

## Application of Mid-Infrared Spectroscopy in Milk Nutrient Prediction and Dairy Cow Trait Analysis: A Postprint

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**Date:** 2017-10-10T00:00:00+00:00

### Abstract

In recent years, numerous studies have demonstrated that mid-infrared spectroscopy (MIRS) analysis technology can perform real-time, rapid, and accurate quantitative analysis of various nutritional components in milk (such as fatty acids, proteins, and minerals) and potentially harmful substances (such as adulterants and antibiotics), based on which predictive models can be established to identify, evaluate, and screen dairy cows' nutritional status (such as feed conversion efficiency, energy utilization efficiency, and methane emissions), health conditions (such as mastitis and metabolic diseases), and reproductive physiology and breeding status, thus providing effective technical support for optimizing livestock production methods, developing innovative circular economy models for animal husbandry, and achieving low-carbon, healthy, and sustainable development. Therefore, this paper primarily reviews the latest international research progress in recent years regarding MIRS analysis technology for milk nutrient determination and analysis of dairy cow-related traits.

### Full Text

## Application of Mid-Infrared Spectrometry in Milk Nutrient Component Prediction and Related Trait Analysis of Dairy Cows

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**Abstract:** In recent years, mid-infrared spectrometry (MIRS) has emerged as a powerful analytical technique for the real-time, rapid, and accurate quantification of various milk nutrients—including fatty acids, proteins, and minerals—as well as potential harmful substances such as adulterants and antibiotics. Beyond compositional analysis, MIRS-based prediction models enable the assessment and screening of dairy cow nutrition (feed conversion efficiency, energy utilization, and methane emissions), health status (mastitis and metabolic diseases), and reproductive physiology. These capabilities provide effective technical support for optimizing livestock production systems, developing innovative circular economy models in animal husbandry, and achieving low-carbon, healthy, and sustainable development. This review synthesizes recent international advances in MIRS applications for milk nutrient determination and dairy cow trait analysis.

**Keywords:** mid-infrared spectrometry; prediction model; milk composition; dairy cows

Since the 2008 melamine incident, limitations of conventional milk testing methods have become increasingly apparent. Traditional techniques such as the Kjeldahl method cannot effectively identify non-protein nitrogen compounds (urea nitrogen, free ammonia, inorganic ammonium salts) in milk. While methods like Coomassie brilliant blue and spectrophotometry enable quantitative analysis, they share common drawbacks with other conventional approaches: high equipment requirements, time-consuming procedures, and inability to perform rapid, accurate measurements outside laboratory settings. Milk fat determination has relied on traditional methods including Soxhlet extraction, Gerber, and Babcock tests, as well as gas chromatography, high-performance liquid chromatography, and GC-MS. However, these methods suffer from either low accuracy or complex, time-consuming procedures, limiting their application to small-scale laboratory analyses rather than widespread production use.

Recent research demonstrates that mid-infrared spectrometry (MIRS) can rapidly, accurately, and non-destructively quantify milk nutrients including proteins, fatty acids, and minerals, along with potential harmful substances. Compared with conventional chemical methods, MIRS requires minimal sample preparation, eliminates the need for chemical reagents, and enables fast, non-destructive detection of multiple components simultaneously. Furthermore, MIRS prediction models for specific milk constituents facilitate rapid analysis and identification of cow nutrition, health, and reproductive status.

International collaboration has accelerated MIRS adoption. The EU Robust-Milk project (2008-2012) employed MIRS to quantify fatty acids, proteins, and carbohydrates, integrating genomic analysis to enhance specific milk nutrients and increase product value. As part of a regional development initiative, six European countries (Belgium, UK, Ireland, Germany, France, and Luxembourg) collaborated in 2011 to analyze fatty acids, proteins, urea, and somatic cell counts using MIRS, developing prediction models for pregnancy detection, health status, and energy utilization to improve management and profitability.

In France, the PhenoFinlait consortium of farmers, breeding companies, milk recording organizations, and research institutions established nutrient prediction models through large-scale MIRS analysis to support nutritional regulation and genetic breeding. In contrast, domestic Chinese research on MIRS quantification of milk nutrients remains scarce, with no reported applications for assessing cow nutrition, health, or fertility.

This review first introduces MIRS fundamentals and measurement methods, then synthesizes recent advances in developing prediction models for milk nutrients and their application in evaluating dairy cow nutrition, health, and reproduction, providing a foundation for understanding MIRS effectiveness in research and production.

### 1.1 Basic Principles

Infrared spectroscopy lies between visible light and microwave regions, typically divided into three zones: near-infrared (0.75–2.50  $\mu\text{m}$ ), mid-infrared (2.50–25.00  $\mu\text{m}$ ), and far-infrared (25.00–1000.00  $\mu\text{m}$ ). The mid-infrared region spans 400–4000  $\text{cm}^{-1}$ , representing the fundamental absorption bands for most organic and inorganic ions. Since fundamental vibrations exhibit the strongest absorption in infrared spectra, the mid-infrared region is considered optimal for both qualitative and quantitative analysis. Combined with modern chemometric methods and computing technology, MIRS enables rapid, accurate, and non-destructive analysis of organic molecular composition and concentration, with broad applications in research and production.

MIRS performs qualitative or quantitative analysis by examining specific absorption characteristics in the mid-infrared region. Qualitative analysis compares sample spectra with standard references, evaluating peak position, shape, and relative intensity for identification. Quantitative analysis applies the Beer-Lambert law to determine component concentrations through characteristic absorption band intensities. The analytical workflow is illustrated in Figure 1 [Figure 1: see original paper].

The procedure involves: (1) collecting representative samples as a calibration set and measuring their MIRS data; (2) determining reference chemical values using standard methods; (3) establishing calibration models using appropriate chemometric techniques; (4) validating model accuracy with validation set spectra and reference data to select optimal models; and (5) predicting composition and content from sample spectra. Model accuracy depends on multiple factors, including variation in fatty acid and protein content across samples from different regions, breeds, and feeding regimes; instrumental errors during spectral collection; and methodological effects on reference data. Spectral preprocessing method selection critically influences prediction accuracy.

## 1.2 Data Preprocessing

Reliable MIRS quantitative analysis depends first on accurate spectral and chemical measurement data. Identifying and removing outliers from both MIRS and chemical analyses eliminates invalid data interference and improves model accuracy. However, spectral data contain not only valid sample information but also random noise, baseline drift, background spectra from interfering components, and environmental effects that cannot be eliminated through outlier removal alone. Appropriate preprocessing or transformation of full spectral data before chemometric modeling can significantly reduce non-target influences, simplify subsequent calculations, and improve prediction accuracy.

Derivative operations (first and second derivative) reduce peak shift and drift while enhancing spectral resolution. Multiplicative scatter correction eliminates spectral deviations caused by particle size and uniformity effects. Standard normal variate transformation reduces unwanted spectral interference, while normalization simultaneously eliminates absolute intensity and light scattering effects.

De Marchi et al. analyzed fatty acid composition in 267 Swiss Brown milk samples using gas chromatography and MIRS, comparing five preprocessing approaches: raw data, first derivative, second derivative, normalization, and multiplicative scatter correction plus first derivative. First derivative and MSC+first derivative preprocessing yielded the most accurate prediction models. First derivative preprocessing achieved cross-validation correlation coefficients of 0.75 and 0.77 for C12:0 and C14:0, respectively. MSC+first derivative preprocessing produced correlation coefficients of 0.74 and 0.76 for C8:0 and long-chain fatty acids. Bonfatti et al., analyzing Simmental cow milk proteins, similarly found that first derivative preprocessing significantly improved model accuracy using modified partial least squares regression. Soyeurt et al. examined 1,609 milk samples from Belgium, Ireland, and UK, demonstrating that first derivative preprocessing produced superior fatty acid prediction models with higher cross-validation correlation coefficients compared to raw data. In a larger study of 2,499 samples investigating six preprocessing methods for lactoferrin prediction, Soyeurt et al. confirmed that derivative processing yielded optimal models with cross-validation correlation coefficients reaching 0.72.

## 2. Analysis of Milk Fatty Acids

Milk contains approximately 70% saturated fatty acids, 25% monounsaturated fatty acids, and 5% polyunsaturated fatty acids. Fatty acid composition and content vary with breed, genetic merit, and feeding management. Real-time acquisition of fatty acid information supports effective dairy cattle management and improves profitability. While gas chromatography provides accurate results, its material requirements and time-consuming procedures limit large-scale application. MIRS enables rapid, accurate, and non-destructive fatty acid analysis, though prediction accuracy depends on analyte concentration, expression units,

and chemometric models.

Soyeurt et al. quantified fatty acids in 275 cows across six Belgian breeds using MIRS, developing prediction models in the 1736–1805  $\text{cm}^{-1}$  and 2823–3016  $\text{cm}^{-1}$  regions. Model accuracy correlated positively with analyte concentration: correlation coefficients between MIRS and GC data reached 0.95 for individual fatty acids, with high-concentration C12:0, C14:0, C16:0, and C18:1 exceeding 0.90, while low-concentration C10:1cis-9 and C14:1cis-9 showed coefficients of only 0.05 and 0.12. For fatty acid groups, saturated and monounsaturated fatty acids achieved correlation coefficients of 0.98 and 0.89, respectively, compared to 0.43 for polyunsaturated fatty acids.

In a subsequent study of 1,060 samples from Belgium, Ireland, and UK using six preprocessing methods, high-concentration C4:0, C6:0, C8:0, C10:0, and C12:0 achieved cross-validation correlation coefficients of 0.88–0.95 and external validation coefficients of 0.82–0.90. Saturated and unsaturated fatty acid groups showed cross-validation coefficients of 0.99 and 0.97, respectively, with external validation coefficients of 0.98 and 0.96, demonstrating high accuracy for specific milk selection applications.

Rutten et al. analyzed 3,622 Dutch milk samples using MIRS and GC, developing prediction models for 16 individual fatty acids and four groups. Model accuracy depended on expression units: when expressed as g/dL milk, C4:0, C6:0, C8:0, and C10:0 achieved correlation coefficients of 0.91, 0.96, 0.94, and 0.92, respectively, with fatty acid group coefficients of 0.91–0.97—significantly higher than models using percentage of total fat. De Marchi et al. compared expression units in 1,200 Swiss Brown cows, finding that g/kg milk expression yielded higher precision, with cross-validation coefficients of 0.74, 0.73, and 0.74 for C18:0, C10:0, and C12:0, respectively, and 0.63–0.76 for fatty acid groups. Lower accuracy in this study likely resulted from limited breed and sample diversity.

Ferrand et al. compared partial least squares (PLS) and genetic algorithm-PLS models using 317 samples from four breeds. PLS rapidly established models with external validation coefficients of 0.82–0.95 for C4:0–C12:0, and 0.97 and 0.89 for saturated and unsaturated fatty acids. Genetic algorithm-PLS slightly improved accuracy (0.98 and 0.91) but required more complex, time-consuming calculations, making PLS the preferred method for routine MIRS analysis. Maurice-Van Eijndhoven et al. developed MIRS prediction models from 41,404 samples across 24,445 cows, achieving high accuracy while confirming breed-related fatty acid differences. Thus, MIRS prediction accuracy for both individual and grouped fatty acids correlates positively with analyte concentration and exceeds traditional GC methods, with mass-based expression units providing superior correlation coefficients compared to volume-based units. Chemometric model selection and sample size also influence accuracy.

### 3. Analysis of Milk Proteins

Milk proteins comprise approximately 80% caseins ( $\alpha$ -,  $\alpha$ s1-,  $\alpha$ s2-, and  $\beta$ -casein) and 20% whey proteins ( $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin). Traditional protein analysis relies on chemical methods such as liquid chromatography, capillary electrophoresis, and capillary isoelectric focusing. Recent studies demonstrate that MIRS enables non-destructive analysis with reduced measurement time and improved accuracy.

Etzion et al. established prediction models using specific MIRS absorption regions ( $1500\text{--}1700\text{ cm}^{-1}$  and  $1060\text{--}1100\text{ cm}^{-1}$ ) for milk proteins, achieving low prediction errors suitable for rapid analysis. De Marchi et al. analyzed 1,336 Simmental cow milk samples using reversed-phase liquid chromatography and MIRS, developing quantitative models via partial least squares. Total protein and casein predictions correlated with chemical analysis at  $r = 0.58$ , with cross-validation root mean square errors of 3.11 and 2.76 g/L. Whey protein,  $\alpha$ S1-casein, and  $\beta$ -lactoglobulin achieved correlations of 0.50–0.58. However,  $\alpha$ -lactalbumin showed poor correlation ( $r = 0.29$ ) due to low concentration, consistent with findings that prediction precision correlates linearly with analyte concentration.

Bonfatti et al. analyzed 13 protein components in Simmental milk via MIRS, achieving higher correlations: total protein (0.80), casein (0.79), whey protein (0.65),  $\alpha$ S1-casein (0.69), and  $\beta$ -lactoglobulin (0.67). Spectral preprocessing (first or second derivative) significantly improved low-concentration  $\alpha$ -lactalbumin prediction ( $r = 0.39$  vs. 0.29).

Expression units also affect protein prediction accuracy. Rutten et al. used capillary zone electrophoresis and MIRS on 5,545 Holstein samples, pioneering percentage-of-total-protein expression units. This approach yielded lower accuracy for casein ( $r = 0.25$ ), whey protein ( $r = 0.53$ ), and  $\beta$ -lactoglobulin ( $r = 0.56$ ) compared to g/dL units which achieved correlations  $>0.90$  in previous studies.

Lactoferrin enhances iron transport and absorption while exhibiting broad antimicrobial properties. Immunoenzymatic assays require sample pretreatment and complex procedures, while HPLC demands high purity and expensive equipment. Soyeurt et al. analyzed 1,609 samples from 475 cows using MIRS, achieving a lactoferrin prediction correlation of 0.83 (cross-validation  $r = 0.75$ ), surpassing traditional immunoenzymatic methods. A subsequent study of 2,499 samples from Ireland, Scotland, and Belgium evaluated six preprocessing methods, demonstrating high correlations (0.69–0.74). Since somatic cell count commonly assesses mastitis and immunity, and lactoferrin relates to immune function, combining lactoferrin predictions with somatic cell equations enables rapid mastitis detection. As with fatty acids, MIRS protein prediction accuracy correlates with analyte concentration and varies with expression units. Compared to chromatographic and immunological methods, MIRS significantly improves prediction precision and efficiency while enabling integrated health assessment

and management enhancement.

#### 4. Analysis of Milk Minerals and Other Harmful Substances

Beyond proteins and fatty acids, milk contains abundant minerals, trace elements, and vitamins. MIRS has become a research hotspot for these components due to its rapid, non-destructive nature. Soyeurt et al. analyzed 1,543 samples from six breeds using atomic absorption and MIRS, demonstrating high predictive capacity for calcium ( $r = 0.96$ ), sodium ( $r = 0.83$ ), and phosphorus ( $r = 0.99$ ), consistent with Toffanin et al. Potassium and magnesium showed moderate accuracy (cross-validation  $r = 0.76$  and  $0.85$ ). Stocco et al. analyzed buffalo milk from 173 animals across five populations, achieving correlations of 0.71, 0.70, 0.72, and 0.55 for calcium, phosphorus, magnesium, and potassium, confirming MIRS applicability across milk sources. Wu et al. compared NIRS and MIRS for calcium prediction in milk powder, finding superior MIRS performance ( $r = 0.94$  vs.  $0.64$ ) in the  $671\text{--}3698\text{ cm}^{-1}$  region.

Production requires real-time detection of adulteration and drug residues beyond nutrient analysis. Chromatographic, mass spectrometric, immunological, and microbiological methods are complex, costly, and slow. MIRS offers higher accuracy for rapid, real-time adulterant quantification. Sivakesava et al. used NIRS and MIRS to quantify tetracycline in milk via partial least squares, with MIRS achieving superior accuracy (maximum  $r = 0.93$ ), improving with tetracycline concentration. Santos et al. quantified milk adulterants (water, whey, hydrogen peroxide, urea, synthetic milk, synthetic urine) using MIRS, achieving correlations of 0.90–0.98 and standard errors of 0.014–2.33. A subsequent comparison of NIRS and MIRS for these six adulterants revealed more distinct absorption peaks in the MIRS region, enabling more accurate quantitative models. Thus, MIRS accurately quantifies not only fatty acids and proteins but also minerals and harmful substances, offering simple operation, high accuracy, and real-time analysis capability as an effective alternative to traditional methods.

#### 5. Prediction and Assessment of Cow Nutrition, Health, and Reproduction

Beyond nutrient analysis, MIRS prediction models enable effective monitoring of cow nutrition, health, and reproduction through appropriate biomarker selection. During early lactation, high-producing cows in negative energy balance exhibit elevated blood and milk ketone bodies ( $\beta$ -hydroxybutyrate, acetone, acetoacetate), leading to ketosis and fatty liver. Heuer et al. quantified milk acetone using MIRS and GC, identifying strong absorption at  $1239\text{--}1370\text{ cm}^{-1}$  with correlation  $r = 0.66$  and prediction error 0.21 mmol, significantly outperforming GC for ketosis detection. Hansen identified acetone absorption peaks at  $964\text{--}1581\text{ cm}^{-1}$ ,  $1697\text{--}1812\text{ cm}^{-1}$ , and  $2699\text{--}2969\text{ cm}^{-1}$ , achieving  $r = 0.81$  and prediction error 0.27 mmol via partial least squares. Van Kneegsel et al. and Van Der Drift et al. compared MIRS with test kits for acetone and  $\beta$ -hydroxybutyrate, confirming MIRS provided more accurate and rapid predictions for hyperketonemia

screening.

Chronic negative energy balance impairs production, disease resistance, and reproduction. Traditional energy balance prediction methods include: (1) calculating intake-output differences, (2) monitoring body weight and condition score changes, and (3) measuring blood/milk metabolites (ketones, insulin, fat, protein, carbohydrates). The first two approaches are labor-intensive and inaccurate, while the third has gained popularity through MIRS applications.

McParland et al. analyzed 1,145 Scottish milk samples using MIRS to predict body energy status, developing models for energy balance, tissue energy content, and effective energy intake based on milk fat and protein content, yield, dry matter intake, body weight, and condition score. The models achieved correlations of 0.78, 0.64, and 0.88, respectively, demonstrating utility for large-scale assessment. A subsequent study of 1,218 UK and 1,586 Irish Holstein cows yielded similar correlations (0.88, 0.75, and 0.62), confirming MIRS effectiveness for energy status prediction. Since residual feed intake correlates significantly with energy balance ( $r = 0.85$ ), MIRS can also predict feed efficiency. McParland et al. predicted residual feed intake from milk nutrients and body traits, achieving  $r = 0.70$ . Thus, MIRS prediction models combined with animal characteristics enable comprehensive nutrition, health, and reproductive management.

## 6. Summary and Outlook

MIRS enables rapid, accurate quantification of nutrients and harmful substances in large-scale milk samples, facilitating assessment of nutritional, health, and reproductive status to enhance milk value and industry profitability while promoting efficient, healthy, and green livestock production.

Although MIRS model development requires substantial data, detailed processing, and thorough validation, advances in computing technology and mathematical optimization will expand its practical impact. MIRS can also quantify other nutrients like vitamins A, B, and C, and predict rumen methane emissions. Future research should expand sample diversity across breeds, genetics, and management conditions to improve model accuracy and applicability. Integrating MIRS with feed efficiency and genetic selection will undoubtedly advance efficient, low-carbon, and sustainable development in China's livestock industry.

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