Effect of homogenizer performance on accuracy and repeatability of mid-infrared predicted values for major milk components¹

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ABSTRACT

Our objective was to determine the effect of mid-infrared (MIR) homogenizer efficiency on accuracy and repeatability of Fourier transform MIR predicted fat, true protein, and anhydrous lactose determination given by traditional filter and partial least squares (PLS) prediction models. Five homogenizers with different homogenization performance based on laser light-scattering particle size analysis were used. Repeatability and accuracy were determined by conducting 17 sequential readings on milk homogenized externally to the instrument (i.e., control) and unhomogenized milk. Milk component predictions on externally homogenized milks were affected by variation in homogenizer performance, but the magnitude of effect were small (i.e., <0.025%) when milks were pumped through both efficient and inefficient homogenizers within a MIR milk analyzer. Variation in the in-line MIR homogenizer performance on unhomogenized milks had a much larger effect on accuracy of component testing than on repeatability. The increase of particle size distribution [d(0.9)] from 1.35 to 3.03 μm (i.e., fat globule diameter above which 10% of the volume of fat is contained) due to poor homogenization affected fat tests the most; traditional filter based fat B (carbon hydrogen stretch; -0.165%), traditional filter-based fat A (carbonyl stretch; -0.074%), and fat PLS (-0.078%) at a d(0.9) of 3.03 μm . Variation in homogenization efficiency also affected traditional filter-based true protein test ($+0.012\%$), true protein PLS prediction (-0.107%), and traditional filter-based anhydrous lactose test ($+0.027\%$) at a d(0.9) of 3.03 μm . Effects of variation

in homogenization on anhydrous lactose PLS predictions were small. The accuracy of both traditional filter models and PLS models were influenced by poor homogenization. The value of 1.7 μm for a d(0.9) used by the USDA Federal Milk Market laboratories as a criterion to make the decision to replace the homogenizer in a MIR milk analyzer appears to be a reasonable limit, given the magnitude of effect on the accuracy of fat tests. In the future, as new PLS models are developed to measure other components in milk, the sensitivity of the accuracy of the predictions of these models to factors such as variation of homogenizer performance should be determined as part of the ruggedness testing during PLS model development.

Key words: mid-infrared, homogenization, testing accuracy

INTRODUCTION

Mid-infrared (MIR) milk analysis is based on the principle that each specific chemical bond absorbs MIR energy at a specific wavelength, and the measurement of the intensities of the absorption peaks makes it possible to quantify milk components (Goulden, 1964; Biggs, 1967; Biggs et al., 1987). The MIR energy passing through inefficiently homogenized milk can be distorted by the Christiansen light-scattering effect, which causes a shift in the apparent wavelength of maximum absorption by the carbonyl and carbon-hydrogen groups to a longer wavelength affecting the accuracy of MIR readings (Goulden, 1961). This shift in wavelength or light absorbance may have a negative effect on the accuracy of the determination of the concentration of the major components of milk. As a result, quality-assurance programs for MIR milk analysis often include a test to determine if the homogenizer in a MIR milk analyzer is working properly (Lynch et al., 2006). Normally, a laboratory would need to pump an unhomogenized milk through the homogenizer on their infrared milk analyzer, collect the instrument-homogenized milk,

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and send the milk to another laboratory for a laser light-scattering particle size analysis to determine if the homogenizer was functioning properly (Lynch et al., 2006). Di Marzo and Barbano (2016) reported that the systematic shift in the MIR absorbance spectra due to the Christiansen effect could be modeled using partial least squares (PLS) and enable a prediction of particle size distribution $d(0.9)$ in real-time operation of the MIR milk analyzer.

Commercial Homogenization of Milk

The recommended milk temperature for commercial milk homogenization is between 60 to 75°C, which achieves breakage of milk fat into smaller fat globules and reduce the tendency of fat globules to aggregate and rise to the top of container of fluid milk (Trout, 1950; Walstra et al., 2005). If the temperature of homogenization of milk is below the melting point of milk fat (i.e., <40°C), fat will be in the solid state, resulting in incomplete fat dispersion and ineffective homogenization (Trout, 1950; Bylund, 1995). In a typical commercial homogenizer, a high-pressure positive displacement pump forces heated milk through a narrow gap in the homogenizer valve (Mulder and Walstra, 1974; Phipps, 1985). As the milk is forced through the gap at high pressure, the linear velocity of the milk and shear forces increase. Often, the high-velocity milk is projected against a surface to create high turbulence and more shear, leading to a reduction in fat globule size (Mulder and Walstra, 1974; Walstra et al., 2005). The high-pressure positive displacement pump can be equipped with a single piston or with multiple pistons (3, 5, or 7). In a single-piston homogenizer, the valves open and close with every stroke of the piston and the flow pressure goes from zero to the set pressure for that stage and back to zero as the valve opens and closes. The multiple pistons are operated intentionally out of phase to achieve a constant applied pressure, continuous flow of milk, and uniform homogenization. The higher the number of pistons, the more the pressure fluctuations are minimized (Phipps, 1985) and the homogenizer valves are running open continuously with relatively constant pressure decrease across the gap. In this way, the valve wear is minimized and consistent particle size is achieved (Walstra, 1975).

Typical homogenization of pasteurized fluid milk is done with a 2-stage homogenizer with a first-stage pressure of 20 MPa and second-stage pressure of 5 MPa (Walstra et al., 2005). The second valve should always operate at lower pressure (i.e., 20% of the total pressure; Walstra, 1975). The function of the high-pressure first stage is to break fat globules to smaller sizes. The newly formed small fat globules are no longer

exclusively covered with the original milk fat globule membrane. Instead, they are also covered with protein adsorbed from the milk plasma (Walstra et al., 2005). In the turbulent environment created by velocity of the milk and shear forces in the milk exiting the first stage, the small fat globules may start to collide before they are completely covered with protein, leading to fat globule coalescence (Mulder and Walstra, 1974). The first stage creates up to a 10-fold increase of the milk fat-plasma interfacial surface area. If the surface of the newly formed fat globules lacks protein, the small fat globules may easily come together to share protein at their interface, forming clusters (Mulder and Walstra, 1974). The function of the low-pressure second stage is to break up the fat globule clusters (Walstra, 1975; Phipps, 1985; Walstra et al., 2005). At the second stage the pressure is low so that the new surface created is insignificant and new clusters are not formed (Mulder and Walstra, 1974). Enough time needs to be given for the newly formed fat globules to cluster after milk passes through the first-stage valve so that the second-stage valve will be able to fulfill its role in breaking clusters (Walstra et al., 2005). The typical $d(0.9)$ in a commercially homogenized milk is about 1.2 to 1.8 μm (Caplan and Barbano, 2013).

Homogenization in a MIR Milk Analyzer

The MIR homogenizer designs are slightly different than a commercial homogenizer. All homogenizers within MIR milk analyzers are single-piston homogenizers; thus, the pressure across the homogenizer stages is going from zero to full pressure and back to zero with every pump stroke during the pumping of a single milk sample. Some MIR homogenizer designs have the springs in the milk flow, and this is different than commercial homogenizers. A 2-stage homogenizer with the springs in the milk flow is shown in Figure 1. This design of homogenizer has been used by Multispec (no longer in business), Bentley Instruments (Chaska, MN), and Delta Instruments (Drachten, the Netherlands). This type of homogenizer includes the 2 stages connected in series within a single homogenizer housing, which is mounted in the MIR as shown in Figure 1A. In Figure 1B, the internal parts of the homogenizer (#1) are shown. The strength of the first- (#8) and second-stage (#13) springs are different (Figure 1B). Heated milk (about 40°C) is pumped through the homogenizer and reaches the first-stage seat (#4) and the ball (#7). Higher milk temperatures are not used in MIR milk analyzers because of the negative effect of high milk temperatures on the cuvette. The high milk pressure (about 15 MPa) operating against the spring (#8) forces the ball off the seat and opens a narrow

Figure 1. Two-stage homogenizer design with the springs in the milk flow: (A) homogenizer mounted in the instrument, and (B) parts of the 2 stages of the homogenizer. Parts include the (1) assembled homogenizer, (2) homogenizer housing, (3) first-stage support disc, (4) first-stage seat, (5) O-ring, (6) cylindrical spring guide, (7) first-stage ruby ball, (8) first-stage spring, (9) second-stage support disc, (10) second-stage seat, (11) second-stage ruby ball, (12) O-ring, (13) second-stage spring, and (14) homogenizer outlet cap. The strength of the spring (8) and (13) are different and provide pressures of 15 MPa in the first stage and 3 MPa in the second stage when they are compressed in the assembled homogenizer. Color version available online.

gap between the seat and the ball, completing the first stage of homogenization. Then, milk flows through the spring (#8) and reaches the seat (#10) and the ball (#11). In the second stage, a lower pressure (about 3 MPa) is needed against the spring (#13) to open the gap between the seat and the ball; thus, flow pressure decreases between stage 1 and 2. Homogenized milk passes through the spring (#13) and flows to the cuvette in the MIR flow system.

A 2-stage homogenizer designed with springs outside the milk flow is shown in Figure 2. This type of homogenizer has 2 separate homogenizer valve housings connected in series in the MIR (Figure 2A). In Figure 2B, the parts of one homogenizer stage housing (#1) are shown. In this type of homogenizer the 2 stages are identical and set at equal pressures. Heated milk is

pumped through the homogenizer and reaches the seat (#3), the ball (#4), and the piston (#9; Figure 2B). The high pressure operating against the spring (#10) opens a narrow gap between the seat and the ball, allowing milk to flow through the first stage of homogenization and exit to the second stage. Next, milk flows to the second homogenizer housing. At this second stage, an equal pressure (about 10 MPa on each stage) is applied against the spring to open the gap between the seat and the ball in the Foss Electric (Hillerød, Denmark) model FT 6000 and FT 120 MIR milk analyzers. In the newer Foss Electric model FT+ and the new Delta FT600 Combi, the first-stage pressure is higher than the second-stage pressure. In both homogenizer designs, the seats and balls open and close with every pump stroke, resulting in variation in the applied pres-

Figure 2. Two-stage homogenizer design with springs outside the milk flow: (A) homogenizer mounted in the instrument with 2 identical homogenizer stages, and (B) parts of 1 of the 2 stages of the homogenizer. Parts include (1) first-stage homogenizer valve assembled, (2) screw-in end cap, (3) first-stage seat, (4) first-stage ball, (5) aligner to keep ball centered on seat, (6) O-ring, (7) O-ring around piston that puts pressure on the ball, (8) first-stage homogenizer housing, (9) first-stage piston, (10) first-stage spring, and (11) first-stage end cap that is turned in to compress the first-stage spring to apply the appropriate loading pressure of the piston against the first-stage ball. In this brand and model of homogenizer, the stage 1 and 2 springs are set at the same pressures (i.e., 10 MPa each). Color version available online.

sure and intermittent flow of milk. Mechanical failures of seats, balls, and springs over time are more likely to happen with this rapid opening and closing during pumping of each sample. This may result in differences in homogenization efficiency from one pump stroke to the next, which will influence repeatability. The reality is that only a portion of homogenized milk from a single pump stroke is actually scanned in the cuvette.

Since 1995, our laboratory has provided a service to the dairy industry of running laser light-scattering particle size analysis of milk homogenized by infrared analyzers to determine if homogenizers in MIR milk analyzers are homogenizing properly. We also rebuild

some homogenizers. Our experience has been that all types of MIR homogenizers wear and degrade in homogenization performance across time and, therefore, need to be checked and controlled. In the literature, no specific information exists comparing the effect of variation in homogenization efficiency on the accuracy of major milk component prediction by MIR using traditional filter versus PLS models. Our objective was to determine the effect of MIR homogenizer efficiency on accuracy and repeatability of Fourier transform MIR predicted fat, true protein, and anhydrous lactose determination given by traditional filter and PLS prediction models.

MATERIALS AND METHODS

Experimental Design

Five in-line 2-stage MIR homogenizers (Delta Instruments) with different homogenization efficiency (i.e., produced different milk fat globule size distributions) were used to homogenize unpreserved, pasteurized, externally homogenized whole milk and unhomogenized whole milk (Cornell Dairy, Ithaca, NY). Prior to the experiment, the slopes and intercepts for fat, protein, and lactose predictions were adjusted using modified milk calibration samples (Kaylegian et al., 2006) with a homogenizer that produced homogenized milk with fat globule $d(0.9)$ of about 1.28 μm . Each homogenizer used in the study was connected in-line to a MIR milk analyzer [LactoScope FTIR Advanced (FTA), Delta Instruments], and 18 component test predictions for traditional filter-based fat B (carbon hydrogen stretch), traditional filter-based fat A (carbonyl stretch), fat PLS, traditional filter-based true protein, true protein PLS, traditional filter-based anhydrous lactose, and anhydrous lactose PLS were collected for both externally homogenized and unhomogenized milk samples at 40 to 42°C. Homogenized milks were collected from the MIR outlet tube and then analyzed with a laser light-scattering particle size analyzer (Mastersizer 2000, model MS2000; Malvern Instruments, Worcestershire, UK). Both externally homogenized and unhomogenized milks were run through all 5 different homogenizers. Repeatability and accuracy of fat, true protein, and anhydrous lactose determination using traditional filter models and PLS prediction models were evaluated.

Evaluation of Repeatability

The repeatability test was performed with the LactoScope FTIR Advanced (FTA) milk analyzer equipped with a BMX optical bench (ABB Bomem, Montreal, Canada). A CaF_2 cuvette (36 μm) was used with a fixed virtual filter calibration approach (Kaylegian et al., 2006). Precalibration was performed according to the procedures described by Lynch et al. (2006), and modified milks were used to adjust slope and intercept of fat, protein, and lactose predictions (Kaylegian et al., 2006). Traditional virtual filter models used the optimized filter wavelengths determined by Kaylegian et al. (2009) for fat A, fat B, protein, and lactose. The gain (i.e., scale factor) and intercorrection factors used in the current study are summarized in Table 1. In addition, PLS models were also used to predict fat, true protein, and anhydrous lactose using Delta Instruments PLS model parameter numbers 9,507, 9,508, and 9,509, respectively.

Table 1. Traditional sample and reference filter center wavenumbers (cm^{-1}), bandwidths (cm^{-1}), powers, scales, offsets, and intercorrection factors used in a Fourier transform mid-infrared milk analyzer for fat B (carbon hydrogen stretch), lactose ($-\text{OH}$ stretch), protein ($-\text{NH}$ stretch), and fat A (carbonyl stretch)

Center	Sample				Reference				Intercorrection factors				
	Bandwidth	Power	Scale	Offset	Center	Bandwidth	Power	Scale	Offset	Fat B	Lactose	Protein	Fat A
2,851	26	0	34.810	0	2,812	24	0	-34.810	0	1.000	-0.149	-0.053	0.000
1,048	20	0	17.947	0	1,293	14	0	-17.947	0	0.045	1.000	0.022	0.000
1,541	20	0	22.256	0	1,491	12	0	-22.256	0	0.066	0.051	1.000	0.000
1,748	16	0	22.171	0	1,791	16	0	-22.171	0	0.000	0.029	0.023	1.000

On test day, unpreserved, pasteurized, externally homogenized and unhomogenized whole milks were provided by the Cornell University Dairy (Ithaca, NY) and split into clear 90-mL vials (Capitol Plastic Products, Amsterdam, NY). Homogenized and unhomogenized milks and 0.01% (vol/vol) Triton-X 100 surfactant solution (G000071020, Delta Instruments) were warmed to $42 \pm 1^\circ\text{C}$ using a water bath (GCA Corporation, Chicago, IL). The 5 different homogenizers were tempered to 42°C in the same water bath so they would quickly come to temperature equilibrium when attached to the MIR system. The first homogenizer was connected to the MIR system and the instrument was cleaned using Decon 90 (Decon Laboratories Ltd., East Sussex, UK) and adjusted to read zero using a 0.01% (vol/vol) Triton-X 100 solution. The first vial of homogenized milk was mixed by inversion, the temperature was checked and the milk from that vial was pumped through the MIR system, 3 corrected readings were collected, and the MIR homogenized milk from all 3 sequential readings from that vial was collected at the instrument's outlet tube into a clear 60-mL vial (Capitol Plastic Products). The MIR-homogenized milk collected into the 60-mL vial was analyzed by a laser light-scattering particle size analyzer (Mastersizer 2000, model MS2000; Malvern Instruments) using the data kill function to exclude particles smaller than $0.195\ \mu\text{m}$ to remove the effect of casein micelles from the final results, as described by Di Marzo and Barbano (2016). This procedure was performed for 6 vials of homogenized milk followed by 6 vials of unhomogenized milk, resulting in a total of 18 corrected readings for the homogenized samples and 18 corrected readings for the unhomogenized samples, for each homogenizer. The first of the 18 readings was discarded to avoid carry over from the Triton-X 100 solution. In between the change of homogenized milk to unhomogenized milk, the instrument flow system was rinsed with 0.01% (vol/vol) Triton-X 100 surfactant solution. At the end of the test, the MIR flow system was rinsed, cleaned, zeroed, the homogenizer was changed, and the procedure described above was repeated. The mean of the MIR predicted values, standard deviation (**SD**), and range were calculated for traditional filter-based fat B, traditional filter-based fat A, fat PLS, traditional filter-based true protein, true protein PLS, traditional filter-based anhydrous lactose, and anhydrous lactose PLS.

Effect of Homogenizer Performance on Accuracy of MIR Readings

Data collected during the repeatability test was used to evaluate the effect of change in homogenization per-

formance on accuracy of MIR-predicted values given by traditional filter models and PLS models. The mean of 17 MIR readings calculated for traditional filter-based fat B, traditional filter-based fat A, fat PLS, traditional filter-based true protein, true protein PLS, traditional filter-based anhydrous lactose, and anhydrous lactose PLS was used to calculate the residual difference between homogenizers for unhomogenized milks. The calculation was done as follows:

$$\begin{aligned} \text{Absolute residual difference (\%)} = \\ (\text{mean MIR predicted component using test} \\ \text{homogenizer}) - (\text{mean MIR predicted component} \\ \text{using reference homogenizer}), \end{aligned}$$

where MIR predicted component = traditional filter-based fat B, traditional filter-based fat A, fat PLS, traditional filter-based true protein, true protein PLS, traditional filter-based anhydrous lactose, and anhydrous lactose PLS; test homogenizer = 1, 2, 3, 4, 5; and the reference homogenizer was the homogenizer that had the best homogenization performance [i.e., lowest $d(0.9)$]. Residual difference of corrected readings for the unhomogenized milks were plotted (Y) as a function of laser light-scattering particle size reference values (X) for each MIR parameter to help visualize and determine the effect of variation in homogenizer performance on the accuracy of MIR predicted values for major milk components.

Statistical Analysis

A PROC GLM LMEANS analysis was performed using SAS (version 8.02, SAS Institute Inc., Cary, NC) to determine if the means for MIR-predicted traditional filter-based fat B, traditional filter-based fat A, fat PLS, traditional filter-based true protein, true protein PLS, traditional filter-based anhydrous lactose, anhydrous lactose PLS, and $d(0.9)$ were different among the 5 homogenizers for externally homogenized milks and unhomogenized milks.

An ANCOVA test was performed using JMP (version Pro 12, SAS Institute Inc.) to determine differences ($P < 0.05$) in the slopes of the regression lines among MIR-predicted fat B and fat A using traditional filter models (Kaylegian et al., 2009) versus PLS-predicted fat, predicted true protein using a traditional filter model (Kaylegian et al., 2009) versus PLS-predicted true protein, and predicted anhydrous lactose using a traditional filter model (Kaylegian et al., 2009) versus PLS-predicted anhydrous lactose plotted as a function of reference $d(0.9)$.

Table 2. Mean fat globule size distribution [d(0.9)] and mean component (%), SD, and range of 17 mid-infrared readings for traditional filter fat B (carbon hydrogen stretch), traditional filter fat A (carbonyl stretch), fat partial least squares (PLS), traditional filter true protein, true protein partial least squares (T. protein PLS), traditional filter anhydrous lactose (An. lactose), and anhydrous lactose partial least squares (An. lactose PLS) on homogenized and unhomogenized whole milks using 5 different homogenizers

Parameter	Homogenizer	Homogenized				Unhomogenized			
		d(0.9) (μm)	Component (%)	SD	Range	d(0.9) (μm)	Component (%)	SD	Range
Fat B	1	0.98 ^c	3.856 ^a	0.004	0.015	1.35 ^d	3.764 ^a	0.005	0.017
Fat B	2	1.01 ^{cb}	3.851 ^b	0.006	0.022	1.34 ^d	3.755 ^b	0.006	0.023
Fat B	3	1.04 ^b	3.847 ^c	0.004	0.015	1.82 ^c	3.718 ^c	0.007	0.022
Fat B	4	1.02 ^b	3.842 ^d	0.006	0.022	2.53 ^b	3.667 ^d	0.018	0.052
Fat B	5	1.11 ^a	3.831 ^e	0.005	0.017	3.03 ^a	3.599 ^e	0.007	0.026
Fat A	1	0.98 ^c	3.802 ^a	0.002	0.009	1.35 ^d	3.718 ^a	0.004	0.017
Fat A	2	1.01 ^{cb}	3.800 ^a	0.003	0.010	1.34 ^d	3.711 ^b	0.004	0.015
Fat A	3	1.04 ^b	3.800 ^b	0.002	0.009	1.82 ^c	3.692 ^c	0.005	0.019
Fat A	4	1.02 ^b	3.787 ^c	0.004	0.013	2.53 ^b	3.667 ^d	0.010	0.033
Fat A	5	1.11 ^a	3.782 ^d	0.003	0.009	3.03 ^a	3.644 ^e	0.007	0.026
Fat PLS	1	0.98 ^c	3.840 ^a	0.009	0.032	1.35 ^d	3.743 ^a	0.011	0.040
Fat PLS	2	1.01 ^{cb}	3.827 ^{bc}	0.007	0.024	1.34 ^d	3.731 ^b	0.007	0.029
Fat PLS	3	1.04 ^b	3.830 ^b	0.008	0.029	1.82 ^c	3.714 ^c	0.013	0.055
Fat PLS	4	1.02 ^b	3.822 ^{cd}	0.008	0.029	2.53 ^b	3.694 ^d	0.014	0.050
Fat PLS	5	1.11 ^a	3.820 ^d	0.009	0.027	3.03 ^a	3.665 ^e	0.011	0.041
True protein	1	0.98 ^c	3.017 ^b	0.003	0.010	1.35 ^d	3.008 ^d	0.003	0.015
True protein	2	1.01 ^{cb}	3.014 ^c	0.003	0.011	1.34 ^d	3.008 ^d	0.004	0.017
True protein	3	1.04 ^b	3.020 ^a	0.005	0.015	1.82 ^c	3.017 ^b	0.004	0.014
True protein	4	1.02 ^b	3.015 ^c	0.004	0.015	2.53 ^b	3.013 ^c	0.005	0.017
True protein	5	1.11 ^a	3.015 ^{bc}	0.003	0.011	3.03 ^a	3.020 ^a	0.004	0.013
T. protein PLS	1	0.98 ^c	3.023 ^a	0.006	0.019	1.35 ^d	3.003 ^a	0.006	0.016
T. protein PLS	2	1.01 ^{cb}	3.025 ^a	0.004	0.012	1.34 ^d	3.006 ^a	0.003	0.013
T. protein PLS	3	1.04 ^b	3.025 ^a	0.006	0.023	1.82 ^c	2.989 ^b	0.003	0.012
T. protein PLS	4	1.02 ^b	3.023 ^a	0.005	0.018	2.53 ^b	2.927 ^c	0.016	0.061
T. protein PLS	5	1.11 ^a	3.017 ^b	0.005	0.015	3.03 ^a	2.896 ^d	0.004	0.014
An. lactose	1	0.98 ^c	4.611 ^c	0.006	0.023	1.35 ^d	4.597 ^c	0.008	0.033
An. lactose	2	1.01 ^{cb}	4.621 ^a	0.004	0.020	1.34 ^d	4.601 ^c	0.006	0.024
An. lactose	3	1.04 ^b	4.617 ^b	0.005	0.017	1.82 ^c	4.608 ^b	0.008	0.034
An. lactose	4	1.02 ^b	4.617 ^b	0.005	0.020	2.53 ^b	4.619 ^a	0.007	0.026
An. lactose	5	1.11 ^a	4.622 ^a	0.004	0.015	3.03 ^a	4.624 ^a	0.007	0.023
An. lactose PLS	1	0.98 ^c	4.602 ^b	0.004	0.010	1.35 ^d	4.597 ^b	0.006	0.020
An. lactose PLS	2	1.01 ^{cb}	4.606 ^a	0.005	0.010	1.34 ^d	4.596 ^b	0.005	0.010
An. lactose PLS	3	1.04 ^b	4.604 ^{ab}	0.005	0.010	1.82 ^c	4.596 ^b	0.005	0.010
An. lactose PLS	4	1.02 ^b	4.602 ^b	0.006	0.020	2.53 ^b	4.602 ^a	0.006	0.020
An. lactose PLS	5	1.11 ^a	4.604 ^{ab}	0.005	0.010	3.03 ^a	4.599 ^{ab}	0.006	0.020

^{a-c}Means not sharing a common superscript differ ($P < 0.05$).

RESULTS

Effect of Homogenizer Performance on Accuracy of MIR Readings

Externally Homogenized Milks. The effect of variation in homogenizer performance on the MIR-predicted values for major milk components of externally homogenized milks is shown in Table 2. If well-homogenized milk [i.e., d(0.9) = 1.12 μm] is pumped through homogenizers with very different homogenization performance, what happens to the d(0.9) and the predicted component values? The in-line instrument homogenization of externally homogenized milk produced very little change (i.e., 0.98 to 1.11 μm) in d(0.9) values (Table 2). Component predictions on externally homogenized milks were affected ($P < 0.05$) by varia-

tion in homogenizer performance, but the magnitude of effects were small (i.e., <0.025%).

Unhomogenized Milks. Typically in payment testing, raw unhomogenized milk [i.e., d(0.9) = about 6 to 7 μm] is analyzed and the homogenizer within the instrument flow system is expected to reduce d(0.9) to less than 1.7 μm . The homogenization of unhomogenized milks by the same 5 in-line MIR homogenizers as above produced 5 d(0.9) levels from 1.35 to 3.03 μm (Table 2), which would include homogenizers with acceptable and unacceptable [i.e., d(0.9) > 1.7 μm] homogenization performance. No difference was detected in d(0.9) (i.e., <1.35 μm) between homogenizers 1 and 2, but homogenizers 3, 4, and 5 were different ($P < 0.05$) from each other and from homogenizers 1 and 2. Accuracy of component predictions on unhomogenized milks were affected more by variation in homogenizer

performance than for externally homogenized milk (Table 2). Poor homogenization affected the accuracy of fat predictions ($P < 0.05$) at all $d(0.9)$ levels. The increase of $d(0.9)$ with poor homogenization produced lower ($P < 0.05$) fat tests for traditional filter-based fat B (-0.165%), traditional filter-based fat A (-0.074%), and fat PLS (-0.078%) at a $d(0.9)$ of $3.03\ \mu\text{m}$ (Table 2). The actual change in the MIR absorbance spectra produced by these variations in $d(0.9)$ were reported previously by Di Marzo and Barbano (2016). Variation in homogenization efficiency produced a slightly higher (0.012%) traditional filter-based true protein test ($P < 0.05$) and a 0.107% lower ($P < 0.05$) true protein PLS prediction at a $d(0.9)$ of $3.03\ \mu\text{m}$. For traditional filter-based anhydrous lactose test, the poor homogenization efficiency produced a 0.027% higher anhydrous lactose at a $d(0.9)$ of 2.53 and $3.03\ \mu\text{m}$ (Table 2), with the effect of variation in homogenization on accuracy of anhydrous lactose test being lower than for fat or true protein, as expected. After homogenization, the fat globule diameter of milk should be less than one-third of the wavelength at which the measurement is made [i.e., for fat B wavelength; $d(0.9) < 1.16\ \mu\text{m}$] to minimize light scattering caused by large milk fat globules. Fat B is the shortest wavelength used for fat analysis (Goulden, 1964; Smith et al., 1993). For anhydrous lactose, the effect of light scattering on MIR measurements would be larger with the milk fat globule $d(0.9)$ larger than $3.20\ \mu\text{m}$. In general, repeatability (i.e., SD) was smaller for externally homogenized milk than for unhomogenized milks (Table 2).

Homogenization Efficiency: Traditional Filter Versus PLS Models

No specific information exists in the literature on the effect of variation in homogenization efficiency on the predictions of the major milk components by traditional filter versus PLS models. The slopes of the regression lines for MIR traditional filter model (Kaylegian et al., 2009) for predicted fat B and fat PLS plotted as a function of reference particle size distribution (Figure 3A) were different ($P < 0.05$). On the contrary, no difference was detected ($P > 0.05$) in the slope of the regression lines for MIR traditional filter model for predicted fat A and fat PLS plotted as a function of reference particle size distribution (Figure 3A). According to Smith et al. (1994), fat B may be more affected by the poor homogenization performance than fat A because of its shorter wavelength. The slopes of the regression lines for traditional filter model (Kaylegian et al., 2009) MIR-predicted true protein and true protein PLS (Figure 3B) and for traditional filter model MIR-predicted anhydrous lactose and anhydrous lactose PLS (Figure

3C) as a function of reference particle size distribution were different ($P < 0.05$). In the case of true protein, the specific PLS model used in the present study was much more sensitive to variation in homogenizer performance than the traditional filter model, and the effect on accuracy of the true protein test at a $d(0.9)$ of $3.03\ \mu\text{m}$ was relatively large. The anhydrous lactose traditional filter model results were affected more than the anhydrous lactose predicted with a PLS model, but the effect of homogenization efficiency on lactose was much smaller than on fat and true protein.

DISCUSSION

Methods for Homogenizer Evaluation

Precalibration tests are currently used to detect if the MIR homogenizer is working properly (Barbano and Clark, 1989; Lynch et al., 2006). In the recycle test, if the difference between the average MIR readings for fat test on the unhomogenized milk and instrument homogenized milk is $>0.05\%$ for a milk containing a mass fraction of 3.5% milk fat, the homogenizer fails homogenization efficiency (IDF, 2000). Although this method is very rapid and practical, a MIR with a very poor homogenizer will pass the evaluation, as the readings before and after homogenization will be the same (Barbano and Clark, 1989; Smith et al., 1993; Lynch et al., 2006). Another method to track homogenizer efficiency is by checking the MIR absorbance at wavenumber $3,750\ \text{cm}^{-1}$. As particle size increases when the same unhomogenized milk is being homogenized by a deteriorated homogenizer, light scattering increases and absorbance at $3,750\ \text{cm}^{-1}$ increases. The increase of light scattering will lead to a pronounced rise in baseline at shorter wavelengths, where the fat globule sizes are of the same order as the wavelength (Goulden, 1964). The limitation of this measure is that sample-to-sample variation in concentration of fat, protein, and lactose also affect the absorbance at $3,750\ \text{cm}^{-1}$; thus, unless you test the same sample over a period of months and compare readings, the results are difficult to interpret. Absorbance at $3,750\ \text{cm}^{-1}$ is useful to compare the relative homogenization efficiency among different homogenizers on the same milk sample. The test to evaluate homogenization efficiency that is more accurate, but is less practical to perform daily, is the laser light-scattering particle size analysis to determine milk fat globule $d(0.9)$ after unhomogenized milk is homogenized within the MIR (Lynch et al., 2006). If $d(0.9)$ of the milk homogenized through the MIR is larger than $1.7\ \mu\text{m}$, then the homogenizer is deteriorating and needs to be replaced (Smith et al., 1995). This approach is used on a monthly basis to evaluate

homogenizer performance by the USDA Federal Milk Market laboratories, whereby instrument-homogenized milks are sent to central testing laboratory for a laser light-scattering test to determine if the $d(0.9)$ is $<1.7 \mu\text{m}$.

Recently, a MIR PLS $d(0.9)$ prediction model was developed (Di Marzo and Barbano, 2016) as an alternative method for use in routine quality assurance to determine if the homogenizer within a MIR milk analyzer was near the failure level [i.e., $d(0.9) >1.7 \mu\text{m}$]. The advantage of this method is that the homogenizer performance can be monitored daily using the mean $d(0.9)$ given by each MIR instrument each day. Additionally, the method can be used for milk with a wide range of fat content (Di Marzo and Barbano, 2016).

Causes of Homogenizer Failure

The deterioration of a homogenizer's mechanical components over time needs to be detected by the MIR operator to minimize the negative effect on ana-

lytical accuracy and repeatability. What causes MIR milk analyzers to have poor homogenization efficiency? There are 3 common problems for a 2-stage homogenizer designed with springs in the milk flow. First, if the coil spring has a rough spot on the end that is in direct contact with the ball it can chip the ball (Figure 4), preventing it from forming perfect closure against the seat (Trout, 1950; Walstra et al., 2005). On this type of homogenizer, the ball rotates, so the same area is not always contacting the seat, and the homogenizer performance may oscillate between getting better and worse depending on whether the place on the ball with the chip is in contact with the seat. Second, because the spring is in direct contact with milk, pieces of foreign material present in milk and ball chips can get stuck in the spring, affecting homogenization performance and purging efficiency (Walstra et al., 2005). Third, if the spring is bent it can cause an unequal opening between the ball and the seat. When these things happen it is common to hear a high-pitched squeaking noise produced by the homogenizer. If the bent spring is allowing

Figure 3. Mid-infrared predicted residual difference for (A) traditional filter-based fat B (carbon hydrogen stretch), traditional filter based fat A (carbonyl stretch), and fat partial least squares (PLS), (B) traditional filter-based true protein and true protein PLS, and (C) traditional filter-based anhydrous lactose and anhydrous lactose PLS, plotted as a function of fat globule size distribution [$d(0.9)$]. Residual difference (%) = (mean mid-infrared predicted component using test with homogenizer 1, 2, 3, 4, and 5) – [mean MIR predicted component using homogenizer 1 (i.e., the best homogenizer)]. Slopes of lines with different superscripts (a,b) differ ($P < 0.05$). Color version available online.

Figure 4. Common failure of a 2-stage homogenizer design with the springs in the milk flow: chip of the sapphire ball caused by a spring with a rough end. Color version available online.

the opening between the seat and ball to happen only on one side, accumulation of material between ball and the seat will diminish homogenization performance.

For a 2-stage homogenizer designed with springs outside the milk flow, 2 problems are common. First, it is possible to see a wear ring (Figure 5A) form on the ball over time where the ball makes contact with the seat. This happens as consequence of a pulsing single-piston pump forcing milk against the ball, resulting in repeated opening the narrow gap between the ball and the seat and bringing the ball to its original position against the seat when pressure is not applied (Wenrich, 1946; Trout, 1950; Walstra et al., 2005). In this type of homogenizer, it appears that the balls do not rotate, so the same area on the ball is always opening and closing against the seat. A localized erosion of the ball lets milk flow through this point with a lower pressure decrease across the seat and will cause poor homogenizer performance. Second, damage to the seat occurs on the edge that contacts the ball, leading to the accumulation of material between the ball and seat (Figure 5B).

Fat Globule Particle Size-Based Decision to Replace a Homogenizer

Is the fat globule $d(0.9)$ value currently used as a criterion to replace homogenizer reasonable? The USDA Federal Milk Market laboratories uses a $d(0.9)$ value of $1.7 \mu\text{m}$ as a criterion to make the decision to replace the homogenizer in an infrared milk analyzer (Smith et al., 1995; Lynch et al., 2006). A $d(0.9)$ of $1.8 \mu\text{m}$ affected the accuracy of MIR fat readings ($P < 0.05$), lowering the results for traditional filter-based fat B (-0.046%),

Figure 5. The failures of mechanical components of the 2-stage homogenizer with springs outside the milk flow: (A) wear ring formed on the ball at the point where the ball contacts the seat, and (B) showing the accumulation of material on the seat that will not allow the ball and seat to form a tight seal when the valve closes. Color version available online.

traditional filter-based fat A (-0.026%), and fat PLS (-0.028%) and increasing SD of repeatability (Table 2). New commercial homogenizers for MIR milk analyzers are capable of producing a $d(0.9)$ in the range of 1.1 to $1.5 \mu\text{m}$, and the time of use of homogenizer before it reaches a $d(0.9)$ of $1.7 \mu\text{m}$ is usually 6 mo or longer based on experience in the USDA Federal Milk Markets. Thus, a $d(0.9)$ of $1.7 \mu\text{m}$ has been used in practice and appears to be a reasonable limit, given the magnitude of effect on the accuracy of fat test results.

Effect of Homogenization Efficiency: Traditional Filter Versus PLS Models

The effect of variation in homogenizer performance on the results of traditional filter model-predicted values for main components are well established (Smith et al., 1995), and the detail of standard optimum sample and reference wavelengths, setting primary slope, approximate intercorrection factors are well understood and documented in the literature. The situation with respect to the effect of variation in homogenizer performance on the predicted values for PLS models is not so clear, as shown in Figure 3. Users need to recognize that the structure of each PLS model for prediction of the same milk component is unique and dependent on several factors (data preprocessing before modeling, baseline correction, mean centering, scaling, ranges of wavelengths used, outlier removal, optical bench resolution, and so on) and, most importantly, the exact population of samples (i.e., MIR spectra) used for the PLS modeling. All PLS models are not the same. Thus, 2 different PLS models developed for the same model of MIR milk analyzer may have different analytical performance characteristic and also different sensitivities to external factors, such as variation in homogenization or variation in type or concentration of preservative system (i.e., both the active and inert ingredients). Generally, a laboratory that is using a PLS model has no tools to diagnose these performance characteristics of PLS models, and, in general, the instrument manufacturers do not provide this type of information. At best, a user (or researcher using results from PLS model) should know and report the equipment manufacturer PLS model identification and version number used for production of results used in the study. Consider the dilemma that could happen: A research study to determine the effect of a management or feeding practice on milk fat production is conducted and the results of the same study come out significantly different depending on which PLS model was used to analyze the same spectra to predict fat. Documentation and reporting of the specific manufacturer's identification numbers for PLS model and version used for every MIR measured parameter is warranted for published research studies.

In the future, as new PLS models are developed to measure other components in milk (e.g., phenotypic traits, blood chemistry parameters, fatty acids, citrate, and so on), the sensitivity of their prediction accuracy to factors such as variation of homogenizer performance should be determined as part of the ruggedness testing during the PLS model development process. The PLS prediction models for other milk components not addressed in the current paper may be more or less sensitive to light scattering of fat globules depending on the

specific wavelengths used in each PLS model and magnitude of the β -coefficients used at those wavelengths.

CONCLUSIONS

Repeatability and accuracy of Fourier transform MIR predicted fat, true protein, and anhydrous lactose given by traditional filter and PLS prediction models were determined. Component predictions on externally homogenized milks were affected by variation in homogenizer performance, but the magnitude of effects were small (i.e., $<0.025\%$) when milks were pumped through both efficient and inefficient homogenizers within a MIR analyzer. Variation in the in-line MIR homogenizer performance on unhomogenized milks had a much larger effect on accuracy of component testing than on repeatability. The increase of particle size distribution $d(0.9)$ from 1.35 to 3.03 μm due to poor homogenization affected fat tests the most: traditional filter-based fat B (-0.165%), traditional filter-based fat A (-0.074%), and fat PLS (-0.078%) at a $d(0.9)$ of 3.03 μm . Variation in homogenization efficiency also affected traditional filter-based true protein test ($+0.012\%$), true protein PLS prediction (-0.107%), and traditional filter-based anhydrous lactose test ($+0.027\%$) at a $d(0.9)$ of 3.03 μm . Effects of variation in homogenization on anhydrous lactose PLS predictions were small. The accuracy of both traditional filter models and PLS models was influenced by poor homogenization. The effect of homogenization on accuracy of traditional virtual filter models on a mid-Fourier transform infrared will be the same on all mid-Fourier transform infrared milk analyzers if the primary slope and intercorrection factors are properly set. These are defined in the public domain. The same is not true for PLS models, and each PLS prediction model for every measured parameter may differ in its sensitivity to variation in homogenizer performance. The value of 1.7 μm for a $d(0.9)$ used by the USDA Federal Milk Market laboratories as a criteria to make the decision to replace the homogenizer in a MIR milk analyzer appears to be a reasonable limit, given the magnitude of effect on the accuracy of fat test. In the future, as new PLS models are developed to measure other components in milk, the sensitivity of the accuracy of the predictions of these models to factors such as variation of homogenizer performance should be determined as part of the ruggedness testing during PLS model development.

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