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Prediction of fatty acid chain length and unsaturation of milk fat by mid-infrared milk analysis[1]

Karen L. Wojciechowski and David M. Barbano[2]
Department of Food Science, Northeast Dairy Foods Research Center, Cornell University, Ithaca, NY 14853

ABSTRACT

Our objective was to develop partial least squares (PLS) models to predict fatty acid chain length and total unsaturation of milk fat directly from a mid-infrared (MIR) spectra of milk at 40°C and then determine the feasibility of using those measures as correction factors to improve the accuracy of milk fat determination. A set of 268 milks (modified milks, farm bulk tank milks, and individual cow) were analyzed for fat, true protein, and anhydrous lactose with chemical reference methods, and in addition a MIR absorption spectra was collected for each milk. Fat was extracted from another portion of each milk, the fat was saponified to produce free fatty acids, and the free fatty acids were converted to methyl esters and quantified using gas-liquid chromatography. The PLS models for predicting the average chain length (carbons per fatty acid) and unsaturation (double bonds per fatty acid) of fatty acids in the fat portion of a milk sample from a MIR milk spectra were developed and validated. The validation performance of the prediction model for chain length and unsaturation had a relative standard deviation of 0.43 and 3.3%, respectively. These measures are unique in that they are fat concentration independent characteristics of fat structure that were predicted directly with transmission MIR analysis of milk. Next, the real-time data output from the MIR spectrophotometer for fatty acid chain length and unsaturation of milk were used to correct the fat A (C=O stretch) and fat B (C–H stretch) measures to improve accuracy of fat prediction. The accuracy validation was done over a period of 5 mo with 12 sets of 10 individual farm milks that were not a part of the PLS modeling population. The correction of a traditional fat B virtual filter result (C–H stretch) for sample-to-sample variation in unsaturation reduced the Euclidean distance for predicted fat from 0.034 to 0.025. The correction of a traditional fat A virtual filter result (C=O stretch) modified with additional information on sample-to-sample variation of chain length and unsaturation gave the largest improvement (reduced Euclidean distance from 0.072 to 0.016) and the best validation accuracy (i.e., lowest Euclidean distance) of all the fat prediction methods.
Key words: mid-infrared, carbon number, unsaturation

INTRODUCTION

Mid-infrared (**MIR**) transmission spectrophotometry is used for both milk payment testing and dairy herd improvement record keeping worldwide. The accuracy of measurement of fat and protein content of milk is extremely important because it has a direct effect on the payment to individual dairy farmers (Lynch et al., 2004; Barbano and Lynch, 2006). Development of harmonized protocols for interlaboratory studies aided in the improvement of method performance and provided a harmonized set method performance statistics (AOAC, 1989; AOAC International, 1995) that provide metrics of expected method performance. The practical interpretation of statistical metrics of method performance parameters was described by Lynch (1998). As farms in the United States have increased in size, the effect of small errors in testing have a large financial effect when applied to large milk volumes. Therefore, continued improvement of the accuracy of milk fat and protein testing become more important as farms get larger and when the value of milk fat and protein are high. Historically, the measurement of fat by MIR has used the carbonyl stretch (C=O), which has been called fat A, and the symmetrical carbon hydrogen stretch (C–H), which has been called fat B in the MIR region (Biggs et al., 1987; Barbano and Clark, 1989).

Kaylegian et al. (2009a) demonstrated that sample-to-sample differences between MIR predictions of fat content of a milk and the ether extraction reference analysis of the same sample are explained by sample-to-sample variation in the differences in mean fatty acid chain length (**CL**) and mean unsaturation (**UN**)

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[2]Corresponding author: dmb37@cornell.edu

of the milk fat. The CL and UN of milk fat can vary systematically among farms due to differences in feeding and management, and these differences can produce systematic over- or underestimation of milk fat content for a farm. The magnitude of the error in fat estimate for an individual farm milk increases as a function of the difference between the average CL and UN of the individual farm milk and the mean of the set of milks used to calibrate (i.e., adjust slope and intercept) the specific infrared milk analyzer being used. Kaylegian et al. (2009a) reported a range of mean fatty acid CL across 45 farms from 13.83 to 15.06 carbons and a range of mean fatty acid UN from 0.25 to 0.42 double bonds per fatty acid. This range in CL and UN can cause systematic errors of ±0.1% fat relative to the ether extraction reference value on a milk sample from an individual farm.

A description of basic calibration equations (Barbano and Clark, 1989; Lynch et al., 2006) and reference and sample wavelengths used for measurement was provided by Kaylegian et al. (2009b). The traditional fat A and fat B measurements can be made either with optical filters or with virtual filters produced from a Fourier transform MIR spectra. With time, optical-filter-based MIR milk analyzers are being replaced with MIR spectrophotometers. The MIR spectrophotometers can be used with optimized virtual filter versions of the traditional fat A and fat B wavelengths (Kaylegian et al., 2009b), or spectral prediction models for fat and protein can be produced by partial least squares (**PLS**) statistical modeling (Luinge et al., 1993). When the traditional MIR filter models are set up and managed correctly, they provide excellent and reliable performance for measurement of the major components of milk and are easier for the instrument operator to understand than PLS models. The PLS models are generally developed by each equipment manufacturer and provide more of a "black box" approach to MIR milk testing. The PLS prediction models can differ in performance from one equipment manufacturer to another; they can differ in their sensitivity to variation in homogenizer performance, preservative type and concentration, and sample temperature variation. Over the years the accuracy of MIR milk testing has been improved due to improved reference chemistry performance (Lynch et al., 1994, 1997), participation in routine proficiency testing for laboratories running reference chemistry, and more care being taken in producing sets of reference samples that have a better range of component concentrations with fewer high leverage samples (Kaylegian et al., 2006). Even with these improvements, there are still factors that vary in milk that cause fat reference chemistry values and instrument values not to agree. Kaylegian et al. (2009b) demonstrated the sensitivity of MIR fat measures to variation in fatty acid composition from sample to sample. Sample-to-sample variation in fatty acid CL and UN were identified as 2 factors that limit the accuracy of fat testing (Kaylegian et al., 2009a). Variation in fatty acid CL and UN in bulk tank milk is caused by variation in mean DIM for the herd due to variation in milk fatty acid composition caused by stage of lactation (Lynch et al., 1992), and the feeding of rumen bypass fat used in lactating dairy cattle feeding to increase the energy density of the diet for high-producing dairy cows.

Soyeurt et al. (2006) reported the development of PLS prediction models using MIR spectra to measure the concentration of a range of individual milk fatty acids and groups of fatty acids (e.g., saturated, monounsaturated, polyunsaturated) present in milk fat, expressed as grams of fatty acid per 100 mL of milk. De Marchi et al. (2014) reviewed and compared the performance of fatty acid prediction models reported in the literature. Ferrand-Calmels et al. (2014) expanded the use of MIR for milk fatty acid measurement to ewe and goat milks. We have not found any reports in the literature where MIR has been used to directly measure metrics of global fatty acid structure in milk. Our objective was to develop PLS models from MIR spectra to predict fatty acid CL (i.e., mean carbon number per fatty acid) and total UN (i.e., mean double bonds per fatty acid) of milk fat directly from a MIR spectra of milk at 40°C and then determine the feasibility of using those measures as real-time correction factors to improve the accuracy of classical MIR fat A and fat B measures of fat content using optimized virtual filters.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

Two hundred sixty-eight milks were selected from a total population of 201 individual farm milks, 29 individual cow milks, and 18 sets (14 milks per set) of modified milk calibration samples (Kaylegian et al., 2006). Modified milk calibration samples were produced as described by Kaylegian et al. (2006) using the general procedure described by the International Dairy Federation (IDF, 2000) with a modification to increase the range of lactose concentrations in the calibration sample set. Within the 268 samples, there were 3 sub-populations: modified milks (18 sets of 14 samples per set), individual farm bulk milks (n = 190), and individual cow milks (n = 29). The bulk tank milks used in the modeling were from different regions of the United States from farms feeding a wide range of diets and using various commercial rumen bypass fat supplements. The individual cow milks represented different