portion in each reading the instrument performs. Poorly mixed samples will show poor repeatability. These elements of the process are important in identifying and solving problems as temperature deviations and poor quality samples may lead to issues being attributed to the instrument instead of to the poor sample handling.

Precalibration involves a multi-step procedure designed to check the mechanical and electronic soundness of the instrument. The first step in precalibration is to check the flow system. This involves evaluating the temperature of the homogenizer which should be between 38°C-42°C (Lynch et al. 2006). Next homogenization efficiency is evaluated. Large fat globules impact the accuracy and repeatability of the machine. This step ensures that fat globules are being reduced to a small enough size to minimize the extent of light scattering (Lynch et al. 2006). If fat globules are not reduced enough in size, then they scatter light and cause poor repeatability and analytical error due to spectral effects caused by the large fat globules (Lynch et. al. 2006). Homogenizers degrade over time and need to be replaced periodically. This check allows instrument operators to track homogenizer

performance and determine when a replacement is necessary. Homogenizer performance is checked by collecting milk that has been homogenized by the instrument and measuring particle size of the milk after homogenization. The d(0.9) of the particle size distribution of fat droplets should be <1.7 microns. When the d(0.9) exceeds 1.7 microns (Lynch et al 2006, DeMarzo et al. 2016), the homogenizer should be replaced. Recently, DeMarzo and Barbano (2016) develop a partial least squares (PLS) prediction model that allow the MIR instrument to predict the d(0.9) of every sample that is run through the instrument. This tool provides immediate feed back to the operator about how well the homogenizer is working.

After that, water repeatability checks are conducted. This is an evaluation of the repeatability of the zeroing solution. It allows for a check on the mechanical and electronic aspects of the instrument as poor repeatability of zeroing solution indicates that the results from the instrument are unreliable (Lynch et al. 2006). The next step is the zero shift check which determines if the instrument's cuvette windows are deteriorating and accumulating deposits on the windows and therefore impacting accuracy. Cuvette deterioration shows as a shift in the readings, seen in protein channels before the other channels, that occurs because of the accumulation of milk solids on the windows (Lynch et al. 2006). After that, the linearity of the instrument is evaluated. Linearity checks show whether linear changes seen in the concentration of the components are also seen in the uncorrected signals. This is important because nonlinearity will negatively impact the standard deviation of difference (SDD) between the MIR results and the reference chemistry results (Lynch et al. 2006). Ultimately, nonlinearity leads to errors in determining the intercorrection factors.

Next in the precalibration process is the primary slope step. This evaluates how responsive changes in the uncorrected signals are to changes in the concentration of fat, protein, and lactose (Lynch et al. 2006). Ideally this relationship would be completely equal meaning that any changes in the concentration of the components are also seen in the uncorrected signal, giving a primary slope of 1. As the primary slope varies above or below 1, corrections must be made by the intercorrection factors and secondary slope and intercept (Lynch et al. 2006). This is not ideal because the purpose of intercorrection factors is to correct for the absorbance of the other components, not the component that is being measured. Primary slope is impacted by cuvette path length, light intensity, and linearity of the uncorrected signal (Lynch et al. 2006). As the cuvette path length increases over time due to window erosion, the primary slope will reflect this change and increase for all of the components. Maintaining a stable calibration and ensuring agreement with reference chemistry are very important to instrument accuracy. Therefore managing primary slope and ensuring a one to one relationship between uncorrected measurements on fat A, fat B, protein and lactose and the reference chemistry are the most important factors influencing machine accuracy.

After that, milk repeatability is evaluated. It is important that this step follows linearity and primary slope, as poorly optimized primary slop settings can easily cause repeatability issues (Lynch et al. 2006). The final step before the creation of the intercorrection factors is to check purging efficiency. Purging efficiency evaluates the amount of carryover from one sample to the next, as described by Lynch et al. 2006. Problems with purging efficiency leads to inaccurate results and poor repeatability as

samples are contaminated by the prior samples that were run on the instrument. Finally, the intercorrection factors are determined. These intercorrection factors allow the instrument to compensate for the influence of water displacement and the absorbance of other components at the wavelength of the component of interest (Lynch et al. 2006). Prior to establishing intercorrection factors, linearity and primary slope of the must be set. This is critical because the setting of intercorrection factors is a function of linearity, primary slope, and the wavelength and bandwidth of the sample and reference wavelengths (Lynch et al. 2006). For each component channel, there are separate intercorrection factors for each of the other components (Lynch et al. 2006). For example, in the protein channel, there are factors applied to compensate for the impact of sample-to-sample variation of background lactose on protein and for the impact of sample-to-sample variation of background fat on protein. This same pattern is true for all of the other channels. Unless major hardware changes are made to the instrument, the primary slope, or linearity, the intercorrection factors should remain fconstant. All of the steps described play an integral role in ensuring the mechanical and electrical reliability and health of the instrument.

The above discussion of primary slope and intercorrection factors applies to basic filter pairs for measuring the main components using tradition filter wavelengths. When using PLS models for measuring other characteristic of milk composition (e.g. MUN, fatty acids, particle size, etc.) the primary slope and intercorrection factors are embedded in the PLS model and cannot be adjusted by the user.

Calibration. Following precalibration, once the instrument has shown that it is in good working order, calibration of the instrument can take place. Calibration of MIR instruments is done using linear regression to establish a secondary slope and intercept that convert the intercorrected MIR signal for each major component to a corrected signal (Lynch et al. 2006). This is done using a calibration set composed of samples with known composition, determined using validated reference chemistry methods. These samples provide a range of values for each component that cover the expected range that samples tested in the product group would fall into.

Kaylegian et al. (2006a) determined that formulated modified calibration milk sets made in a central location performed better than locally made producer milk calibration sets. Modified calibration sets are made in a central location in which the milk is broken down into its various components (cream, whey, skim milk, etc) and then combined back together into samples with varying amounts of the components of interest (fat, protein, lactose). These sets can then be shipped out to laboratories to be used to calibrate any MIR instrument. Being able to purchase pre-made calibration sets is an easy and cost-effective way for laboratories to develop an accurate and robust calibration on their MIR instrument. These sets provide better calibration performance on their instrument when compared to a laboratory building their own local calibration set samples. Once an instrument has undergone precalibration and calibration procedures, it is optimized for the rapid analysis of liquid milk products. Everything in the discussion above about precalibration and calibration procedures for basic filter models applies to measurement of the composition of non-milk samples (e.g., whey, whey protein concentrate, creams, cheeses, etc.) by MIR.

IMPORTANCE OF THE ANALYSIS OF CHEDDAR CHEESE BY PROCESSING FACILITIES

Cheddar Cheese Standard of Identity

In dairy foods processing, the Food and Drug Administration (FDA) sets a standard of identity for the composition and processing steps necessary for a product to fall into a specific category. This means that in order for a product to legally be labeled and considered Cheddar cheese, there are specific processing and composition requirements that need to be met. According to the Code of Federal Regulations (CFR) Title 21, Cheddar cheese must have a minimum fat content of 50% with a moisture no higher than 39% (FDA 2018a). The CFR Title 21 also describes what ingredients can be used in Cheddar cheese manufacturing including annatto coloring, rennet and calcium chloride, among other ingredients (FDA 2018a). The FDA also sets requirements for pasteurization and aging. The dairy products used for the manufacturing of Cheddar cheese need to be pasteurized, and if they are not pasteurized the cheese must be aged at no less than 35°C for 60 days (FDA 2018a).

Standards are also in place for Cheddar cheese for manufacturing. This product differs from Cheddar cheese in that it is intended to be used in the production of a different cheese product like process cheese. As Cheddar cheese for manufacturing will undergo further processing and heat treatments, there are slight differences in the standard of identity. Cheddar cheese for manufacturing does not need to be made using pasteurized dairy ingredients or undergo the 60-day aging period (FDA 2018b).

These regulations are one of the main reasons that production facilities need the ability to rapidly analyze cheese samples. Moisture, fat, and other composition results are used to ensure a product is meeting the necessary standards. If the composition is found to be out of specifications, then facilities are able to make in process corrections and adjustments to prevent large quantities of product from not meeting all of the necessary standards. If a final product is found to not meet the necessary standard of identities it risks having to be thrown out or downgraded resulting in large amounts of food waste and a loss of profits. Cheese sample composition results need to be accurate, so that individuals have the necessary information to make sound decisions and have the ability to make in process adjustments in order to prevent food waste and profit loss.

Impact of Chemical Composition on Cheddar Cheese Quality and Yield

Chemical Composition and Cheese Yield. Analysis of cheese, whey, and milk is an important for controlling the cheese making process in manufacturing plants. Plants rely on this data to make processing and management decisions and therefore need accurate and reliable results in order to make sound decisions. Performing reference chemistry on all the necessary cheese samples in a large cheese plant, while accurate, is not a feasible option due to the large amount of time needed to perform the tests as well as the need for highly skilled workers to get accurate results. However, cheese plant management needs these compositional results in order to operate efficiently.

The efficiency of cheese production is impacted by many factors, however one of the most important factors is the quality and composition of the milk. Milk

composition has a direct impact on cheese yield. When milk has higher fat and casein content, there is more fat and casein available to incorporate into the cheese structure (Barbano et al. 1991). This means that the seasonal and regional variation in milk composition (Barbano 1990) has a large impact on cheese yield.

Accurate milk composition and weight measurements play an important role in manufacturing Cheddar cheese. A system to track cheese yield and fat recovery in Cheddar and mozzarella cheese plants and to measure the current performance of composition analysis in these factories was developed by Margolies et al. (2017). The traditional formula used to predict the theoretical yield of Cheddar cheese is the Van Slyke formula (Van Slyke and Publow 1909) which predicts cheese yield based on fat and casein content of the milk used at any given moisture value. Figure 1.1 below shows the Van Slyke formula for the prediction of the theoretical yield of Cheddar cheese.

$$\text{Yield} = \frac{\left[\left(\text{milk fat } \% \times 0.93\right) + \left(\text{milk casein } \% - 0.1\right) \times 1.09\right]}{1 - \left(\text{target cheese moisture } / 100\right)},$$

Figure 1.1 Van Slyke formula for theoretical Cheddar cheese yield (reproduced from Maroglies et al. 2017)

The Van Slyke formula has given accurate predictions of the theoretical yield of Cheddar cheese (Barbano and Rasmussen 1992), however the 1.09 value in the formula does not provide accurate yield predictions for low fat Cheddar cheese or other types of cheeses when fortified milk is used (Margolies et al. 2017). To predict yield for various cheese types, target compositions, and whey draining pH values, the Barbano theoretical yield formula is a more appropriate formula to use (Rudan et al.

1999, Metzger et al. 2000). This formula is shown below in Figure 1.2, and was the formula used by Margolies et al. (2017).

$$\text{Theoretical cheese yield} = (\text{A} + \text{B} + \text{C})/\{1 - [(\text{target cheese moisture} + \text{target cheese salt})/100]\},$$

where A is the milk fat recovered in the cheese:

$$\text{A} = [\%\ \text{fat in the milk} \times (\%\ \text{fat recovery in cheese}/100)];$$

B is the milk casein plus calcium phosphate recovered in the cheese:

$$\text{B} = [(\%\ \text{casein in the milk} - 0.1) \times \text{calcium phosphate retention factor}];$$

and C is the other milk solids recovered in the cheese (i.e., nonfat, noncasein, non-calcium phosphate–milk solids):

$$\text{C} = \{[(\text{A} + \text{B})/(1 - \text{actual cheese moisture}\ \%/100)] - (\text{A} + \text{B}) \times (\text{separated whey solids}\ \%/100)\} \times (\text{solute exclusion factor}).$$

Figure 1.2 The Barbano theoretical cheese yield formula (reproduced from Margolies et al. 2017)

Unlike the Van Slyke formula the Barbano theoretical yield formula uses the parameters A, B, and C. These parameters allow the formula to predict the theoretical

yield for any type of cheese using the composition of the milk. Fat recovered in the cheese is represented by A, casein and calcium phosphate retention in the cheese is represented by B, and the retention of nonfat whey solids in the water phase of the cheese is represented by C (Margolies et al. 2017). The formula also allows for the target cheese salt content and moisture content to be specified (Margolies et al. 2017). The Barbano formula, however, is only as accurate as the accuracy of the milk composition data and separated whey solids data. Without accurate compositional results, the theoretical yield will not be accurate.

Milk composition differences and uncertainty in milk weight influences cheese quality and the overall profitability of the cheese plant. Margolies et al 2017 reported a ± 0.1% fat or protein change of the milk in a vat causes a range of theoretical cheddar cheese yield from 10.05% to 10.37% with an uncertainty of the efficiency of cheese yield of ± 1.5%. This translates to ± 1,451 kg of cheese per day in a plant processing 907,185 kg of milk per day (Margolies et al. 2017). While 0.1% may seem like a miniscule variation in composition, the magnified impact of this variation has a large influence on revenue and on management's ability to make sound and accurate processing decisions.

Uncertainty in analyzing the composition of the final cheese product also impacts revenue. Being low 0.5% on moisture or fat of cheese translates to 484 kg of missed profits from cheese production for one day in a plant processing 907,185 kg of milk per day (Margolies et al. 2017). This uncertainty, on top of influencing profitability, also impacts other cheese composition analysis. Inaccurate moisture results causes the calculations for measurements like fat on dry basis and salt in

moisture to be off as well. Variation of salt in the moisture content of the cheese will cause variation of cheese flavor development during aging and cheese quality.

Accurate performance in quality assurance laboratories plays a vital role in ensuring that profitability is at its optimum level and management has accurate data to make decisions with. Margolies et all (2017) found a 0.5% bias in the analysis of fat and moisture across cheese factories. Uncertainty at this level leads to huge changes in cheese yield and revenue. While reference chemistry methods are accurate, they are very time consuming and not practical for large cheese plants to use in measuring in process cheese samples. Finding a more accurate rapid method for analyzing cheese is important in improving cheese yield and the consistency of cheese from vat to vat.

Impact of Cheese Moisture and Salt on Cheese Quality. Salt and moisture content of cheese have a large influence on the quality of the cheese. Salt plays multiple roles in the final characteristics of Cheddar cheese. Historically salt was used as a food preservative, and this role is still important today in cheese manufacturing. Along with other factors including water activity and pH, salt helps to prevent spoilage and the growth of harmful pathogens (Farkye and Guinee 2017). Salt also has a large impact on sensory characteristics. The typical range of salt in the moisture content of Cheddar cheese is about 4.5 to 6%. When salt concentration in the moisture phase of cheese is too low, starter culture growth during cheese cooling after salting may produce cheese that is too low in pH resulting in pasty texture, allow growth of undesirable gas producing bacteria in the cheese may causing texture defects, and a change in the specificity of chymosin for degradation of casein shifts from αs_1- casein to β-casein resulting in an increase in bitter off-flavors. The flavor of cheese and our

perception of flavor is influenced by the presence of salt. Reducing salt in cheese leads to an insipid and watery tasting product (Farkye and Guinee 2017).

Along with the dietary and sensory roles that salt plays in cheese, it also impacts cheese quality during the ripening process. The addition of salt helps to regulate the pH of cheese which has an impact on cheese ripening and texture (Farkye and Guinee 2017). Cheddar cheese curd has approximately 0.6-1% (w/w) lactose at the time of hooping (Farkye and Guinee 2017). This lactose is fermented during the beginning of ripening by the starter culture which is influenced by the ratio of salt to moisture and the level of salt tolerance of the starter culture (Farkye and Guinee 2017). In 1974, O'Connor determined that the pH of the cheese decreased due to the starter culture activity when amount of salt was <0.5%, but when the amount increased the starter culture activity decreased.

Upreti and Metzger (2007) determined that a low ratio of salt to moisture created a larger increase in acid production during ripening compared to a high ratio of salt to moisture. This translates to a low pH which has a large impact on cheese quality. A low pH (<5) leads to whey expulsing defects during ripening and an increase in proteolysis (Upreti and Metzger 2007). A lower pH also causes contraction of the protein matrix (Pastorino 2003). Altering the interactions between proteins in the cheese matrix influences the functionality and quality of the cheese. Salt content of cheddar cheese plays a role in the pH and acid production of the cheese during ripening. Starter culture activity and curd pH are important aspects of the early stages of ripening, so regulating salt levels is important in creating high quality cheese as salt levels has a large impact on both of these. This means that processors need fast,

yet accurate results on the salt and moisture content of cheese samples in order to make informed decisions to ensure proper cheese aging and in turn cheese quality.

Product consistency

Cheddar cheese compositional analysis and raw ingredient compositional analysis are important in ensuring product consistency from day to day and year to year. Milk is known to vary in composition across different seasons and different regions and areas of the country and world (Barbano 1990). The type of feed the animal received (Palmquist et al. 1993) and the stage of lactation (Lynch et al 1992) also impact the composition of the milk. At large manufacturing plants, all of the milk coming from different farms is typically combined to smooth out farm to farm variation (Lawrence et al. 2004) but seasonal variation is still a challenge. The overall fat content of the bulked milk can then be standardized to the same amount regardless of the time of year or farm to farm variations in milk. This standardization is typically done by separating the skim milk and cream portions and then recombining the portions back into the desired proportion. The typical casein to fat ratio targeted for Cheddar cheese is 0.67 to 0.72 (Lawrence et al. 2004). Today, ultrafiltration (or possibly microfiltration) can also be used to concentrate protein in skim milk and the UF skim milk retentate and can be combined to keep both constant concentration and ratio of casein to fat ratio in the milk for cheese making throughout the year (Papadatos et al. 2003). This consistency in milk ingredient going into the cheese vat will help reduce vat to vat variation in cheese composition. Analyzing incoming milk, standardizing it, and ensuring these results are accurate greatly impacts the consistency of Cheddar cheese because of the variation in milk composition.

Processing factors can also influence the consistency of Cheddar cheese composition. Starter activity uniformity from vat-to-vat and day-to-day is an important factor in creating a consistent Cheddar cheese. To achieve the required chemical composition of the cheese, the rate of acid development is key (Lawrence et al. 2004). Ensuring the proper amount of starter culture added and length of milk ripening time plays an important role in this acid development. The length of time Cheddar cheese curds spend on the salting belts also impacts product consistency. Spending too much time on the belts or too little time will impact the amount of salt in the curd. The relationship between salt and moisture in the cheese and its role in the ripening process and final cheese quality was discussed above.

Product consistency has a large influence on the functionality and quality of Cheddar cheese. In order for Cheddar cheese to perform adequately in secondary processing steps like slicing and shredding, the cheese needs to consistently meet chemical composition specifications. Differences in composition like moisture, salt, calcium and fat content, in addition to proteolysis during cheese aging, will impact how the cheese performs. Cheese that gums up slicing or shredding equipment will cause large issues for processors and can impact yield and revenue. Controlling all of the factors that impact Cheddar cheese composition like milk composition, starter culture activity, and salting are key to ensuring consistent product throughout the day and year.

Measurement of Chemical Composition of Cheese

Fat. Reference chemistry for cheese is usually done according to standards established by the Association of Analytical Chemists (AOAC) and Standard Methods for the Evaluation of Dairy Products (SMEDP). For fat, the reference methods for cheese are the Babcock method and the Mojonnier ether extraction method (SMEDP methods 15.083 and 15.086). The Mojonnier ether extraction method is widely accepted by the dairy industry as a more accurate reference method for fat as it has been shown to be more precise than the Babcock method (Hooi et al. 2004). Due to the relative simplicity of the Babcock method when compared to Mojonnier ether extraction, Babcock is more widely used as routine method in cheese plants however the calibration lines on a Babcock bottle are every 0.5% fat.

For analysis, cheese samples need to be ground or shredded into a smaller particle size. In Babcock analysis, the sample is mixed with concentrated sulfuric acid which results in an exothermic reaction to release the free fat (Hooi et al. 2004). This free fat is collected in the graduated neck of the bottle which gives the fat content of the tested product as a percentage of fat by mass (Hooi et al. 2004).

Mojonnier ether extraction is a gravimetric method of analysis. The basis of this method is the extraction of fat using a combination of ethyl ether and petroleum ether (Barbano et al. 1988, Hooi et al. 2004). The ether fraction is separated into a dry weighing pan, the ether is evaporated, and then the pans, with the fat residue, are dried

(Hooi et al. 2004). Fat content is determined as the difference between the pans before extraction and after drying, with the result being calculated to the nearest 0.01% fat.

As the Mojonnier method is best for milk analysis, when using it to analyze cheese samples, some precautions need to be taken to ensure accurate results. The main difference to account for is ensuring that the cheese is completely dissolved in order to ensure complete recovery of the fat from the cheese. There are a variety of options when running this analysis to ensure this happens including adding ammonium hydroxide, increasing shaking times, and adding 60°C water (Hooi et al. 2004). Amount of sample and fat in each test flask is also important in the accuracy of the test. The sample size should not exceed 10 g and should be determined based on yielding 0.3 to 0.6 g of extracted fat (Hooi et al. 2004). When analyzing products that contain stabilizers and emulsifiers, like processed cheese, it is important to account for these ingredients if they are soluble in ether (Hooi et al. 2004). These ingredients, if soluble in ether, can cause an overestimate of the fat content of the sample when using the Mojonnier method.

Protein. The determination of protein/nitrogen content of cheese is done using the Kjeldahl method (AOAC International 2012 method 920.123, SMEDP method 15.131). The principle of this method is the digestion of the sample using sulfuric acid and potassium sulfate with copper (II) sulfate as the catalyst to convert organic nitrogen into ammonium sulfate. The addition of sodium hydroxide to the cooled digest frees the ammonia which is distilled with boric acid solution and titrated with hydrochloric acid. The amount of ammonia produced is used to calculate the nitrogen (Hooi et al. 2004).

Moisture. For determination of moisture content of cheese, a forced air oven or vacuum oven should be used (AOAC International 2012 method 926.08, SMEDP method 15.114). Cheese samples should be ground into a smaller particle size before being heated. When using a forced air oven, cheese samples should be dried for 24 hours at 100°C (Hooi et al. 2004).

Salt. The Volhard method is the reference method used for the determination of salt in cheese samples (AOAC International 2012 method 935.43, SMEDP method 15.052). The principle of this method is the digestion of the sample with nitric acid and potassium permanganate in the presence of a known number of moles of silver nitrate (Hooi et al. 2004). Acid digestion frees the chloride from the sample and allows it to react with the silver to form AgCl. The excess silver nitrate is titrated with potassium thiocyanate using ferric ammonium sulfate as the indicator (Hooi et al. 2004). The amount of chloride in the sample is determined based on how much silver remains during the titration versus the total amount of silver added.

Cheese Analysis Using Near Infrared Spectroscopy

NIR Mechanics. As food production demands increased, the need for faster, more efficient routine methods of analysis has also increased. Methods of analysis like near infrared (NIR) spectroscopy have become popular in cheese production facilities as a tool to meet this need. NIR is similar to MIR in that they both rely on molecular vibrations and the absorption of radiation, however NIR is different from MIR in many ways (Siesler 2008). Most notably, NIR uses different wavelengths than MIR. While MIR operates from 4,000-200 cm^{-1}, NIR uses the range of wavelengths from 12,500-4,000 cm^{-1} (Siesler 2008). Within the NIR region, the absorption bands are

mostly overtone and combination bands which causes a low specificity in NIR, when compared to MIR, due to the overlap of these bands (Siesler 2008). These low band intensities do offer some advantage to NIR. NIR analysis allows very easy sample handling and when using total reflectance measurement approaches can handle a larger sample thickness than MIR (Siesler 2008). NIR instruments routinely utilizes reflectance measurements which contributes to the ease of sample handling and sample presentation for analysis. However, the results of NIR are influenced by the uniformity of the size distribution during grinding of cheese samples and the consistency of the cheese surface that NIR light is reflected from (Williams 2008). Due to the ease of sample handling and preparation, NIR instruments have become very common in cheese manufacturing plants.

Another large difference between MIR and NIR is the variety of product types that can be analyzed. MIR instruments, commonly used in the dairy industry, are not designed for the analysis of solid samples. They can only be used to analyze liquid samples as that is what the flow system is designed for. NIR instruments, however, can be used to measure solid samples, like Cheddar cheese and dairy powders, with only a small amount of sample preparation and handling. NIR and MIR spectroscopy are similar in fundamental background principles, however they are very different in many ways that alter the calibration and practicality of these instruments when used in the analysis of cheese.

Sample Preparation and Presentation. Easy sample preparation and presentation are some of the main reasons that NIR became a common method utilized for the rapid analysis of cheese. Sample preparation is one of the most vital principles

in successful analysis using NIR as not providing a representative sample would lead to poor results. Sample preparation can include identification and documentation, cleaning, drying, subsampling, grinding, and blending (Williams 2008). For NIR analysis of cheese, grinding is an important step. This reduces the particle size and allows for accurate readings. When preparing cheese for NIR analysis temperature differences of samples will have a negative impact on the precision of the results (Frankhuizen 2008). The high milkfat content of cheese also introduces a potential source of error in sample preparation. Fat may undergo changes in its distribution within the sample due to melting during sample preparation which can impact the reflectance (Frankhuizen 2008).

Samples are presented to most NIR instruments using some sort of cell that are available in many different shapes and sizes (Frankhuizen 2008). Loading the sample cell, while it may seem like an easy step, can still have an impact on the accuracy and precision of the results. The bottom of the layer of sample is what the instrument actually senses, so stratification while filling the cell can impact results (Frankhuizen 2008). The most important guidelines to proper cell loading is ensuring even distribution of the sample against the cell window (Frankhuizen 2008). Ensuring that the same amount of sample is loaded into the cell is also important. The degree of compaction at the surface changes if the cell is underfilled or overfilled which changes the reflectance and therefore the final result (Frankhuizen 2008). NIR sample preparation and presentation both play an important role in the accuracy of the final result.

NIR Calibration. NIR instruments are a secondary testing method which means they rely on calibration using reference chemistry in order to provide accurate measurements. The main steps in the calibration process include selecting the calibration set samples, collecting NIR spectra of the samples, and creating the mathematical PLS model. First, samples need to be selected for use in calibrating the instrument. It is important to select samples that provide an evenly distributed and complete concentration range of the component being calibrated (Workman, Jr. 2008). A uniform distribution is a vital part of creating a robust and accurate calibration model. Even small differences in background composition can cause large changes in the spectra (Workman, Jr. 2008). Sample-to-sample variance in composition within the sample set also plays an important role in developing a good calibration. By choosing a calibration set that has large variation in sample type and a large range of constituents, the resulting model will be able to analyze a large range of materials with relatively low accuracy (Workman, Jr. 2008). In contrast, a set of samples with small variance in one sample type (e.g., Cheddar, Mozzarella, Gouda, etc.) and a small range of constituents will create a model with increased accuracy over a smaller range (Workman, Jr. 2008). A separate calibration PLS model development is required for each cheese type on each NIR instrument. Each PLS model for each measured parameter (e.g., moisture, fat, protein, salt, etc.) will need reference chemistry on 200 to 400 samples for model development. Sample selection is a very important first step in the creation of a NIR calibration model.

The second step in NIR calibration is the collection of data. The overall goal of calibration is to develop a model that responds to changes in constituent

concentrations while not responding to non-concentration related factors like instrument and physical variables (Workman, Jr. 2008). One way to ensure that a model is calibrated to be sensitive to changes in component concentration is to not run data in the order of concentration. This eliminates the possibility of the instrument correlating unrelated instrument changes with changes in sample concentration (Workman, Jr. 2008). Calibration samples should also be run on different days and in different temperatures representative of the temperature fluctuations seen in the lab. Again, this decreases the chances of the instrument correlating instrument and environmental changes with concentration changes within the sample.

Finally, once the data has been collected, the mathematical PLS model is created. Multiple linear regression is the general statistical modeling form used for the calibration of NIR instruments (Workman, Jr. 2008). Specifically, partial least squares (PLS) models are commonly used to calibrate NIR instruments (Bjorsvik and Martens 2007). The goal in the creation of the statistical model is to develop an equation that can provide accurate results on the composition of any unknown sample. An ideal model should be sensitive to changes in concentration of the component of interest without being impacted by variation in the instrument, temperature, or background noise (Workman, Jr. 2008). Each step in the calibration process is important, however sample selection is the most crucial. A computer is only able to analyze the data that it is provided with and is unable to turn poor calibration sample selection into a robust calibration model (Workman, Jr. 2008). Choosing samples that represent the population of sample that will be routinely analyzed by the instrument for calibration

is the only way to develop a model that provides accurate and reliable results on any unknown sample.

Accuracy of NIR Instruments in Determining the Composition of Cheese Samples

NIR is a versatile method that can be used to analyze a variety of products, solid or liquid. Cheese is one of the most difficult products to analyze due to differences in composition and the high level of moisture and fat (Frankhuizen 2008). This means that sampling and sample prep are more critical in cheese analysis than analysis of other products to get an accurate measurement that is representative of the whole lot of cheese. Differences in temperature have a large impact on the precision of the results (Frankhuizen 2008). For this reason, the ground cheese should not be exposed to room temperature any longer than necessary. The high fat content of cheese is sensitive to structural changes (i.e., melting and coalescence) which can lead to differences in reflectance ultimately impacting the accuracy of the results (Frankhuizen 2008). This means that samples should be run in a timely fashion in order to minimize the potential of this issue influencing the cheese results.

Frankhuizen (2008) studied the accuracy of a cheese calibration model on NIR. He analyzed a combined calibration model for gouda and edam cheeses in which 125 cheese samples were used in the creation of the model. The cheeses ranged in fat from 22.89 to 43.06%, in protein from 23.23 to 28.45%, and in moisture from 34.4 to 46.06%. For fat, protein, and moisture Frankhuizen found high correlation coefficients (0.995, 0.995, and 0.993 respectively) between the reference methods and NIR for the combined calibration. The required accuracy for the standard error of calibration (SEC) is 0.2% for moisture and 0.25% for fat and protein (Frankhuizen 2008). For the

major constituents, Frankhuizen (2008) created calibration models for gouda and edam cheese with relatively low SEC values when compared to the above benchmark. The SEC values were 0.26%, 0.37%, and 0.32% for fat, protein, and moisture respectively (Frankhuizen 2008). These values were improved when the calibration was split up into two calibrations – one for each type of cheese. A decrease in the SEC of about 30% was seen when the calibration was split into two separate models (Frankhuizen 2008).

These results show the effectiveness of NIR as a method for the rapid analysis of cheese. Frankhuizen (2008) created a NIR calibration model with repeatability and reproducibility similar to that of the reference methods. However there are a variety of problems that need to be addressed. First, calibration set sample selection is key in creating an accurate and robust model. In order to get accurate results, cheese samples that are representative of the samples to be tested need to be used to create the calibration model. Second, sample prep also impacts the measurement abilities of the instrument. Particle size, temperature, and sample presentation all need to be standard across the running of all calibration samples and test samples. Lastly, the accuracy of the reference chemistry used in the calibration is important in the performance of the instrument. NIR measurements are dependent on the accuracy and precision of the reference methods used for determining the reference chemistry for the calibration of the instrument. The NIR models can only be as accurate as the reference chemistry for the population of cheese samples used in the modeling set and producing accurate reference chemistry on large populations of cheese samples by a routine cheese factory lab to use for modeling is a challenge. If samples are sent out to a third-party

reference chemistry laboratory for analysis the cost of development of PLS calibration models can be very high. Currently NIR is the best option for the rapid analysis of solid dairy products where mid infrared has not an option due to the solid state of the sample.

Cheese Analysis Using Mid Infrared Spectroscopy

Current Methods for Rapid Analysis of Cheese. In most cheese plants, analysis of cheese samples and multiple other dairy products happens frequently throughout the day. Chemical reference methods, while accurate, are incredibly slow and are not a feasible option for analysis in large scale cheese plants. This led to a need for more rapid methods of analysis like infrared spectroscopy. NIR is an accepted method for the determination of fat, protein and moisture of cheese (McKenna 2001), and the coulometric method has become an accepted rapid method for the determination of salt (Varcoe 2001). While these methods may improve the speed of analysis, accuracy and reliability cannot be compromised.

NIR methods use a partial least squares (PLS) model for calibration that need to be made in each cheese factory for each instrument. These models require a large amount of cheese samples, ranging from 200 to 400 samples, of each type of cheese to be tested by reference chemistry for each parameter (i.e., fat, moisture, protein, salt, etc.) and by the NIR instrument of that factory (McKenna 2001; Barbano and Lynch 2006). These instruments might require a very simple sample prep and a high speed of analysis, however they require a lot of work to calibrate each cheese type accurately. This makes creating an accurate NIR calibration not practical in most cheese plants where workers capable of performing large amounts of accurate reference chemistry

are not available. Accurate reference chemistry is a vital part of creating an accurate model along with the concentration range and distribution of the fat, protein, and moisture of the samples (McKenna 2001; Barbano and Lynch 2006b). NIR seems to be a simple and easy to perform rapid method of analysis, however there are many factors to consider when determining machine accuracy and reliability.

Cheese plants commonly use MIR instruments for the rapid analysis of milk, cream, and liquid whey samples. In fact, the use of MIR in measuring fat and protein in milk is very well understood (Kaylegian et al. 2006b, 2009). MIR is also an accepted official method for the analysis of milk (AOAC International 2012 method 972.16). MIR is distinctly different from NIR because it uses well documented wavelengths for measuring fat, protein, and lactose (Kaylegian et al. 2009). While there are a few challenges, this same principle could be applied to the analysis of cheese.

Cheese Preparation and Analysis. The main challenge in analyzing cheese using an MIR milk analyzer with a flow through cuvette is sample preparation. Solid cheese needs to be converted into a homogenous, particle free liquid in order to be pumped through the flow system of the MIR milk analyzer. Sjaunja 1999 described a method for dissolving cheese using a solution made of disodium metasilicate and pentasodium triphosphate. Margolies and Barbano (2017) utilized this solution to create and validate a model for the analysis of cheese using a MIR milk analyzer. To prepare cheese for analysis, the cheese was cut into 1 cm cubes and ground in a blender to a homogenous consistency (Margolies and Barbano 2017). Creating a homogenous blended cheese sample is important in ensuring that sub-samples from this cheese are

representative of the entire cheese core/block. The cheese dissolver solution was created using 12.7 g of pentasodium triphosphate and 5.3 g of disodium metasilicate in 1,982 g of distilled water at 40C (Margolies and Barbano 2017). To prepare the ground cheese for analysis on the MIR, the cheese sample is diluted about 10:1 (wt/wt) with the solution, blended, and then pumped through the MIR instrument. (Margolies and Barbano 2017).

MIR Accuracy. After prepping the cheese sample and running it through the MIR milk analyzer, the next step is to interpret the results. These results need to be accurate and meaningful in order to benefit cheese plants. Margolies and Barbano (2017) evaluated the performance of MIR milk analyzers in the analysis of cheddar cheese. In a calibration using 34 full fat Cheddar cheeses, the SEP values for fat A, fat B, fat A+B, moisture, protein, and salt were 0.115, 0.110, 0.110, 0.092, 0.074, and 0.034, respectively (Margolies and Barbano 2017). These low SEP values indicate the potential for MIR being used to accurately measure the composition of cheese. To validate the calibration, 36 full fat cheddar cheese samples were analyzed for reference chemistry values, analyzed on the MIR, and analyzed using NIR in their cheese plant of origin (Margolies and Barbano 2017).

When comparing the MIR results to the reference values and the NIR values to the reference values, the MIR showed better predictability for most components. Moisture regression produced the most similar R^2 values for both MIR and NIR of about 0.93 (Margolies and Barbano 2017). However all of the other components showed stark differences in R^2 values for the two types of rapid analysis. Fat R^2 values for MIR compared to the reference and NIR compared to the reference were 0.93 and 0.70,

respectively. This difference in predictability of fat between the MIR and NIR instruments could be caused by poor calibration models made at the cheese plant. Inaccurate reference chemistry values from the cheese plant or not using a robust and representative calibration sample set could lead to poor fat predictability by the NIR instrument.

Salt R^2 values also showed starkly different results. In this study, the salt values measured in the cheese plants were produced using the coulometric method (Margolies and Barbano 2017). The MIR and coulometric R^2 values when compared with reference chemistry values were 0.94 and 0.16, respectively (Margolies and Barbano 2017). A protein calibration model for MIR was also created, however most cheese factories do not have models for protein on their NIR instruments and do not routinely test for protein in cheese samples. The large differences between the predictability of the MIR and methods of analysis used in cheese plants is unsurprising based on previous research analyzing the performance of these plants in accurately determining cheese composition. Margolies et al. (2017) determined that some cheese factories may underestimate fat and moisture by as much as 0.5%. There is an opportunity to provide cheese plants with a more accurate method for the rapid analysis of cheese that requires a less extensive calibration and utilizes equipment likely already present in their quality assurance laboratory.

Calibration of MIR Instruments for Cheese Analysis

MIR, like NIR, is a rapid secondary method of analysis which means that it requires calibration using accurate reference chemistry. In order for these methods to be practical in a commercial cheese plant setting, the calibration needs to be feasible

for quality assurance laboratory workers to create accurately. The calibration approach for MIR instruments is very different from the calibration approach for NIR.

NIR instruments require an extensive calibration. Each cheese variety within each plant requires the development of a PLS model for each parameter to be measured. These PLS models need accurate reference chemistry on 200-400 cheese samples for each type of cheese and each parameter that a plant intends to measure (McKenna 2001; Barbano and Lynch 2006). To perform accurate chemical analysis, a lab needs skilled staff and all of the necessary equipment. Collecting all of the necessary reference values is difficult for cheese plant laboratories. NIR calibrations are also not transferable between instruments, even within the same cheese plant. This means that these calibrations are very cheese type and cheese plant specific. Holroyd (2011) reported the potential for a "global" PLS base calibration developed by an equipment manufacturer. The "global" base calibration model would only require 10 to 20 samples which would greatly reduce the amount of reference chemistry needed to be performed by the cheese plant. However this global calibration approach is not widespread in industry, so cheese plant employees still need to perform reference chemistry in house on 200 to 400 cheese samples in order to create an accurate calibration model for their NIR instruments.

MIR is not currently being used in industry to analyze cheese samples. However, the work done by Margolies and Barbano (2017) lays out the potential approach to calibrating MIR instruments for cheese analysis. The added blending stage reported for cheese prior to running on the MIR does mean that MIR requires a longer sample preparation than NIR. However, the inconsistency of sample grinding

used for NIR analysis has been shown to influence the results (Holroyd 2011). Blending the cheese into a completely homogenous and particle free liquid for MIR analysis makes sample preparation consistency less of a concern in the accuracy of the MIR result. Margolies and Barbano (2017) developed an accurate Cheddar cheese calibration to predict fat, protein, moisture, and salt for the MIR milk analyzer using 34 full fat cheddar cheese samples. Purchasing and utilizing modified milk calibration sets from a central location is an accepted method for the calibration of MIR instruments in the US for milk (Kaylegian et al. 2006a, Kaylegian et al. 2006b). Kaylegian et al. (2006b) found that modified calibration sets for milk had more consistent slope and intercept values for all of the components compared to locally made calibration sets. As this approach to calibration of MIR is effective in the analysis of milk, there is a potential for this approach to also be applied to cheese analysis. Plants could purchase a cheese calibration sample set just like they do for milk and whey calibration on MIR milk analyzers currently. If effective, this calibration method would be much easier for cheese plant workers to perform and could lead to improved accuracy in cheese composition analysis on a day to day basis in cheese plants.

ANALYSIS OF THE COMPOSITION OF DAIRY POWDERS

Measurement of the Chemical Composition of Nonfat Dry Milk (NFDM)

Fat. The reference method for the determination of fat in NFDM is the Mojonnier ether extraction method (AOAC International 2012 method 932.06,

SMEDP method 15.086). The Roese-Gottlieb method is very similar to the Mojonnier method, however the Mojonnier method is accepted as the best option (Hooi et. all 2004). The Roese-Gottlieb method differs from the Mojonnier method in one distinct way. In the Roese-Gottlieb method the weight of the fat plus the pan after evaporation is determined and then the fat is removed from the pan, the pan is dried, and the difference in weight is used to calculate the fat content (Hooi et al. 2004). This method requires extra time due to the extra steps at the end and also introduces more sources of error, so it is not commonly used in laboratories.

Protein. The Kjeldahl method is the accepted reference method for the determination of the protein/nitrogen content of NFDM (AOAC International 2012 method 930.29, SMEDP method 15.131). There are slight modifications made to the Kjeldahl method used for milk to apply it to dairy powders. The main difference is in the determination of the amount of test sample used. The ideal amount of protein per flask is 0.25 to 0.2g for any sample (Hooi et al. 2004).

Lactose. The enzymatic method for the determination of lactose is the accepted method for NFDM (SMEDP method 15.092). Lactose is the most prevalent ingredient in nonfat dry milk powders which means that accurate lactose results are important in meeting product quality standards and in utilizing these powders in other applications.

Total Solids. The reference method for the determination of moisture in NFDM is the vacuum oven drying method (AOAC International 2012 method 927.05, SMEDP method 15.111). Samples are heated under vacuum in an oven at 100C for 5 hours (AOAC International 2012, Hooi et al. 2004).

Analysis of Dairy Powders Using Near Infrared Spectroscopy

The official reference analytical methods for moisture fat, protein, and lactose take a long time and require skilled workers. These methods are not practical in controlling manufacturing processes and getting real time composition results on dairy powders. Rapid analysis methods fill this gap and allow dairy plants to get accurate composition data quickly. Infrared absorption has had a large influence on the dairy industry and is a recognized method of analysis for milk (AOAC International 2012 method 972.16, SMEDP method 15.121). This type of analysis, however, requires liquid samples which means that other dairy products like powders would require some type of prep before analysis.

The principle of NIR is reflectance spectroscopy which measures the intensity of the light reflected from the sample surface (Frankhuizen 2008). This eliminates the need for sample prep when analyzing non liquid samples like powder and cheese. The uniform particle size and shape of dairy powders make them one of the best products to analyze using NIR. No grinding is necessary because of their small particle size.

Frankhuizen (2008) evaluated the performance of the calibration of NIR instruments in analyzing dairy powders. Sample selection for milk powder calibration is still important, however it is not as influential of a factor in the calibration accuracy as cheese sample selection. Cheese varies in processing steps, physical and chemical composition, and has high moisture and fat content which makes it difficult to get completely representative samples and measurements. For their calibration, Frankhuizen (2008), collected powders over a two-year period from various countries based on the powder composition, production processes, and production period.

Selecting powders with various production backgrounds and composition produces a more robust calibration model.

Frankhuizen (2008) found a high correlation between the reference chemistry values for protein and the NIR results for protein. They created calibration models for skim milk powder, buttermilk powder, skim milk powder + buttermilk powder, denatured milk powder, and milk powder with nonmilk fat. They saw R^2 values between 0.97 and 0.999 and SEC values of 0.18 to 0.34% for protein of these different dairy powder models with milk powder with nonmilk fat showing the highest R^2 value and lowest SEC. The correlation between reference moisture and the NIR moisture was also high. The average SEC value was 0.12% and the average R^2 value was 0.97. For lactose, the SEC values were acceptable for skim milk powder, buttermilk powder, and milk powder with nonmilk fat (0.38, 0.35, and 0.34%, respectively). However, the SEC values for the calibration models for the other powder types were too high for accurate analysis. NIR analysis has been shown to be an effective and accurate rapid method for the analysis of dairy powders.

Undenatured Whey Protein Nitrogen as an Indicator of the Thermal History of NFDM

Nonfat dry milk is a widely used dairy powder in baking and other food industries. It is manufactured by removing water from pasteurized skim milk resulting in a powder with not more than 5% by weight of moisture and not more than 0.5% by weight of milkfat (FDA 2018c). These parameters define what is legally designated as NFDM, however the thermal history of NFDM also has an impact on the final product functionality.

Undenatured whey protein nitrogen (WPN) is an important compositional measurement of dairy powders. During the processing of dry milk products, serum proteins are partially denatured (Anderson and Bell, Parris and Baginski 1991). Skim milk goes through a variety of heat treatments including being preheated prior to spray drying to improve water absorption and the functional properties of the NFDM powers (Parris and Baginski 1991) for some applications. Other applications may require low heat denaturation of milk proteins. The intensity and duration of these heat treatments impact the amount of whey protein denaturation.

Nonfat dry milk is classified as low heat, medium heat, or high heat powder based on the extent of whey protein denaturation. The USDA sets standards for the official classification of NFDM based on the amount of whey protein denaturation. High-heat NFDM has no more than 1.50 mg of undenatured whey protein nitrogen per gram in the finished product (USDA 2001). Medium-heat classification requires that the finished product have no more than 1.50 mg of undenatured whey protein nitrogen per gram and no less than 6.00 mg of undenatured whey protein nitrogen per gram of nonfat dry milk (USDA 2001). A low-heat classification means that the finished product has no less than 6.00 mg of undenatured whey protein nitrogen in the finished product (USDA 2001). These classifications are an important descriptor of the thermal history of dairy powders, however the overall impact of the heat processing on the functionality of these dairy powders is an essential part of utilizing these products in food applications.

Undenatured Whey Protein Nitrogen and Dairy Powder Functionality

Dairy powders are used widely in the food industry in a variety of applications and for many manufacturing purposes. Milk powders are used as an ingredient in products including baked goods, confections, dairy desserts, ice cream, and infant formula. After drying, milk powder retains most of the nutritional and sensory properties of fluid milk including color, flavor, and solubility (Capuano 2015). Reducing the moisture content of milk by drying it into milk powders offers the main advantages by extending shelf-life and reducing costs associated with transportation and storage. However, the heat processing required to create this shelf-stable product can impact the state of the proteins present in the powder.

The amount of denaturation of a dairy powder impacts its functionality in making other products. Dried whey has many functional properties that are dependent on the heat treatment the product undergoes during processing including whippability, emulsifying capacity, gelling ability, solubility, and water holding capacity (Mahmoud et al. 1990). The denaturation of whey proteins has also been linked with the release of sulfur compounds which cause cooked flavors in heated milk due to their intense flavor profile (Al-Attabi et al. 2009). Hutton and Patton (1952) determined that β - lactoglobulin proteins, which are the most prevalent serum protein in milk, are the main source of sulfhydryl compounds and therefore are the main cause of the cooked flavor that occurs upon heating. However, the complex behavior of whey proteins during processing (Wit 1990) makes it difficult to completely predict and control their functional properties as a result of heat treatments.

Measurement of Undenatured Whey Protein Nitrogen in NFDM Using Spectrophotometry

The routine method for the determination of undenatured whey protein nitrogen in nonfat dry milk is the spectrophotometry method, also called the Harland-Ashworth method (SMEDP method 15.134). In this method the NFDM is rehydrated, heated in a saturated sodium chloride solution, which precipitates the casein and denatured whey protein, and filtered (Hooi et al. 2004). The filter catches the casein and denatured whey protein fractions while the filtrate contains the undenatured whey proteins and the non-protein nitrogen fractions. The filtrate is then diluted in a saturated salt solution and acidified after which the solution is analyzed spectrophotometrically (Hooi et al. 2004). These values are compared to a standard curve that plots the Kjeldahl nitrogen value of NFDM standards with different undenatured whey protein values (i.e., reference method for undenatured whey protein nitrogen) against their transmittance in the spectrophotometric method (Hooi et al. 2004).

The current routine method is based on the turbidimetric method developed by Harland and Ashworth (1947) for determining the baking quality of NFDM. This method does not directly measure denaturation, but it is the accepted method used by the industry to analyze the thermal history of milk powders. This method was originally created to determine if high enough heat treatment was applied in the production of nonfat dry milk in order to create high quality NFDM for baking purposes (Harland and Asworth 1947). This method was modified by Kuramato et al (1959) and by Leighton (1962) in an effort to make it more widely applicable and to

improve agreement across laboratories. One of the largest accuracy issues with the Harland Ashworth method occurs when creating the standard curve. Kuramato et al. (1959) modified the standard curve creation by using a batch of low heat powder and a batch of high heat powder and combining them in different quantities to create the standard curve samples. Leighton (1962) modified this method by creating the whey protein index (WPI) for skim milk and by enhancing the development of turbidity. WPI is the milligrams of whey protein nitrogen that are soluble in saturated sodium chloride solution per gram of milk powder.

While these methods work effectively for NFDM, they are not easily transferable to use for whey protein powders. Whey powders contain about 13% whey proteins while NFDM powders contain about 7 to 8% whey proteins and the pH of whey powder varies more than the pH of NFDM (Mahmoud et al. 1990). This leads to issues in the turbidity development and precipitation of the proteins in the Harland Ashworth method. Mahmoud et al. (1990) studied a modification to this method to determine undenatured whey protein nitrogen in whey powder that avoided the turbidimetric difficulties faced. In this modified method, the undenatured whey protein nitrogen is determined based on the difference between the absorbance of the same filtrate used in the unmodified method before and after deproteinization with 12% TCA. While the modified methods created by Mahoumod et al. (1990) was determined to be reproducible and has the potential to be used for whey powders, there is an opportunity of creating a more universal and rapid method for the determination of undenatured whey protein nitrogen in all dairy powders.

RESEARCH GOALS AND OBJECTIVES

Inaccuracies in the analysis of cheese and dairy powder composition has a large economic impact on dairy processors when this information is used to make important business decisions. Mid infrared analysis is a well understood and accurate rapid method of analysis for milk and has the potential to be used for other dairy products. The objective of our research was to (1) determine how to create a centralized cheddar cheese calibration set that can be shipped to cheese manufacturing facilities to be used to calibrate mid infrared instruments for the rapid analysis of Cheddar cheese produced in different factories and to (2) develop a method for the rapid measurement of fat, protein, moisture, and lactose of reconstituted nonfat dry milk powders using mid infrared spectroscopy.

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CHAPTER TWO

PREPARATION OF CHEDDAR CHEESE CALIBRATION STANDARDS FOR MID-INFRARED TRANSMITTANCE SPECTROSCOPY

ABSTRACT

The objective of our work was to develop approaches for production of liquid Cheddar cheese calibration standards and evaluate the calibration set performance for calibration of a mid-infrared (MIR) milk analyzer for measuring fat, protein, moisture, and salt content of Cheddar cheese. Cheddar cheese was blended with a cheese dissolver solution containing pentasodium triphosphate and disodium metasilicate to create a uniform, particle free dispersion of cheese with a composition similar to milk which could be analyzed using a MIR milk analyzer. The MIR milk analyzer was calibrated with an 8 sample liquid Cheddar cheese reference calibration sample set that was produced by blending different amounts of one Cheddar cheese with the same amount of solver to create an 8 sample calibration set to simulate a range of cheeses containing differing amounts of fat, protein, total solids, and salt. Annatto-colored Cheddar cheese samples (30) from one cheese factory were analyzed using reference chemistry methods for protein (Kjeldahl), fat (Mojonnier ether extraction), moisture (oven drying total solids), and salt (Volhard silver nitrate titration) and used as validation samples. These same cheese samples were also blended using the cheese dissolver solution into a uniform liquid and then run through the MIR milk analyzer and the same cheeses were also analyzed using near infrared (NIR) spectroscopy for

fat, moisture, and salt in the factory where they were produced. The mean differences (MD) from reference chemistry (IR minus reference chemistry) for a set of 30 unknown Cheddar cheeses for MIR for fat, moisture, and salt were -0.23, -0.10, and 0.004%, respectively, while the MD for NIR were -0.87, 0.25, and 0.07, respectively. Standard error of prediction (SEP) values for fat, moisture, and salt of the validation samples on the NIR were 0.304, 0.366, and 0.133, respectively whereas the MIR produced SEP values for fat A+B, moisture, and salt of 0.177, 0.176, and 0.022, respectively. These lower MD and SEP values indicate that MIR calibrated using an 8 sample set of liquid cheese calibration samples could be used as an effective alternative to cheese producers for the rapid analysis of cheese using equipment most dairy processors already have in their facility. For protein on the validation samples, the MIR produced an SEP value of 0.140 which also shows a potential for cheese producers to be able to effectively measure protein to determine protein recovery in cheese making.

INTRODUCTION

In large cheese plants, cheese yield, protein and fat recovery, and cheese compositional control are important factors used in management and decision making. Margolies et al. (2017) developed a system for the evaluation and control of these parameters. The authors determined that in large scale cheese plants, there were mean bias errors in measurement as high as ±0.5% for fat and moisture. The magnitude of these errors indicated the need for improvement of rapid secondary testing methods used in cheese plants.

Chemical reference methods for determination of fat, protein, moisture, and salt, while accurate, are slow and require skilled staff to perform well. This makes these methods impractical for the rapid analysis of a large number of cheese samples in a commercial cheese plant quality assurance laboratory. Near infrared (NIR) spectroscopy has filled the need for a more rapid and cost-effective method for analysis of fat, protein, and moisture (McKenna 2001) in cheese plants.

NIR calibration methods utilize partial least squares (PLS) statistical models. These models are created by analysis (NIR and reference chemistry) of cheeses produced in that factory. A large number of cheese samples (200 to 400) of each cheese type need to be analyzed by reference chemistry and by the NIR instrument in each cheese plant (McKenna 2001; Barbano and Lynch 2006). The reference chemistry is costly. However, NIR offers a large benefit to efficiency to cheese compositional analysis because of its ease of sample preparation and its high speed of analysis. Accuracy of the NIR PLS calibration models depend on the accuracy of the reference chemistry and the concentration range and distribution of fat, protein, and moisture in the calibration samples used in the development of the PLS model (McKenna 2001; Barbano and Lynch 2006). Sample presentation consistency (particle size distribution) is a source of analytical variation in cheese analysis using NIR (Holroyd 2011).

Mid infrared (MIR) milk analyzers are commonly used in cheese plants for analyzing milk, cream, and liquid whey products. In fact, the use of MIR in measuring fat and protein in milk is very well understood (Kaylegian et al. 2006b, 2009). MIR is also an accepted official method for the analysis of milk (AOAC International 2012

method 972.16). While a few instrument manufacturers have created application notes for cheese analysis using MIR instruments, this approach for cheese analysis is not well understood or commonly used.

Unlike NIR instruments, MIR instruments utilize well documented wavelengths for the measurement of fat, protein, and lactose (Kalyelgian et al. 2009). However, the main challenge to overcome is sample preparation as the solid cheese needs to be converted to a particle free liquid to be pumped through the flow system of the MIR. Sjaunja (1999) reported a solution for dissolving cheese for analysis in MIR instruments made of pentasodium triphosphate and disodium metasilicate. Margolies and Barbano (2017) utilized this method of dissolving cheese to evaluate the performance of a MIR instrument for the analysis of cheese. Using 34 annatto colored full fat cheddar cheese samples, the authors created a calibration model on the MIR milk analyzer which produced SEP values for fat A, fat B, fat A+B, moisture, protein, and salt of 0.115, 0.110, 0.110, 0.092, 0.074, and 0.034, respectively (Margolies and Barbano 2017). To validate the calibration, 36 annatto colored full fat cheddar cheese samples were analyzed for reference chemistry, analyzed on the MIR, and analyzed using NIR in their cheese plant of origin (Margolies and Barbano 2017). The authors found a better predictability with the MIR milk analyzer for most components when comparing the MIR results to the reference values and the NIR results to the reference values. The R2 values for MIR compared to the reference values were higher than the R2 values for NIR (coulometric method for salt) compared to the reference values for all of the components measured (fat and salt) except for moisture in which the R2 values were comparable (Margolies and Barbano 2017). Protein is not commonly

measured in large scale cheese plants, so NIR data for this component was not available, however protein content does offer valuable information on the functionality and quality of cheese products. Margolies and Barbano (2017) developed a calibration model on the MIR milk analyzer for protein with an SEP value of 0.19% suggesting the potential for MIR to be utilized as a fast and easy method for the measurement of protein by cheese plants. The large predictability differences between NIR and reference values are unsurprising based on the research by Margolies et al (2017) which showed a difference in fat and moisture results in cheese plants as high as ±0.5%. The objective of our work was to develop approaches for production of liquid Cheddar cheese calibration standards and evaluate the calibration set performance of a rapid using a combination MIR transmittance analysis for measuring fat, protein, moisture, and an in-line conductivity sensor within the MIR milk analyzer for measuring salt content of Cheddar cheese.

MATERIALS AND METHODS

Experimental Design

Margolies and Barbano (2017) demonstrated that Cheddar cheese could be dispersed as a liquid and analyzed using traditional basic filter models commonly used in MIR for milk analysis. This type of equipment is common in quality assurance laboratories in cheese factories and is used for milk and whey analysis.

Three possible approaches to development of a calibration sample set for cheese analysis by MIR were considered. One approach would be to take a large number (10 to 30) of cheese samples from individual vats of cheese, run reference chemistry on each cheese, and prepare these samples for use as a calibration sample

set. This approach was used successfully by Margolies and Barbano (2017). The limitation of this approach is that it would need to be repeated at least monthly and requires a lot of reference chemistry testing to do one calibration of an instrument. Also, the range and distribution of concentrations of fat, protein, moisture and salt from sample to sample within the calibration may not be wide enough or uniform enough (i.e., high leverage samples) to obtain the best adjustment of slope and intercept for each measured parameter.

A second possible approach would be to take a large quantity of one ground composite cheese and make one large batch of cheese dispersed in dissolver to use as a base and then make dilutions (a series of 8 dilutions) of this base with dissolver to simulate a series of cheeses with a range of composition.

A third approach would be to make a set of 8 calibration samples from one batch of composite cheese but vary the amount of cheese per amount of dissolver to simulate a series of cheeses with a range of composition. The advantage of the second and third approaches is the amount of reference chemistry that is needed (one cheese) is minimized while producing a series of liquid cheese reference standards with a systematic range and increments of the components required for a proper adjustment of calibration slopes and intercepts. Both the second and third approaches described above were evaluated. The third approach worked better than the second approach and the results from that approach are reported in this paper.

Cheese Analysis

Cheese Grinding and Storage. Cheese samples were taken at a commercial cheese factory as cores removed from 19 kg blocks of 4 day old Cheddar cheese.

Cheese samples (4°C) were ground to a 3 to 4 mm particle size using a blender (E8442, Eberbach Corporation, 505 S Maple Rd, Ann Arbor, MI 48103) at the cheese factory where the cheese was produced. The ground cheese was packed into whirl bag pack bags (B00992, Nasco,16 Simulaids Drive, Saugerties, NY 12477) and refrigerated at 4°C until testing. To minimize moisture loss from the cheese, bags were sealed with minimal headspace. At the time of analysis, 2 cm of cheese from the top of the bag was discarded to remove the top layer of cheese that may have lost moisture by evaporation.

Cheese Dissolver Solution. As reported by Sjaunja (1999) and Margolies and Barbano (2017), the cheese dissolver solution was prepared using a combination of 12.7 g of pentasodium triphosphate (7758-29-4, Fisher Chemical, 2000 Park Lane Drive, Pittsburgh, PA 15275) and 5.3 g of disodium metasilicate (10213-79-3, Fisher Chemical, 2000 Park Lane Drive, Pittsburgh, PA 15275) in 1,982 g of water at 40°C. The equipment manufacturer also offers this cheese dissolver chemical (MA00090055, Delta Instruments, Kelvinlaan 3, 9207 JB Drachten, Netherlands) in a premixed form.

Individual Cheese Sample Preparation. Each of the 30 cheese samples were blended individually. Cheese dissolver solution was heated to 65 ± 2°C and approximately 81 g of the solution was added to a 300 mL plastic cup (RK10, Fabri-Kal Plastics Place, Kalamazoo, MI 49001). The weight of the dissolver solution plus the cup weight was recorded on a top loading balance (PG5002-S, Mettler-Toledo, LLC 900 Polaris Parkway Columbus, OH 43240) to 2 decimal places. Next, the dissolver solution was poured into the high shear stainless steel bender with a stainless

steel blade assembly (E8580, Eberbach Corporation, 505 S Maple Rd. Ann Arbor, MI 48103). The weight of the cup plus any residual dissolver was immediately recorded and the weight of the dissolver transferred into the blender (81 ± 0.2 g) was calculated by difference. After that 9 ± 0.05 g of ground cheese was placed in a plastic 150 mL cup (RK5, Fabri-Kal Plastics Place, Kalamazoo, MI 49001) and the weight of the cheese plus the weight of the cup was recorded on an analytical balance (AE100, Mettler-Toledo, LLC 900 Polaris Parkway Columbus, OH 43240) to 4 decimal places. Unlike the approach by Margolies and Barbano (2017), antifoam was not added to each sample. The antifoam was eliminated from the method because of batch to batch variation in antifoam. Next, the cheese was added to the same high shear blender as the 65ºC dissolver solution. The weight of the empty cup plus any residual cheese was recorded and the weight of the cheese transferred to the blender was calculated by difference.

The stainless-steel blender jar containing the warm dissolver plus cheese was immediately placed on a 2-speed blender base (E8580, Eberbach Corporation, 505 S Maple Rd. Ann Arbor, MI 48103). The jar was capped and run at low speed for 15 s, followed immediately by high speed for 45 s before being poured into a 150 g snap lid plastic vial (75662, United States Plastics, Corp., 1390 Neubrecht Road, Lima, OH 45801). When pouring the liquid cheese into the vial, the liquid was observed to see if any cheese particles remained. If for some reason particles of cheese remained, the liquid could be poured back into the blender and blended on high speed for another 15 s. The vial containing the blended cheese plus dissolver was closed and then was placed in a 40°C water bath for about 20 minutes to allow the foam to break and any

entrapped air to dissipate. The cheese plus dissolver solution was run through the MIR analyzer and the remaining liquid was portioned out for determination of fat (Mojonnier ether extraction) and protein (Kjeldahl).

Cheese Calibration Sample Preparation. The unused Cheddar cheese remaining from the samples from the 30 vats of cheese were combined and blended together to obtain one cheese that was a homogeneous mixture of cheese from all 30 vats. A series of 8 ratios of cheese to dissolver were prepared with the goal of spanning the range of cheese composition with uniform increments of cheese composition that could be encountered in manufacturing. Sufficient quantity of each of the 8 cheese to dissolver ratio were produced (a composite of 3 blendings of cheese plus dissolver), using the physical procedures described above, to make 4 sets of 8 calibration samples. This would provide material for reference chemistry testing and samples to run on the MIR milk analyzer. To achieve this, a 24 total blends were performed – 3 blends of 8 different ratios of cheese to solver. The first 3 blends started with 9.8 g of cheese to 81 g of solver and from there the amount of cheese was decreased while keeping the amount of solver the same.

The 3 blends at each ratio, after sitting at room temperature for approximately 15 minutes in order for the foam to dissipate, were combined in a 300 mL plastic cup (RK10, Fabri-Kal Plastics Place, Kalamazoo, MI 49001). These blends were mixed by pouring them back and forth between two 300 mL plastic cups (RK10, Fabri-Kal Plastics Place, Kalamazoo, MI 49001). After mixing, the liquid cheese solution was divided evenly into five 36 g snap lid vials (CPP02, Capitol Plastic Products, 1030 Riverfront Center, Amsterdam, NY 12010). The same procedure was used on the 7

different cheese to solver ratio blends in order to create five sets of an 8 sample calibration set. One set of 8 vials was run through the MIR milk analyzer, one set was used for the determination of fat (Mojonnier ether extraction) and protein (Kjeldahl) on each vial, and the rest were frozen in a -80°C freezer. Reference chemistry for salt and total solids were determined on the combined solid cheese sample and the reference values for the diluted liquid cheese samples were calculated using these reference values and the cheese to solver ratio in each vial. The target minimum and maximum fat, protein, salt, total solids, and moisture in the 8 sample cheese calibration set were 30% and 35%, 21% and 25%, 1.5% and 1.7%, 60% and 65%, and 35% and 40% respectively, with component concentrations spaced equally across the range.

Validation Samples

Validation was done using 30 full-fat colored cheddar cheeses from one factory. Reference chemistry for all of the cheese samples was done in the Cornell University laboratory. Validation samples were analyzed for fat, moisture, and salt using the NIR instrument used routinely in the cheese factory that produced the cheeses. Cheese samples were prepped and analyzed using MIR as described above. Reference total solids (oven drying) and salt (Volhard method) analysis were performed on the solid cheese samples and reference fat (Mojonnier ether extraction) and protein (Kjeldahl) were performed on the liquid samples.

Chemical Reference Methods

Fat. Fat determination of the cheese was performed on the liquid cheese samples that had been diluted and blended with the cheese solver solution at 65 ± 2°C.

Fat was determined on the liquid cheese samples for each of the cheese vats used for validation testing and on each of the 8 cheese – dissolver mixtures in the calibration sample set. As is done for milk samples (Barbano et al. 1988), the cheese plus dissolver solution (10g) was weighed into a Mojonnier ether extraction flask and tested in duplicate. To calculate the amount of fat in individual cheese samples, the final result was multiplied by the weight/weight dilution factor of the cheese plus dissolver. To calculate the amount of fat for a reference value in each calibration sample, the final result was divided by grams of cheese per 100 grams of solution that would be seen in a typical blend in which 9 grams of cheese and 81 grams of solver was used. In a typical blend of 9 grams of cheese and 81 grams of dissolver, there would be 10 grams of cheese/ 100 grams of solution. Using this dilution factor simulates the fat level varying while the amount of cheese theoretically staying the same. For example, the following equation was used:

Percent fat in calibration sample = (ether extraction fat value / (10 grams cheese/ 100 grams solution)) x 100.

Protein. The Kjeldahl method was used for determination of the nitrogen content (Barbano and Clark 1990, Lynch and Barbano 1999). The total nitrogen content was then multiplied by 6.38 in order to determine the total (crude TN x 6.38) protein content of the cheese. Just like in the determination of fat, the liquid cheese sample of cheese and dissolver was used for the determination of protein which was also run in duplicate. The official method used for milk (Barbano and Clark 1990) was the same Kjeldahl method utilized for the cheese. Similar to fat, to calculate the amount of protein in each cheese sample, the final result was multiplied by the

weight/weight dilution factor of the cheese plus dissolver. Protein content of the calibration set was determined in the exact same way as for fat. The final result was divided by grams of cheese per 100 grams of solution that would be seen in a typical blend in which 9 grams of cheese and 81 grams of dissolver is used to simulate a decreasing protein content. ***The following equation was used:***

Percent protein in calibration sample = (Kjeldahl protein reference value / (10 grams cheese/ 100 grams solution)) x 100.

Salt. The Volhard method (AOAC 2012, method number 935.43; SMEDP 15.052) was performed in duplicate directly on the solid cheese samples (3g) to determine cheese salt. Cheese salt was determined on each of the individual cheese vat samples and on the combined base solid cheese sample for the calibration set A known number of moles of silver nitrate were added and then the cheese was heated and digested with nitric acid and potassium permanganate. By going through this acid digestion, chloride in the sample is freed and will react with the silver to form AgCl. To determine the final salt content, a back-titration of the remaining unreacted silver was performed using potassium thiocyanate with ferric ammonium indicator. For the individual cheese vat samples, the cheese final result was used as is. To calculate the salt in the first sample of the calibration set, the final result for the combined sample was multiplied by the ratio of the grams of cheese per 100 grams of solution in the first diluted sample to the grams of cheese per 100 grams of solution for a typical blend (10 g of cheese/ 100 g of solution). For example, the following equation was used:

Percent salt in first vial of calibration set = Volhard reference value for combined solid cheese sample x (A/B)

Where:

A = grams of cheese/ 100 grams of solution in the first calibration set vial

B = 10 grams of cheese/ 100 grams of solution which is the grams of cheese per 100 grams of solution in a typical blend with 9 grams of cheese and 81 grams of solver

To calculate all of the following diluted samples, the previous salt content was multiplied by the ratio of the grams of cheese per 100 grams of solution for that sample to the grams of cheese per 100 grams of solution for the previous sample. For example, the following formula was used:

Percent salt in a calibration set vial = calculated reference value for previous vial * C/B

Where:

C = grams of cheese/ 100 grams of solution in the vial that the percent salt is being calculated for

B = 10 grams of cheese/ 100 grams of solution which is the grams of cheese per 100 grams of solution in a typical blend with 9 grams of cheese and 81 grams of solver

Total Solids. The solids content of the cheese was determined using the reference method of 24 h at 100°C in a forced air oven using a 2 g sample (SMEDP, method 15.114). Oven drying was performed in duplicate for each cheese sample for

from the individual cheese vat samples and in duplicate on the combined base cheese sample for the calibration set. The result for the individual vat samples was used as is. Total solids of the diluted cheese samples for the calibration set were determined utilizing the same calculations as the salt content.

Mid-infrared Analysis

The pre-calibration of the MIR and the setting of the primary slopes for fat A, fat B, and protein were set as described by Lynch et al. (2006). For fat B, fat A, and protein the optimized virtual filter wavelengths and bandwidths used were the same as those described by Kaylegian et al. (2009). Similar to milk analysis, fat A+B was determined as [(0.7 x fat B corrected) + (0.3 x fat A corrected)]. The instrument used in this study had the same intercorrection factors (Barbano and Clark 1989, Lynch et al. 2006) as those used for milk analysis. Salt was measured using an in-line conductivity sensor housed in the pumping system of the MIR. Table 2.1 shows the sample and reference wavelengths and bandwidth, scale, and intercorrection factors for fat A, fat B, and protein. To calculate total solids, the sum of [(fat) + (protein) + (salt)] was taken and a slope and intercept adjustment was made using the reference chemistry from oven drying.

Table 2.1 MIR wavenumbers (cm^{-1}), scale, offset, and intercorrection factors for fat B, lactose, protein, and fat A traditional virtual filter wavelengths

Filters		number		
Fat B	2838	2864	34.6352	0.00
Lactose	1038	1058	17.6939	0.00
Protein	1531	1551	21.6284	0.00
Fat A	1740	1756	21.8407	0.00
Reference filters	1st wave number	Last wave number	Scale	Offset
Fat B	2800	2824	-34.6352	0.00
Lactose	1286	1300	-17.6939	0.00
Protein	1485	1497	-21.6284	0.00
Fat A	1783	1799	-21.8407	0.00

Intercorrection Factors				
	Fat B	Lactose	Protein	Fat A
Fat B	1.000	-0.149	-0.057	0.000
Lactose	0.044	1.000	0.016	0.000
Protein	0.064	0.052	1.000	0.000
Fat A	0.000	0.024	0.020	1.000

The 8 sample set of calibration samples was tested on the MIR (Delta Instruments, Model FTA, Drachten, The Netherlands). The slope and intercept adjustment for fat A, fat B, 70% fat B + 30% fat A combination, protein, and total solids were calculated using the reference chemistry values for the 8 sample calibration set to achieve a mean difference of zero between instrument prediction and reference chemistry mean for the set. The standard deviation of residual difference between reference chemistry and instrument prediction was calculated for each parameter. Moisture was calculated as 100% minus percent solids. After adjustment of slopes and intercepts, validation cheese samples were prepared with solver and tested on the calibrated MIR as described above.

RESULTS AND DISCUSSION

Calibration

The 8 sample calibration set created using full-fat annatto colored Cheddar cheese from one factory produced a standard error of prediction (SEP) for fat A, fat B, fat A+B, moisture, protein and salt of 0.099, 0.088, 0.088, 0.182, 0.097, and 0.024, respectively (Table 2.2). The r-squared values were 0.998, 0.998. 0.998, 0.998, 0.996, 0.945, respectively (Table 2.2).

Table 2.2 Regression analysis results for fat A, fat B, fat A+B, protein, lactose, total solids, and moisture from MIR calibration, MIR validation, and NIR

MIR Calibration Set

	N	Fat A	Fat B	Fat A+B	Protein	Salt	Total Solids	Moisture
SDD	8	0.092	0.082	0.018	0.090	0.009	0.169	0.169
SEP	8	0.099	0.088	0.088	0.097	0.009	0.182	0.182
Slope	8	1.069	1.074	1.073	1.050	0.723	0.993	0.993
Intercept	8	-0.113	-0.199	-0.177	0.361	0.486	1.186	-0.470
R^2 value	8	0.998	0.998	0.998	0.996	0.969	0.998	0.998

MIR Validation Set

	N	Fat A	Fat B	Fat A+B	Protein	Salt	Total Solids	Moisture
MD	30	-0.218	-0.239	-0.233	-0.013	0.046	0.104	-0.104
SDD	30	0.182	0.184	0.182	0.144	0.100	0.187	0.187
SEP	30	0.176	0.179	0.177	0.140	0.022	0.176	0.176

NIR Calibration

	N	Fat	Moisture	Salt
MD	30	-0.869	0.249	0.066
SDD	30	0.299	0.374	0.141
SEP	30	0.304	0.366	0.133

MIR = mid infrared
MD = mean difference
SDD = standard deviation of difference
SEP = standard error of prediction

The regression analysis of reference fat values as a function of predicted fat A by MIR had a similar r-squared value to the regression analysis of reference fat values as a function of predicted fat B and as a function of predicted fat A+B by the MIR. The r-squared values for each was 0.998 (Figure 2.1, Figure 2.2, Figure 2.3) which is high and indicates that this model was effective at predicting the fat content. The slopes and intercepts for each of these regression analyses (fat A, fat B, and fat A+B) were close to 1 and 0 (Figure 2.1, Figure 2.2, Figure 2.3), respectively.

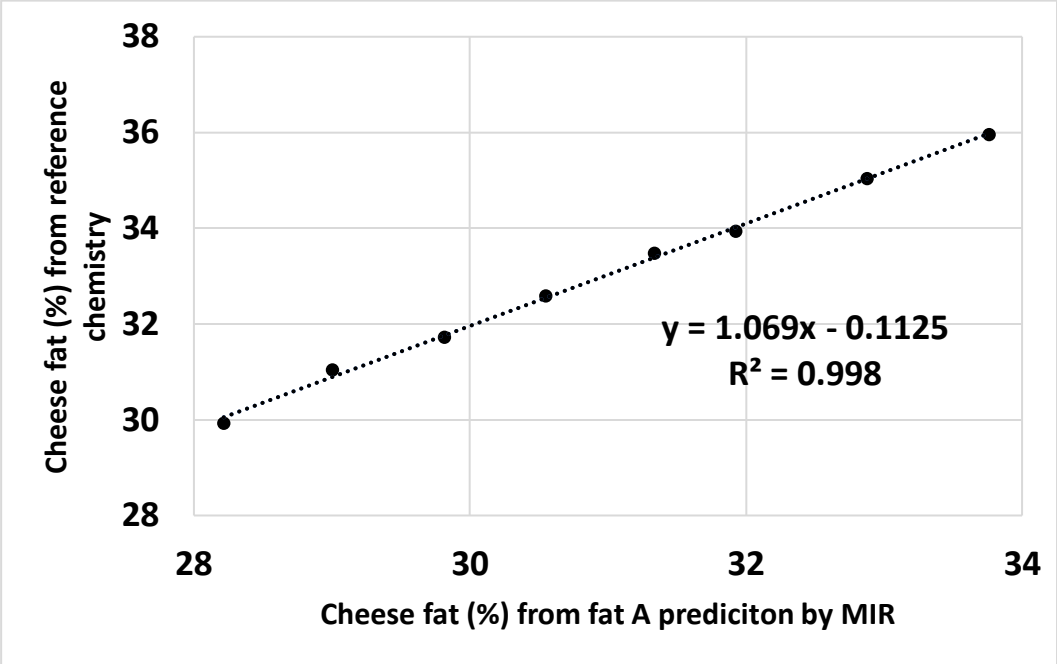

Figure 2.1 Linear regression analysis for reference fat values as a function of predicted fat A by MIR

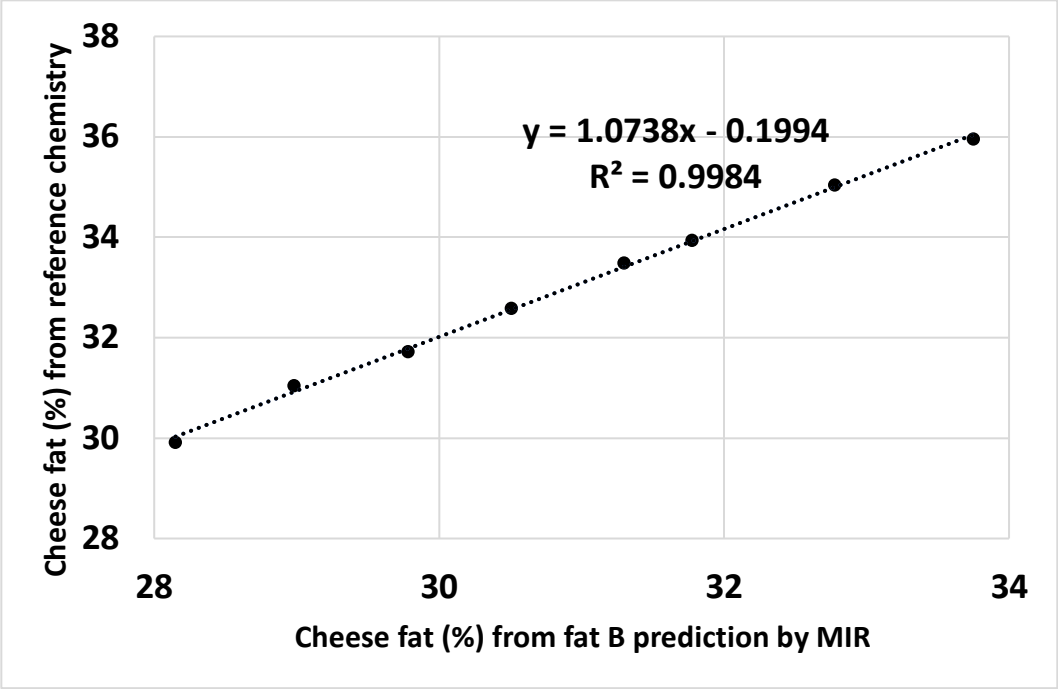

Figure 2.2 Linear regression analysis for reference fat values as a function of predicted fat B by MIR

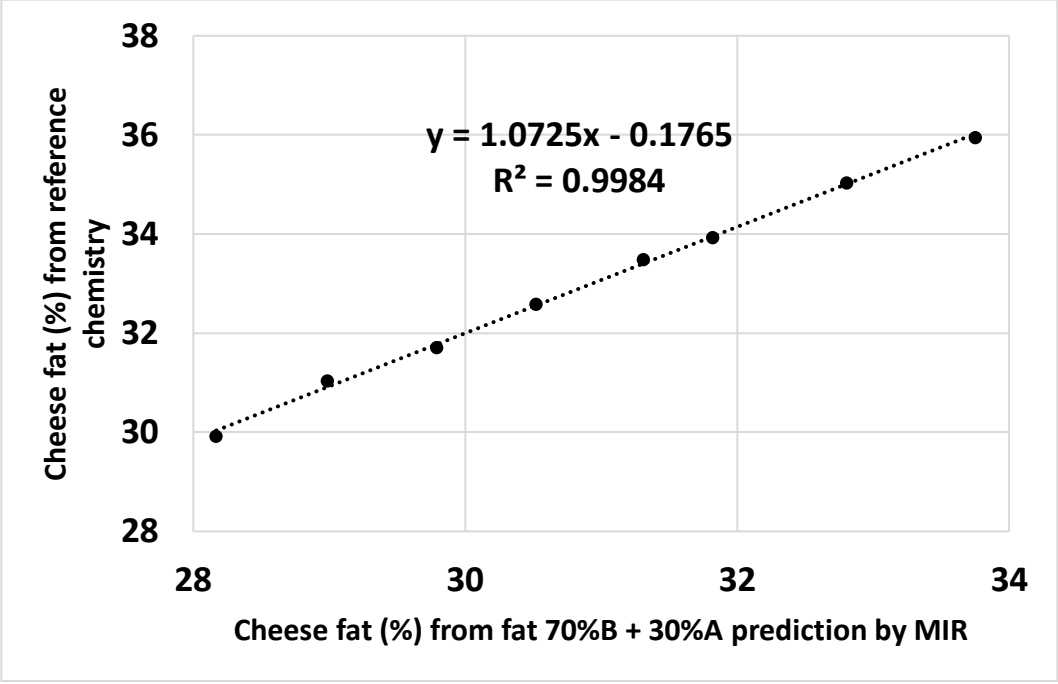

Figure 2.3 Linear regression analysis for reference fat values as a function of predicted fat A+B by MIR

The regression analysis of cheese protein reference values as a function of predicted protein by MIR also had a high r-squared value of 0.996 (Figure 2.4) and a slope and intercept close to 1 and 0 (Figure 2.4), respectively. The same was seen for the regression analysis of the reference values for moisture as a function of predicted moisture by MIR. This regression had a r-squared value of 0.998 (Figure 2.5) and a slope and intercept close to 1 and 0 (Figure 2.5), respectively.

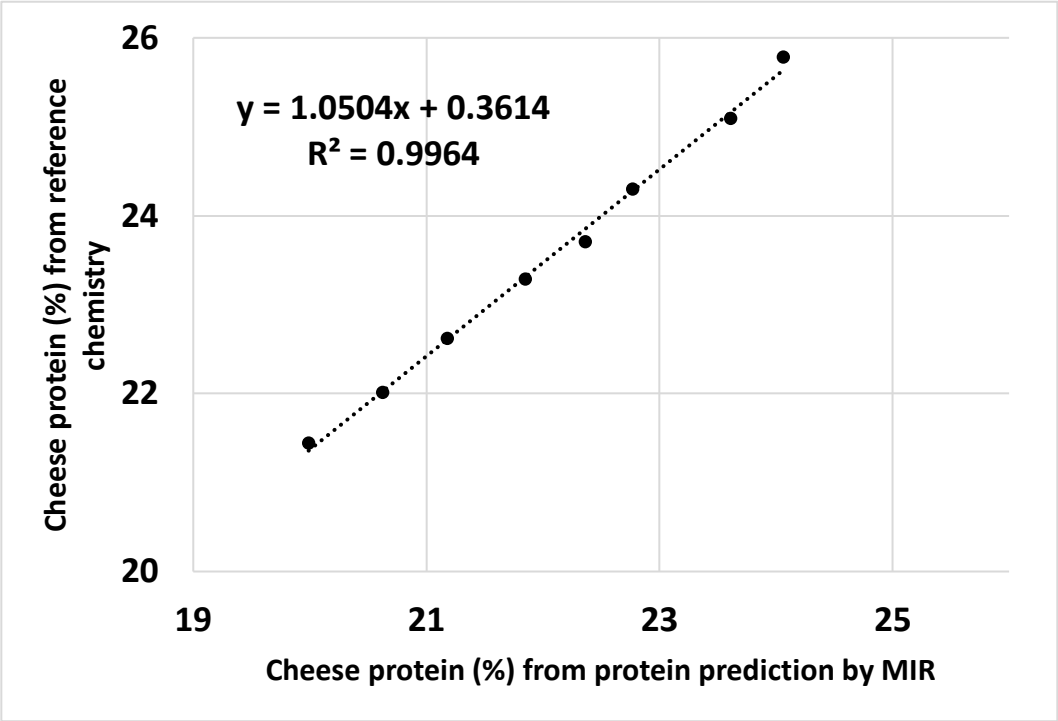

Figure 2.4 Linear regression analysis for reference protein values as a function of predicted protein by MIR

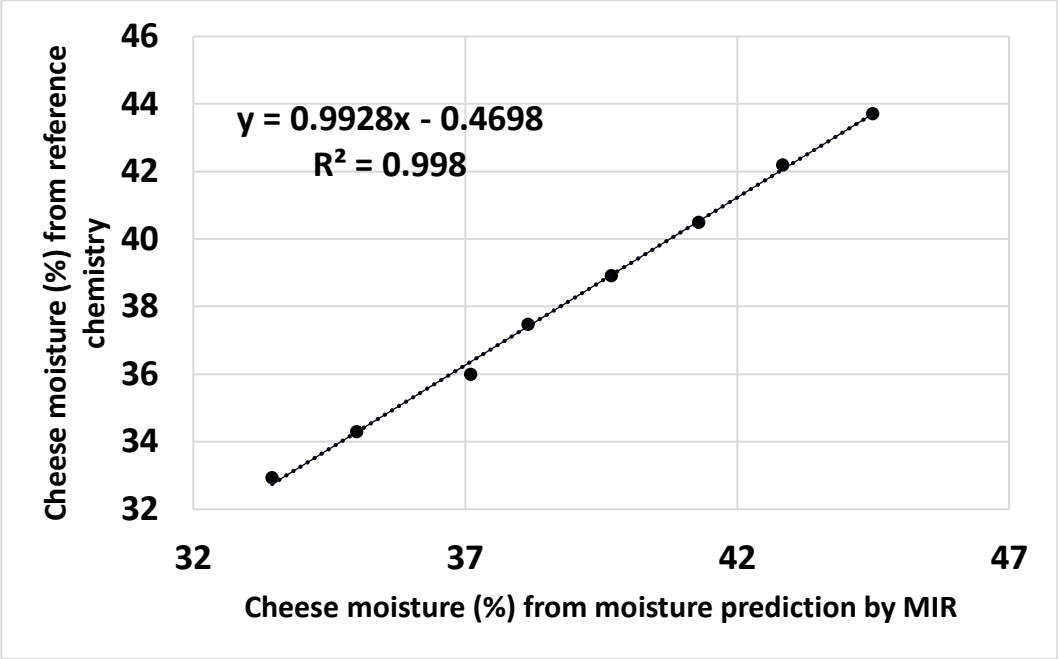

Figure 2.5 Linear regression analysis for reference moisture values as a function of predicted moisture by MIR

The regression analysis of cheese salt reference values as a function of predicted salt by MIR also had a r-squared value of 0.9455 (Figure 2.6) and a slope and intercept close to 1 and 0 (Figure 2.6), respectively. The r-squared value for cheese salt, however, is noticeably lower than the r-squared values for each of the other components. This could potentially be due to an issue with the conductivity sensor that is used to measure salt. Salt is predicted by MIR using an inline conductivity sensor and spectra using an equation developed by Delta Instruments.

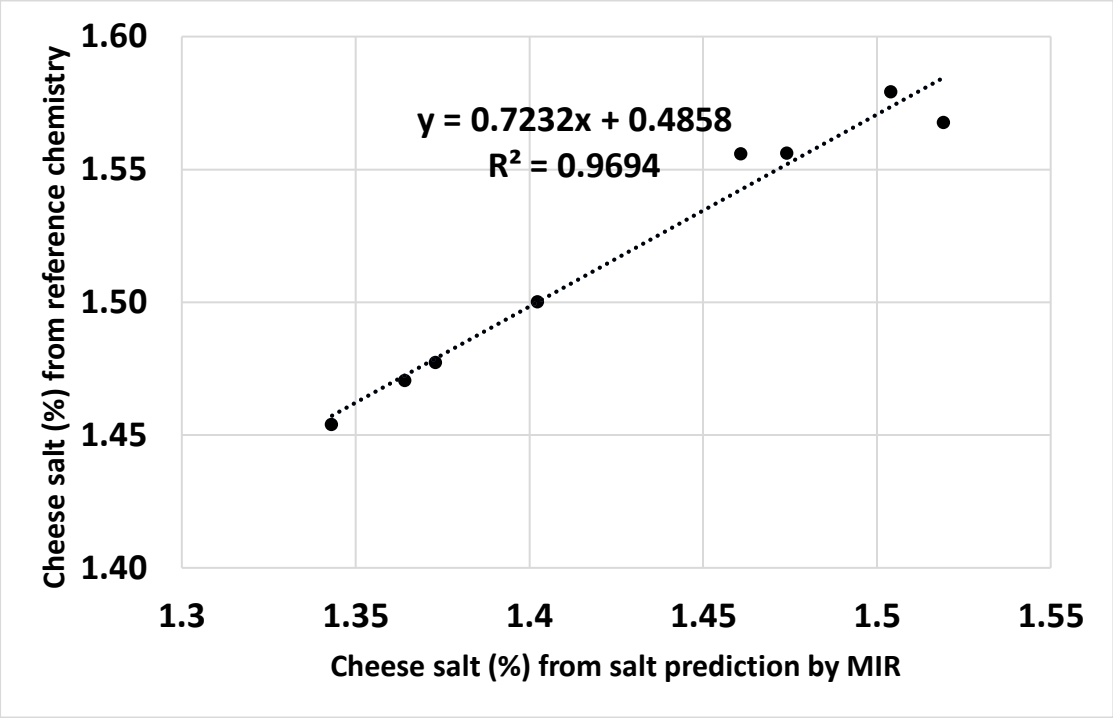

Figure 2.6 Linear regression analysis for reference salt values as a function of predicted salt by MIR

Validation

Validation of the MIR calibration method was done using an independent set of 30 full-fat colored Cheddar cheeses. When testing the same set of 30 validation cheese samples the NIR had standard deviation of difference (SDD) between reference chemistry and instrument production for fat, moisture, and salt of 0.299, 0.374, and 0.141% (Table 2.2), respectively, while the SDD for the MIR for fat A+B (which had the best performance of all the fat measurements on the MIR), moisture, and salt were 0.182, 0.187, 0.04 % (Table 2.2), respectively. The MIR calibration performed better on the validation samples than the NIR calibration in the factory QC laboratory that produced the cheese.

At the time of this study, the cheese plant providing samples was not analyzing their cheese for protein content. The MIR validation set SEP and SDD for protein

were 0.140 and 0.144 (Table 2.2), respectively, suggesting that MIR could be an effective way for cheese plants to easily measure protein in their cheese.

The mean difference (MD) between the MIR prediction and the reference chemistry for fat and moisture in the MIR validation were smaller than the MD for the NIR validation (Table 2.2). The MD for fat in the NIR validation was -0.87% (Table 2.2). The MD between the MIR predicted value and the fat reference for fat A, fat B, and fat A+B were -0.22, -0.24, and -0.23% (Table 2.2), respectively, which were all much lower than the MD for the NIR validation result for fat of -0.87%. The MD from reference chemistry for moisture and salt for the MIR were -0.10 and -0.004%, respectively while the MD from reference chemistry for moisture and salt for the NIR were 0.25 and 0.07%, respectively (Table 2.2).

The MIR results were also compared to the reference chemistry for each sample. The residual plot of the differences between the fat A MIR value minus the fat reference value as a function of the fat reference chemistry does not show a linear relationship (Figure 2.7) Similar plots for fat B, fat A + B, protein, and moisture are shown in Figures 2.8, 2.9, 2.10, and 2.11, respectively. A scattered, nonlinear relationship is also seen for fat B and fat A+B (Figure 2.8, Figure 2.9). The residual graphs for fat A, fat B, and fat A+B have a slope of -0.1135, -0.1329, and -0.1269, respectively, which are close to zero (Figure 2.7, Figure 2.8, Figure 2.9). This indicates that there is little to no linear relationship between the residuals and the reference chemistry. The residual graphs for fat A, fat B, and fat A+B also have r-squared values of 0.0448, 0.0597, and 0.0559, respectively (Figure 2.7, Figure 2.8,

Figure 2.9). The low r-squared values indicate that the calibration set is a good fit for the population of unknown samples.

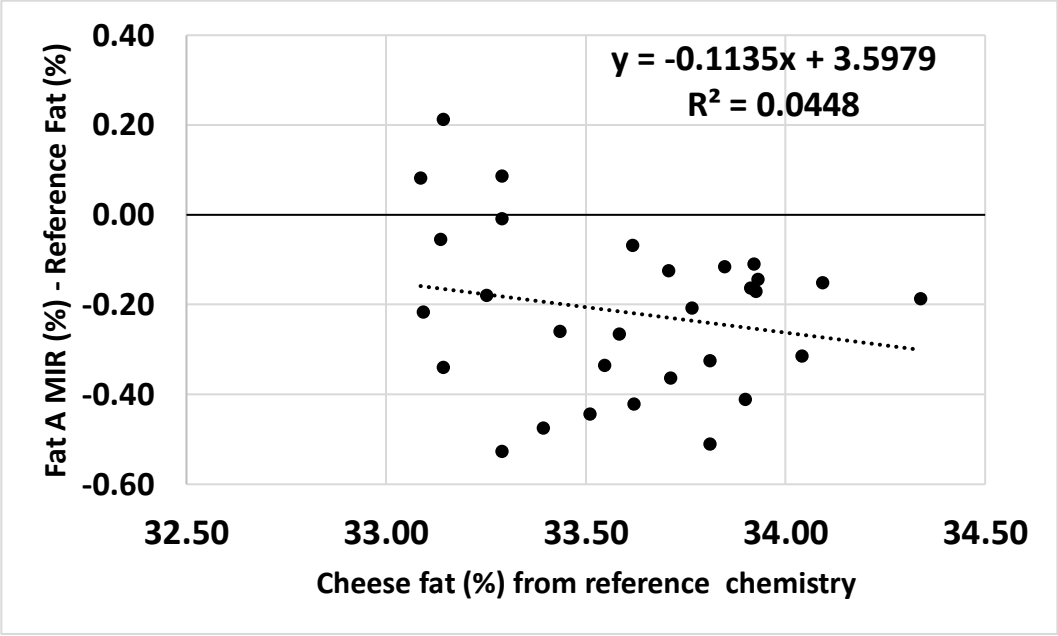

Figure 2.7 Residuals of the difference between fat A MIR values and reference fat values as a function of the reference fat values

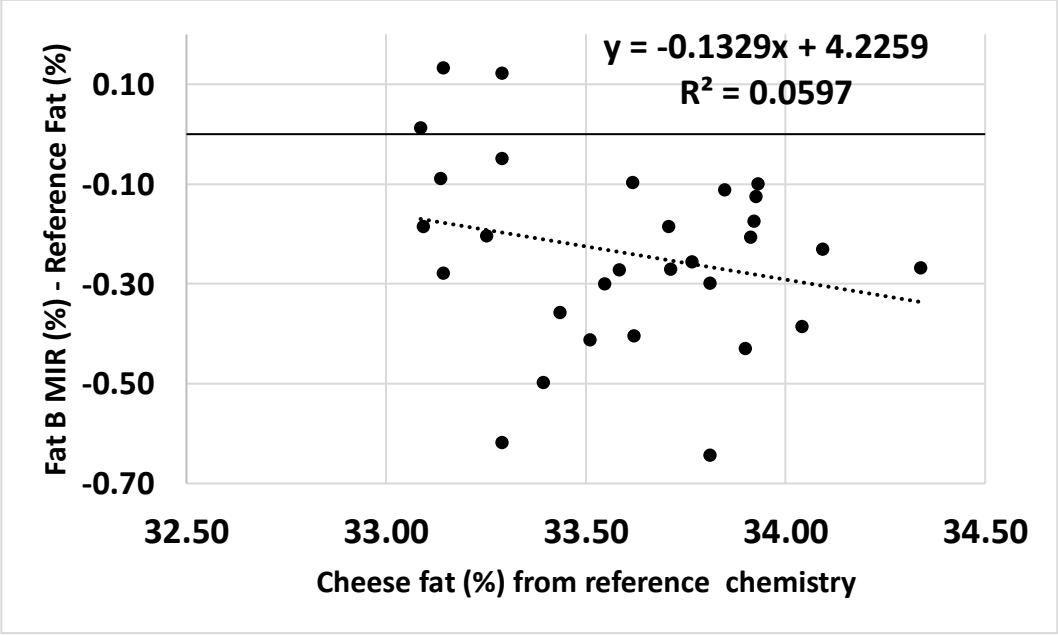

Figure 2.8 Residuals of the difference between fat B MIR values and reference fat values as a function of the reference fat values

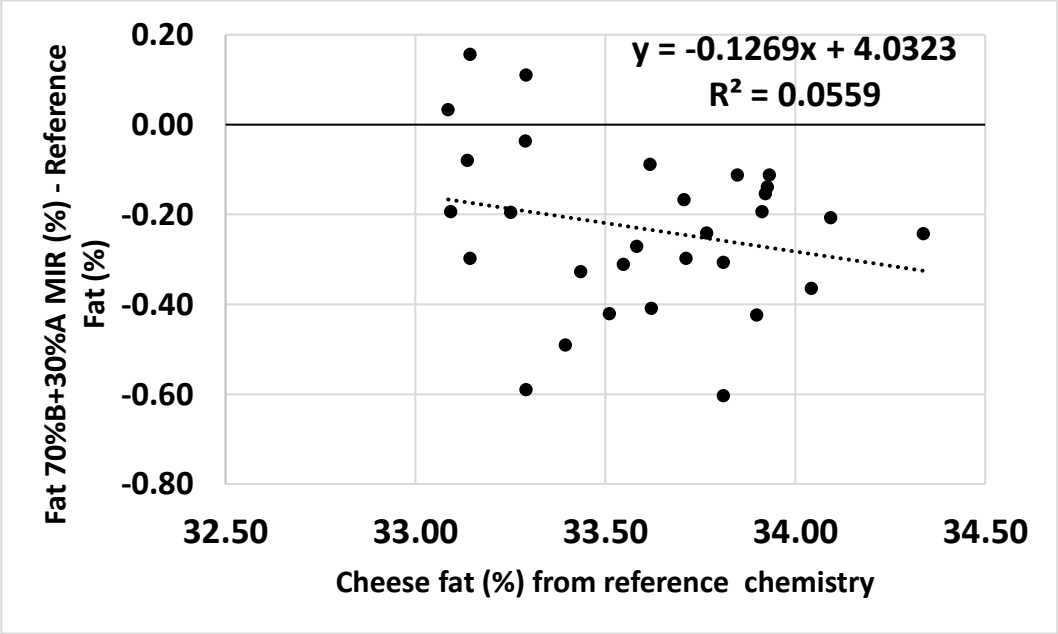

Figure 2.9 Residuals of the difference between fat A+B MIR values and reference fat values as a function of the reference fat values

The residual plot of the differences between the protein MIR value minus the protein reference value as a function of the protein reference chemistry does not show a strong linear relationship (Figure 2.10). The slope of the regression is -0.3944 which is close to zero and the r-squared value is 0.2524 indicating that there is a weak linear relationship between the residuals and the reference chemistry (Figure 2.10).

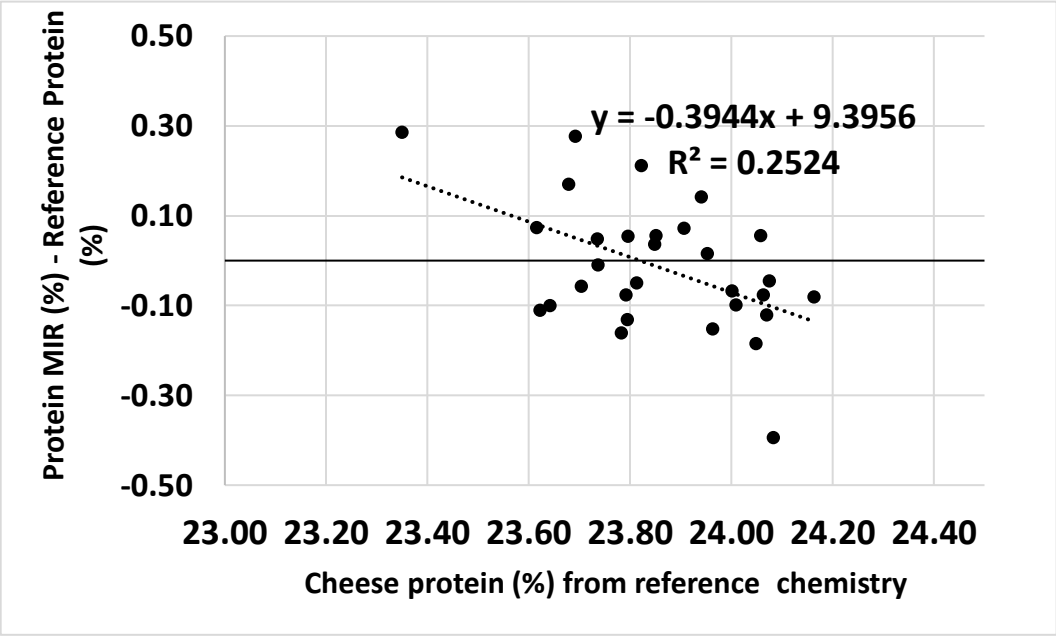

Figure 2.10 Residuals of the difference between protein MIR values and reference protein values as a function of the reference protein values

Similar to fat and protein, the residual plot for the difference between the adjusted moisture MIR value minus the moisture reference value as a function of the moisture reference chemistry is scattered and does not show a linear relationship (Figure 2.11). The slope of this regression is -0.0337 and the r-squared is 0.0051 (Figure 2.11). As the slope is close to zero, and the r-squared is low, it indicates that there is no linear relationship between the residuals and the reference values. This means that the calibration set is a good fit for the population of unknown samples.

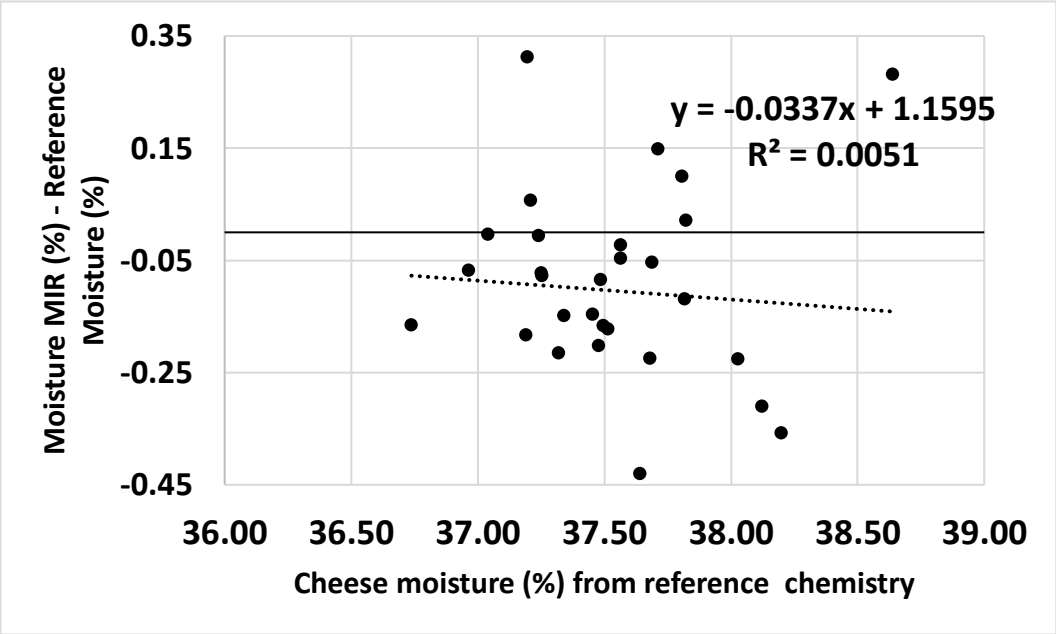

Figure 2.11 Residuals of the difference between moisture MIR values and reference moisture values as a function of the reference moisture values

The residual plot of the differences between the salt MIR value minus the salt reference value as a function of the salt reference chemistry is scattered and does not show a linear relationship (Figure 2.12). The slope of the regression is -0.0961 and the r-squared is 0.2883 (Figure 2.12). The slope close to zero and the low r-squared show that there is a weak linear relationship indicating that the calibration set is a good fit for the population of unknown samples.

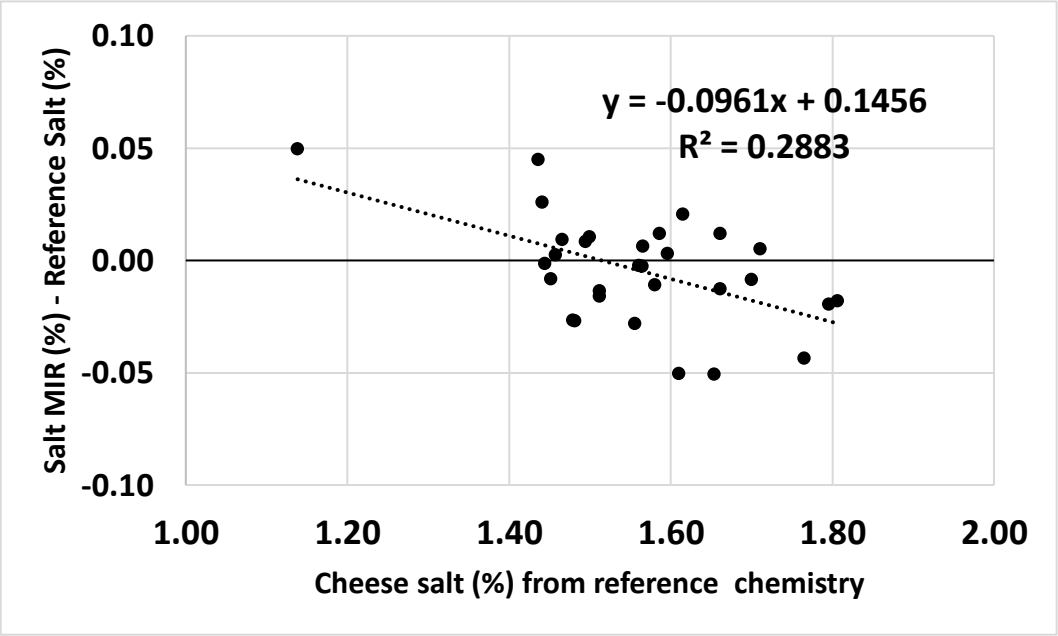

Figure 2.12 Residuals of the difference between salt MIR values and reference salt values as a function of the reference salt values

The NIR results for the 30 full-fat colored Cheddar cheese samples were also available to compare to the MIR results. The SEP for the NIR model for fat, moisture, and salt were 0.304, 0.366, and 0.133 (Table 2.2), respectively. These values were higher than the SEP for the MIR calibration model for fat A+B (which had the best performance of all the fat measurements on the MIR), moisture, and salt which were 0.088, 0.182, and 0.024 (Table 2.2), respectively. It is interesting to note that the cheese plants QC salt analysis was even worse than the MIR results. The SEP for the MIR validation samples were also lower than the SEP for the NIR calibration at 0.177, 0.176, and 0.022 (Table 2.2), respectively. This indicates that the MIR calibration out performs the NIR calibration in predicting fat, moisture, and salt values of unknown samples.

The SEP values for MIR using the calibration set method described in the present study are comparable to or better than those reported by Holroyd (2011) for NIR and those seen on the NIR instrument in the cheese plant from this study. This indicates the effectiveness of MIR for the rapid analysis of cheese using a calibration approach in which calibration sets are made centrally and then distributed to plants, as is currently done with milk calibration sets.

DISCUSSION

Calibrating a MIR milk analyzer for cheese analysis would be very different than calibrating a NIR instrument for cheese analysis. To calibrate a NIR instrument, each cheese type within each cheese plant requires a PLS calibration model to be created for each measured parameter. Developing a PLS model requires reference chemistry data on 200 to 400 cheeses for each cheese variety and for each parameter within those cheese types to be measured (McKenna 2001; Barbano and Lynch 2006). Performing accurate reference chemistry testing requires skilled staff and extra equipment and supplies which makes creating accurate PLS models for NIR calibration in a cheese plant very difficult. Most cheese factories send their calibration samples to an outside commercial lab and pay for reference chemistry analysis on a large number of cheeses. NIR calibrations are also not typically transferable between NIR instruments and are therefore cheese type and cheese plant specific. Some, but not all, NIR manufacturers have produced a global PLS calibration that would only require reference chemistry on 10 to 20 samples (Holroyd 2011). While this global model does reduce the amount of reference chemistry testing that needs to be done by

cheese plants, the effectiveness of the global PLS model still needs to be determined under the conditions for that factory.

In the present study, a standard MIR milk analyzer, that is normally used to test milk, cream, and whey samples in cheese plant, was used. MIR milk analyzers are very common in cheese plants in order to test liquid dairy products. Margolies and Barbano (2017) reported a method for blending the cheese samples into a liquid that could then be tested using a MIR milk analyzer like a milk or whey sample. This liquification step does result in the MIR method for cheese analysis requiring more preparation time than for direct analysis of cheese with NIR, however in NIR analysis, analyst-to-analyst consistency in grinding can impact the results (Holroyd 2011).

Margolies and Barbano (2017) tested the effectiveness of using the classical filter wavelengths (Kaylegian et al 2009) that are used for measurement of fat and protein in milk analysis instead of developing PLS models and determined that use of classical filter wavelengths was effective. We found that an 8 sample liquid cheese calibration sample set used with classical MIR filter wavelengths worked as well as NIR. This liquid cheese calibration set could be prepared centrally with reference chemistry established in a laboratory that specializes in high quality reference chemistry. Then cheese plants could purchase the calibration set for cheese analysis, exactly as currently done for milk and whey calibration sets. This would reduce the burden on cheese plants to run reference chemistry and would also provide cheese plants with better analytical performance in cheese analysis and hopefully improved control of the cheese manufacturing process.

CONCLUSIONS

A method for creating an 8 sample calibration set by varying the amount of cheese in the cheese plus dissolver solution for MIR analysis was developed. The MIR analysis method calibrated using these 8 liquid cheese calibration samples outperformed the NIR analysis for the same full-fat colored Cheddar cheese. The mean differences (MD) from reference chemistry (IR minus reference chemistry) for a set of 30 unknown Cheddar cheeses for MIR for fat, moisture, and salt were -0.23, -0.10, and 0.004%, respectively, while the MD for NIR were -0.87, 0.25, and 0.07, respectively. The SEP values for fat, moisture, and salt on the NIR on validation samples, were 0.304, 0.366, and 0.133, respectively, and were all higher than the SEP values for the MIR results on the validation samples fat A+B, moisture, and salt, 0.177, 0.176, and 0.022, respectively. The SEP value for MIR estimation of protein versus protein measured by Kjeldahl on the validation samples was 0.14, and the MD and SDD for protein were -0.013% and 0.144, which suggests that MIR could be an effective way for cheese plants to determine protein content of their cheese. In the future studies could determine an effective way to make larger batches of calibration samples in order service more cheese producers efficiently.

ACKNOWLEDGEMENTS

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CHAPTER THREE

DETERMINATION OF FAT, PROTEIN, LACTOSE, AND MOISTURE OF NONFAT DRY MILK USING MID-INFRARED TRANSMITTANCE SPECTROSCOPY

ABSTRACT

The objective of our work was to develop and evaluate the performance of the rapid determination of fat, protein, lactose, and moisture of nonfat dry milk (NFDM) powders using mid-infrared transmittance analysis. NFDM powder was blended with lab grade (LG) water to achieve a uniform, particle free dispersion with a composition similar to milk and could be analyzed using a MIR transmittance milk analyzer. NFDM powder samples (14) were reconstituted and analyzed using reference chemistry methods for fat (Mojonnier ether extraction), protein (Kjeldahl), lactose (enzymatic method), and moisture (oven-drying total solids). The reference values were calculated using the weight/weight dilution factor of the powder plus LG water. These same powder samples were run on a Fourier transform mid-infrared (FT-MIR) to create a calibration. Moisture, fat A+B, protein, and lactose had SEP values of 0.283, 0.022, 0.1150, and 0.206, respectively for the calibration. FT-MIR may be used to measure the components of NFDM powders.

INTRODUCTION

Measurement of lactose, protein, fat, and moisture of dairy powders is important in quality control in large scale milk powder manufacturing factories. While the chemical reference methods for measurement of fat (Mojonnier ether extraction), protein (Kjeldahl), moisture (vacuum oven drying), and lactose (enzymatic method) are very accurate, they require lots of time and resources to perform. This makes them not a feasible option for the rapid analysis of a large number of dairy powder samples in a large processing plant. For this reason, more rapid and cost-effective methods for analysis such as near infrared (NIR) analysis (Frankhuizen 2008) are commonly used in dairy plants that manufacture milk powder.

Rapid methods like NIR increase the speed of running analysis on powder samples, however NIR is a secondary testing method. This means that NIR is dependent on calibration with accurate reference chemistry. NIR methods utilize partial least squares (PLS) models that are developed locally in each factory (Workman Jr. 2008) and typically require chemical analysis of several hundred samples to develop each PLS model for each NIR analyzer. NIR utilizes reflectance measurements which means it measures the intensity of the light reflected from the sample surface (Frankhuizen 2008). This makes sample preparation relatively easy for NIR analysis. NIR analysis utilizes wavelengths from 12,500 cm-1 to 4,000 cm-1, however it uses mainly combination and overtone bands (Siesler 2008). Due to the use of combination and overtone bands, NIR has relatively low specificity. While NIR offers a benefit in ease of sample preparation and speed of analysis, the work needed

to develop PLS calibration models for each dairy powder type within each plant is a challenge.

Sample selection is one of the most important aspects of creating an accurate PLS calibration. When selecting samples for calibration, it is necessary to select sample that provide a complete range of the component concentration that is evenly distributed (Workman Jr. 2008). It is essential to utilize calibration samples that provide values across the range that would be expected to be seen in unknown samples for each component because this creates an accurate model with good predictive qualities. In addition, accurate reference chemistry values are need for each sample and typically the reference testing has to be done by an outside laboratory and not by the manufacturing plant. This can be costly.

Mid infrared (MIR) milk analyzers are very common in dairy processing facilities for analyzing liquid dairy products like milk, cream, and liquid whey. Utilizing MIR for the measurement of fat and protein is well developed (Kaylegian et al. 2006b, 2009) and MIR analysis is an accepted official method for milk analysis (AOAC International 2012, method number 972.16). MIR is similar to NIR in that it is a rapid secondary testing method that requires calibration with accurate reference chemistry, however it is different from NIR in many important ways. MIR measures fat, protein, and lactose utilizing well documented wavelengths (Kaylegian et al. 2009). This is distinctly different than the combination and overtone bands used in NIR analysis and causes MIR to have a much higher specificity than NIR analysis.

It is possible that these same principles and equipment used for milk analysis could be applied to the analysis of rehydrated milk powders using MIR. The main challenge is sample preparation as MIR milk analyzer flow systems can only measure liquids. Utilizing an MIR with reconstituted powder samples creates the potential advantage of being able to make liquid calibration samples that are prepared centrally and used by multiple instruments at multiple factories as is already done for milk (Kaylegian et al. 2006a, 2006b). An approach using liquid calibration samples could be attractive for manufacturing plants as it would eliminate the need for large amounts of reference chemistry analysis on powder samples by elimination of the need to develop PLS models for NIR equipment. MIR liquid analyzers are already available in almost all dairy processing plants. The objective of this work was to develop and evaluate the performance of MIR analysis for measuring lactose, protein, fat, and moisture in reconstituted dairy powders.

MATERIALS AND METHODS

Experimental Design

Nonfat dry milk (**NFDM**) powders were diluted 10:1 (w/w) with 40°C lab grade (LG) water, reconstituted overnight to allow complete hydration, and then pumped through a MIR milk analyzer (LactoScope FTIR Advanced, Delta Instruments, Kelvinlaan 3, 9207 JB Drachten, Netherlands). Calibration of the MIR milk analyzer was done using 14 NFDM samples that represented different individual batches of NFDM from one dairy processing facility. Low heat and high heat NFDM samples were included in the calibration. For each NFDM sample, reference chemistry

was determined for fat, protein, total solids, and lactose. Using this reference chemistry, a linear slope and intercept adjustment was made for estimation of fat (for fat A, fat B, and 70% B + 30% A), protein, lactose, and total solids. Moisture was calculated as 100 minus total solids.

Nonfat Dry Milk Analysis

Reconstitution of Powders. Lab grade (LG) water was heated to 40 ± 2°C in a water bath. The weight of a 90 g snap lid vial (CPP03EDM-CL, Capitol Plastic Products, 1030 Riverfront Center, Amsterdam, NY 12010) was recorded to four decimal places. Approximately 10 g of NFDM sample was added to it and the weight of the vial plus the powder was recorded to four decimal places. The weight of the powder added was calculated by difference between the weight of the empty vial and the weight of the vial plus powder. Then 90 g of the warm LG water was added slowly to the vial with the powder and the weight of the vial plus powder plus water was recorded to four decimal places. The weight of the water was calculated by difference as described above. Then the vial was inverted to disperse the powder into the liquid and placed in a 40 ± 2°C water bath to complete hydration. Not all of the powder dispersed into the water at first. The vial was kept in the water bath and inverted periodically until all powder was dispersed into the water. Once the powders completed dissolved, the vial was placed in an ice bath and quickly cooled to 4°C before being refrigerated overnight to allow for complete hydration. These steps were repeated for all powder samples that were used for the calibration.

Chemical Reference Methods

Total Solids. The total solids content of the NFDM samples was determined using the reference method of 4 h at 100°C in a forced air oven using 2 g of sample (AOAC 2012, 925.23). Oven drying was performed in duplicate for each NFDM sample. The moisture content was determined as 100 minus the total solids final result for each NFDM sample.

Fat. Fat analysis of the powder samples was performed on the reconstituted powder samples that had been reconstituted in 40 ± 2°C LG water. Fat was determined on all of the NFDM powder samples. As described by Barbano et al. (1988) for milk samples, the reconstituted powders (10g) were weighed into a Mojonnier ether extraction flask and were tested in duplicate. To calculated fat of each individual powder on the dry powder basis, the final result was divided by the reference moisture value determined using oven drying and multiplied by 100.

Protein. Determination of the nitrogen content of the NFDM samples was done using the Kjeldahl method (Barbano and Clark 1990, Lynch and Barbano 1999) on the reconstituted powder samples. Using the total nitrogen content, the total crude protein content was determined by multiplying the total nitrogen content by 6.38 (crude TN x 6.38). Kjeldahl was run in duplicate on the reconstituted powder samples. The official method for milk (Barbano and Clark 1990) was the same Kjeldahl method utilized for analysis of the reconstituted powders. The protein content for each sample on the dry powder basis was calculated exactly as described above for fat.

Lactose. The enzymatic method (AOAC 2012 2006.06) was used to determine the lactose content of the NFDM samples. Lactose determination was performed on

the reconstituted powder samples (2 g) in duplicate. Lactose on a dry powder basis in each of the NFDM samples was calculated as described above for fat.

Mid-infrared Analysis

The 14 NFDM samples were tested on the MIR (Delta Instruments, Model FTA, Drachten, The Netherlands). The pre-calibration of the MIR and the setting the primary slopes for fat A, fat B, lactose, and protein were set as described by Lynch et al. (2006). The optimized filter wavelengths and bandwidths used for fat B, fat A, lactose, and protein were the same as those described by Kaylegian et al. (2009). Like milk analysis, fat A+B was determined as [(0.7 x fat B corrected) + (0.3 x fat A corrected)]. The same intercorrection factors (Barbano and Clark 1989, Lynch et al. 2006) as those used for milk analysis were used on the instrument in this study. Table 3.1 shows the sample and reference wavelengths and bandwidth, scale, and intercorrection factors for fat A, fat B, and protein. Total solids was calculated as the sum of [(fat) + (protein) + (salt)] with a slope and intercept adjustment made using the reference chemistry results from oven drying. The calculated reference values for the powders on a dry powder basis were used to make slope and intercept adjustments for fat A, fat B, 70% fat B + 30% fat A combination, protein, total solids, and lactose to achieve a mean difference of zero between instrument prediction and the reference chemistry mean for all 14 samples. The standard deviation of residual difference between the reference chemistry and the instrument prediction was calculated for each parameter. Moisture was calculated as 100 minus total solids.

Table 3.1 MIR wavenumbers (cm^{-1}), scale, offset, and intercorrection factors for fat B, lactose, protein, and fat A traditional virtual filter wavelengths

Sample Filters	1st wave number	Last wave number	Scale	Offset
Fat B	2838	2864	34.6352	0.00
Lactose	1038	1058	17.6939	0.00
Protein	1531	1551	21.6284	0.00
Fat A	1740	1756	21.8407	0.00
Reference filters	1st wave number	Last wave number	Scale	Offset
Fat B	2800	2824	-34.6352	0.00
Lactose	1286	1300	-17.6939	0.00
Protein	1485	1497	-21.6284	0.00
Fat A	1783	1799	-21.8407	0.00

Intercorrection Factors				
	Fat B	Lactose	Protein	Fat A
Fat B	1.000	-0.149	-0.057	0.000
Lactose	0.044	1.000	0.016	0.000
Protein	0.064	0.052	1.000	0.000
Fat A	0.000	0.024	0.020	1.000

RESULTS AND DISCUSSION

Calibration

The MIR analysis results from 14 NFDM powders had a standard error of prediction (SEP) for fat A, fat B, fat A+B, protein, lactose, and moisture of 0.021, 0.026, 0.022, 0.115, 0.206, and 0.283, respectively (Table 3.2). The r-squared values were 0.910, 0.855, 0.910, 0.991, 0.960, and 0.967, respectively (Table 3.2).

Table 3.2 Regression analysis results for fat A, fat B, fat A+B, protein, lactose, total solids, and moisture from MIR calibration

MIR Calibration with Powder Samples

	N	Fat A	Fat B	Fat A+B	Protein	Lactose	Total Solids	Moisture
SDD	14	0.020	0.025	0.021	0.110	0.211	0.273	0.271
SEP	14	0.021	0.026	0.022	0.115	0.206	0.284	0.283
Slope	14	1.000	1.000	1.000	1.000	1.084	1.000	1.000
Intercept	14	0.000	0.000	0.000	0.000	-4.191	0.013	0.000
R^2 value	14	0.910	0.855	0.910	0.991	0.960	0.967	0.967

The regression analysis of reference fat values as a function of predicted fat B by MIR had a lower r-squared value than the regression analysis of reference fat values as a function of predicted fat A and fat A+B by MIR. The r-squared values for fat A and fat A+B were both 0.910 (Figure 3.2 and Figure 3.3) while the r-squared value for fat B was 0.855 (Figure 3.1). The slope and intercept values for the regression analysis for fat A, fat B, and fat B+A were all close to 1 and 0 (Figure 3.1, Figure 3.2, Figure 3.3), respectively. Fat A, fat B, and fat A+B are responding similarly which is what we would expect to see in a MIR model.

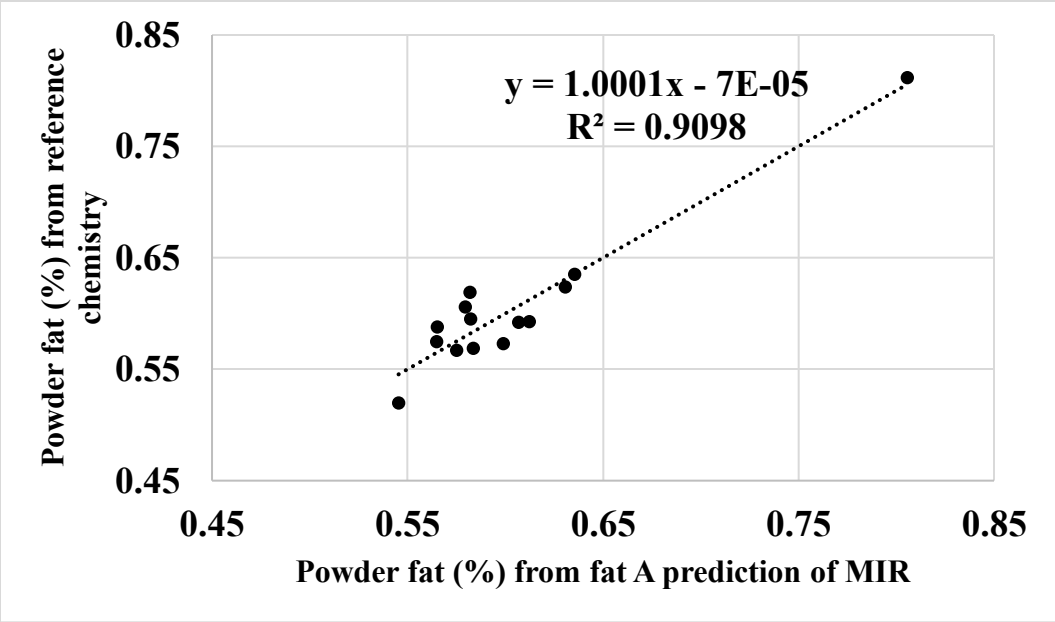

Figure 3.1 Linear regression analysis for reference fat values as a function of predicted fat A by MIR

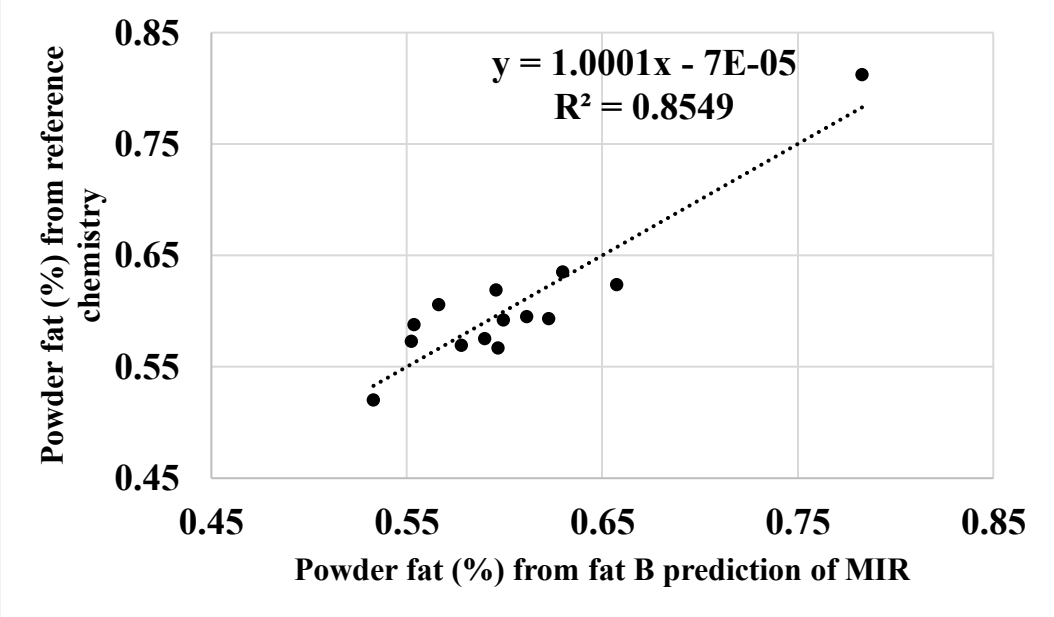

Figure 3.2 Linear regression analysis for reference fat values as a function of predicted fat B by MIR

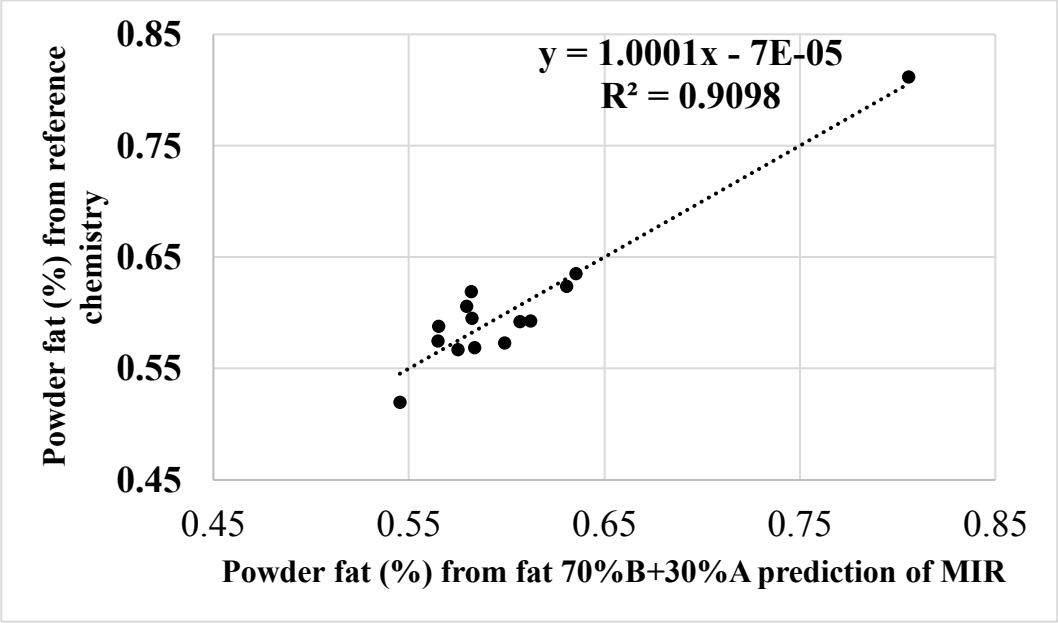

Figure 3.3 Linear regression analysis for reference fat values as a function of predicted fat A+B by MIR

The regression analysis of reference protein values as a function of predicted protein by MIR also had a high r-squared value of 0.991 (Figure 3.4). The slope and intercept of the protein regression analysis were also close to 1 and 0 (Figure 3.4), respectively. The regression analysis of reference moisture values as a function of predicted moisture by MIR showed the same pattern. The r-squared value was very high at 0.967 (Figure 3.6) and the slope and intercept were close to 1 and 0 (Figure 3.6), respectively. A high r-squared value was also seen for the regression analysis of lactose at 0.9597 (Figure 3.5). These values all indicate that the MIR model is effective at predicting fat, protein, lactose, and moisture.

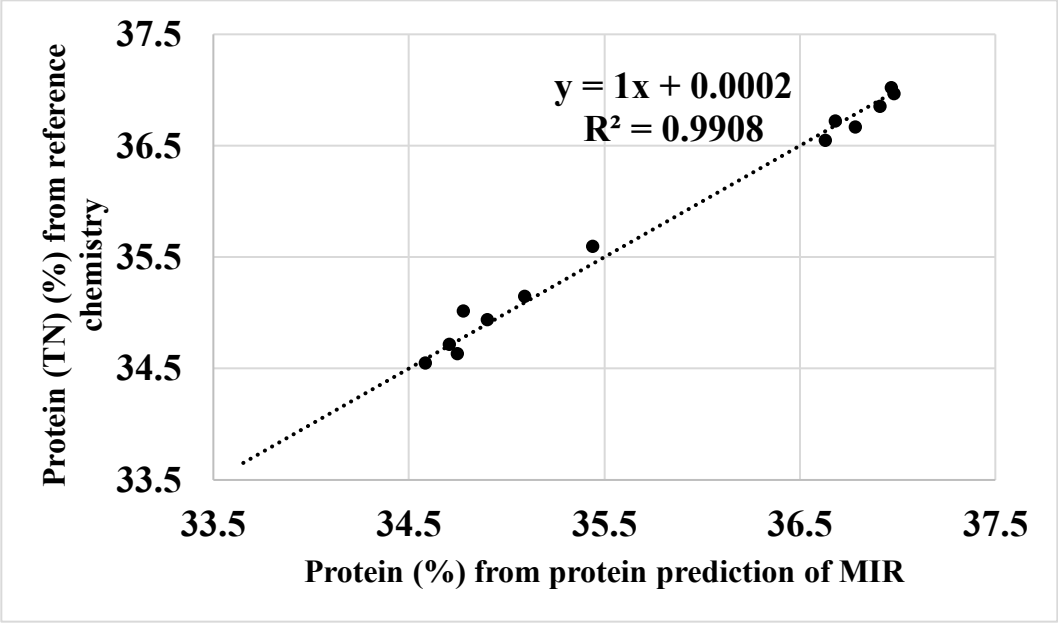

Figure 3.4 Linear regression analysis for reference protein values as a function of predicted protein by MIR

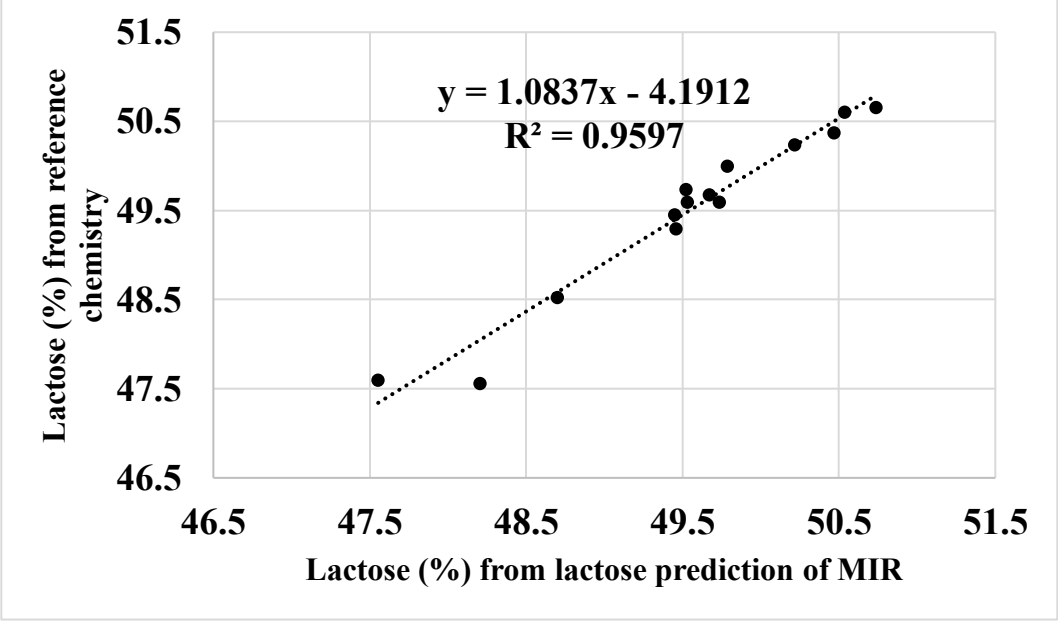

Figure 3.5 Linear regression analysis for reference lactose values as a function of predicted lactose by MIR

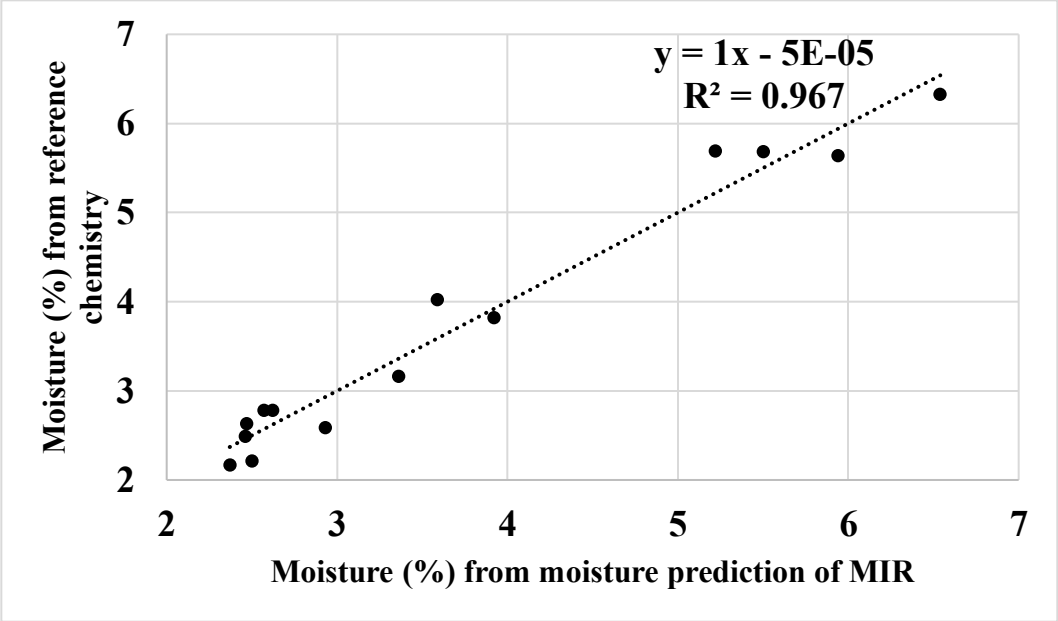

Figure 3.6 Linear regression analysis for reference moisture values as a function of predicted moisture by MIR

The SEP values for the MIR calibration were lower overall than SEP values for direct analysis of skim milk powder by NIR performed by Frankhuizen (2008). Frankhuizen (2008) reported SEP values for a NIR for fat, protein, lactose, and moisture of 0.10, 0.25, 0.44, and 0.11, respectively using 159 skim milk powder samples. The SEP values from the MIR calibration using 14 NFDM powders in the current study for were lower (Table 3.2) for fat, protein, lactose, and moisture (0.022, 0.115, 0.206, and 0.283, respectively). Thus, MIR analysis of reconstituted NFDM powders may be a more accurate alternative to NIR analysis of milk powders.

CONCLUSIONS

Calibration of a MIR milk analyzer using 14 NFDM samples dissolved in water was more effective than literature indicates NIR analysis is. The MIR was

calibrated to provide results of the dry powder. The SEP values for the MIR calibration for fat A+B (which had the best performance of all the fat measurements on the MIR), protein, lactose, and moisture were 0.022, 0.115, 0.206, and 0.283 respectively. A validation study of the method is needed using a population of validation samples collected from many different manufacturers of milk powder. In the future, studies could be done to determine how to create dairy powder calibration samples, similar to what is currently done for MIR milk calibration samples. The use of MIR as a method to determine other components of interest to dairy powder producers like undenatured whey protein could also be explored.

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CHAPTER FOUR

CONCLUSIONS AND FUTURE WORK

A method for creating an 8 sample calibration set by varying the amount of cheese in the cheese plus dissolver solution for MIR analysis was developed. The MIR analysis method calibrated with these samples outperformed the NIR analysis for the same full-fat colored Cheddar cheese. The mean differences (MD) from reference chemistry (IR minus reference chemistry) for a set of 30 unknown Cheddar cheeses for MIR for fat, moisture, and salt were -0.23, -0.10, and -0.004, respectively, while the MD for NIR were -0.87, 0.25, and 0.07, respectively. The SEP values for fat, moisture, and salt on the NIR on validation samples, were 0.304, 0.366, and 0.133, respectively, and were all higher than the SEP values for the MIR results on the validation samples fat A+B, moisture, and salt, 0.177, 0.176, and 0.022, respectively. The SEP value for MIR estimation of protein versus protein measured by Kjeldahl on the validation samples was 0.14, and the MD and SDD for protein were -0.013 and 0.144, which suggests that MIR using the 8 liquid cheese calibration approach could be an effective way for cheese plants to determine protein content of their cheese. This would be more practical, and potentially produce more accurate results, for cheese plants than development of PLS models for NIR calibration for cheese analysis.

A method for analyzing reconstituted nonfat dry milk powders using a MIR milk analyzer gave better SEP values than published literature values for NIR analysis of milk powders. The SEP values for the MIR calibration for fat A+B (which had the

best performance of all the fat measurements on the MIR), protein, lactose, and moisture were 0.022, 0.115, 0.206, and 0.283, respectively.

In the future, methods for larger scale cheese calibration sample set production should be developed and evaluated. This method could be used to create centrally produced cheese calibration sample sets that can be distributed to several factories, similar to MIR calibration for milk analysis

In the future, a validation study of the method used for the analysis of nonfat dry milk powders is needed using a population of validation samples collected from multiple manufacturers of milk powder. Studies could be done to determine how to create dairy powder calibration samples, similar to what is currently done for MIR calibration for milk analysis, where calibration sets are centrally produced and then distributed to multiple processes. The potential for the use of MIR as a method to determine other components of interest to dairy powders, e.g. undenatured whey protein could also be explored