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Performance Evaluation of PLS-Based FT-NIR Calibration Models for Milk Powder Proximate Analysis

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Abstract

Rapid and reliable determination of the proximate composition of milk powder is essential for quality assurance, nutritional labeling, and process control in the dairy industry. Conventional analytical methods, although highly accurate, are labor-intensive, time-consuming, and require extensive sample preparation and chemical reagents. This study aimed to develop and evaluate Fourier Transform Near-Infrared (FT-NIR) calibration models based on Partial Least Squares (PLS) regression for the rapid determination of moisture, protein, fat, and ash contents in milk powder according to the recommendations of ISO 21543|IDF 201:2020. A total of 304–912 representative milk powder samples were analyzed using standard reference methods to generate calibration data, followed by FT-NIR spectral acquisition using a Bruker TANGO spectrometer. Various spectral preprocessing techniques were evaluated, and the optimal models were validated through internal cross-validation using the coefficients of determination (R^2), root mean square error of estimation (RMSEE), root mean square error of cross-validation (RMSECV), bias, and residual predictive deviation (RPD). The combination of Standard Normal Variate (SNV) and first-derivative preprocessing produced the best calibration performance for all analytes. The developed models achieved calibration R^2 values ranging from 0.9245 to 0.9949 and cross-validation R^2 values between 0.8996 and 0.9933, with low prediction errors and RPD values exceeding 3. These findings demonstrated that PLS-based FT-NIR calibration models provided accurate, rapid, and non-destructive proximate analysis, supporting their application as reliable tools for routine quality control in dairy laboratories.

INTRODUCTION

Safe and high-quality food is essential for protecting public health, ensuring food security, and sustaining the growth of the food industry (Ali et al., 2026; Dhal & Kar, 2025; Lisboa et al., 2024; Melnyk et al., 2024; Varzakas & Smaoui, 2024). Dairy products are recognized as important sources of essential nutrients, including proteins, fats, vitamins, and minerals, and therefore play a significant role in maintaining human health and nutrition (Li et al., 2026). Globally, the dairy sector continues to expand, driven by urbanization, rising incomes, and changing consumer lifestyles, resulting in increased per capita dairy consumption (Amenu et al., 2019; D et al., 2018). This trend is also evident in Indonesia, where dairy consumption is projected to increase from 3.02 million metric tons in 2023 to 3.63 million metric tons by 2026, according to the United States Department of Agriculture (USDA), reflecting the growing demand for effective quality assurance systems throughout the dairy supply chain (Carolus Darmawan & Lisa Ahramijan, 2025).

Analytical laboratories play a crucial role in supporting quality assurance by determining the nutritional composition of dairy products, including moisture, protein, fat, ash, and carbohydrate contents, to ensure the physicochemical and organoleptic quality of dairy products (Y. Wang et al., 2026). These parameters are fundamental for process control, product release, and nutritional labeling. Conventional proximate analyses are commonly performed using standardized reference methods, including thermogravimetric analysis for moisture determination (Standar Nasional Indonesia, 2015), Kjeldahl or Dumas methods for protein determination (P. G. Wiles et al., 1998), Mojonnier extraction for fat determination (Standar Nasional Indonesia, 2022), and muffle furnace incineration for ash determination (Standar Nasional Indonesia, 2022). Although these methods provide high analytical accuracy and metrological traceability, they are labor-intensive, require extensive sample preparation, consume chemical reagents, and involve relatively long analysis times, limiting laboratory efficiency as analytical demands continue to increase (X. Wang, Liu, et al., 2026).

Consequently, modern analytical laboratories require alternative methods that not only maintain analytical reliability but also improve operational efficiency through shorter analysis times, minimal sample preparation, higher sample throughput, and reduced chemical consumption. Among available technologies, Fourier Transform Near-Infrared (FT-NIR) spectroscopy has emerged as one of the most promising analytical approaches for routine food analysis (Chen et al., 2018). Unlike conventional reference methods, FT-NIR is a secondary analytical technique that predicts analyte concentrations by establishing mathematical relationships between near-infrared spectral data and reference analytical values using chemometric algorithms. Its ability to simultaneously quantify multiple constituents within seconds, without sample destruction or chemical reagents, has led to widespread application in the dairy industry for quality control and compositional analysis (X. Wang, Liu, et al., 2026).

To support the reliable implementation of this technology, ISO 21543|IDF 201:2020 provides internationally recognized guidance for applying Near-Infrared Spectroscopy to milk and dairy products. The standard describes the analytical workflow, including representative sample selection, determination of reference values using validated primary methods, chemometric model development, and calibration model validation prior to routine implementation. Because FT-NIR predictions depend on the relationship established between spectral information and reference analytical data, the accuracy, robustness, and long-term reliability of this technique are fundamentally determined by the quality of the calibration model. Therefore, calibration model development and validation represent critical stages in implementing FT-NIR as a reliable secondary analytical method (International Organization for Standardization & International Dairy Federation, 2020).

The development of FT-NIR calibration models has been extensively investigated in food analysis due to its ability to correlate spectral responses with reference values through chemometric approaches (Dias et al., 2026). Previous studies have demonstrated that quantitative FT-NIR models can be successfully developed using Partial Least Squares (PLS) regression (Chen et al., 2018; Diana Giannuzzi et al., 2022) and evaluated through statistical indicators, including the coefficient of determination (R^2), Root Mean Square Error of Calibration (RMSEC), Root Mean Square Error of Cross-Validation (RMSECV), and Root Mean Square Error of Prediction (RMSEP) (De Girolamo et al., 2020). However, most published studies have focused primarily on food authentication, classification, and

adulteration detection using FT-NIR spectroscopy (Cai et al., 2025). Comparatively few studies have comprehensively described the development of FT-NIR calibration models specifically for routine proximate analysis of milk powder, including moisture, protein, fat, and ash determination, following the standardized workflow recommended in ISO 21543|IDF 201:2020.

Therefore, this study aimed to develop and validate FT-NIR calibration models for determining moisture, protein, fat, and ash contents in milk powder using Partial Least Squares (PLS) regression in accordance with ISO 21543|IDF 201:2020. Model performance was evaluated using calibration and cross-validation statistical parameters to assess predictive capability and suitability for routine laboratory implementation.

METHOD

Sample Collection

Milk powder samples used in this study consisted of various dairy products, including infant formula, follow-up formula, growing-up formula, and other commercial milk powder products routinely analyzed in the laboratory. All samples were initially analyzed using the corresponding reference methods specified in SNI 2970:2015 to obtain reference values for calibration model development.

The number of samples varied depending on the analyte and the available concentration range. A total of 304 samples were used for ash calibration, 439 samples for fat calibration, 374 samples for moisture calibration, and 912 samples for protein calibration. The concentration ranges of each analyte are summarized in Table 1.

Table 1. Number of samples and concentration ranges used for calibration model development

Parameter	Number of Sample	Calibration Range
Fat	439	0,27–42,04
Moisture	374	1,61–8,36
Ash Content	304	1,02–7,91
Protein	912	0,57–14,12

Reference Analysis

Reference values were obtained using conventional analytical methods according to SNI 2970:2015. Moisture content was determined by the thermogravimetric method, protein by the Kjeldahl method, fat by the Mojonnier extraction method, and ash by muffle furnace incineration. The analytical results obtained from these reference methods were used as calibration targets for FT-NIR model development.

FT-NIR Spectral Acquisition

Spectral measurements were performed using a Bruker TANGO FT-NIR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) operated with OPUS software. Samples were scanned according to the manufacturer's operating procedure, and the acquired near-infrared spectra were used as predictor variables for calibration model development.

Calibration Model Development

Calibration models were developed using the Partial Least Squares (PLS) regression algorithm implemented in OPUS software. PLS is one of the most widely used chemometric

techniques in NIR spectroscopy because it effectively establishes relationships between spectral variables and reference values while overcoming multicollinearity among spectral data (Chen et al., 2018).

Prior to model development, spectral data were subjected to preprocessing to reduce spectral variations unrelated to chemical composition, including baseline shifts, light scattering, and sample thickness variations. Several preprocessing methods available in OPUS including Linear Offset Subtraction, Straight Line Subtraction, Vector Normalization, Min–Max Normalization, Multiplicative Scatter Correction (MSC), First Derivative, and Second Derivative were evaluated during model development. The preprocessing combination providing the best statistical performance was selected as the final calibration model .

Independent calibration models were established for each proximate parameter using samples with known reference values.

Model Validation and Performance Evaluation

The predictive performance of each calibration model was evaluated using internal cross-validation implemented in OPUS software. Cross-validation iteratively excludes subsets of calibration samples from the calibration process and subsequently predicts their reference values, thereby assessing model robustness and generalization capability without requiring an external validation dataset.

Model performance was evaluated using the coefficient of determination (R^2), Root Mean Square Error of Estimation (RMSEE), Root Mean Square Error of Cross Validation (RMSECV), bias, and Residual Prediction Deviation (RPD). The optimum number of latent variables (PLS rank) was selected based on the lowest RMSECV while maintaining a high coefficient of determination, thereby achieving an optimal balance between predictive accuracy and model robustness without underfitting or overfitting.

RESULTS AND DISCUSSION

Calibration Dataset Characteristics

The predictive performance of FT-NIR calibration models is highly dependent on the representativeness of the calibration dataset. Since FT-NIR is a secondary analytical method, the developed models can only reliably predict samples that are adequately represented within the calibration domain. Therefore, calibration samples should encompass the compositional variability expected during routine analysis.

In this study, calibration models were developed using milk powder samples representing various commercial dairy products, including infant formula, follow-up formula, growing-up formula, and other milk powder formulations. The calibration datasets consisted of 304–912 samples with broad concentration ranges for all proximate parameters (Table 2). Such variability provides sufficient spectral information for the PLS algorithm to establish robust relationships between spectral responses and reference values.

According to ISO 21543|IDF 201:2020, calibration samples should represent the variability encountered during routine laboratory analysis. Consequently, the heterogeneous dataset employed in this study is expected to improve model robustness and expand the applicability domain of the developed FT-NIR calibration models.

Development of FT-NIR Calibration Models

Table 2 summarizes the optimum preprocessing methods and the number of latent variables selected for each proximate parameter. Among all preprocessing techniques evaluated in OPUS, the combination of Standard Normal Variate (SNV) followed by the **First Derivative** consistently produced the best calibration performance for fat, moisture, ash, and protein, although the optimum number of latent variables differed among analytes.

Table 2. Selected preprocessing methods and optimum PLS rank

Parameter	Preprocessing	PLS Rank
Fat	SNV + First Derivative	10
Moisture	SNV + First Derivative	9
Ash Content	SNV + First Derivative	10
Protein	SNV + First Derivative	7

Calibration models were established using Partial Least Squares (PLS) regression he consistent selection of SNV combined with the first derivative suggests that correcting physical sources of spectral variation was an important step in developing robust calibration models for milk powder 16/J.(Wiggins et al., 2007). Powdered dairy products commonly exhibit variations in particle size distribution, sample packing density, optical path length, and diffuse light scattering, which may introduce baseline shifts and scatter effects that are unrelated to chemical composition (Andries & Vander Heyden, 2024)If not properly corrected, these physical variations can reduce the correlation between spectral responses and reference values, thereby decreasing calibration accuracy.

SNV is widely applied to compensate for multiplicative scatter effects arising from differences in particle size and sample packing, while the first derivative corrects baseline offsets and enhances subtle absorption features associated with chemical constituents. The combination of these preprocessing techniques improves spectral comparability among samples and facilitates the extraction of chemically relevant information for subsequent PLS modelling (Wiggins et al., 2007; Zeaiter & Rutledge, 2009).

Although the same preprocessing strategy was selected for all proximate parameters, the optimum PLS rank varied from 7 to 10 latent variables. Protein achieved optimal performance with seven latent variables, moisture required nine, whereas fat and ash required ten. This difference indicates that each analyte exhibits a different degree of spectral complexity and therefore requires a different amount of latent information to adequately describe the relationship between spectral responses and analyte concentration (X. Wang, Yu, et al., 2026). Selecting an appropriate number of latent variables is essential to balance model complexity and predictive performance, as too few latent variables may lead to underfitting, whereas too many may incorporate spectral noise and increase the risk of overfitting.

Overall, these findings demonstrate that appropriate spectral preprocessing, combined with optimization of the PLS latent variables, is essential for developing robust FT-NIR calibration models for routine quantitative analysis of milk powder.

Performance of FT-NIR Calibration Models

Table 3 presents the statistical performance of the developed calibration models. Overall, all models demonstrated satisfactory predictive capability, with calibration coefficients of

determination ranging from 92.45% to 99.49%, while cross-validation coefficients ranged from 89.96% to 99.33%.

Table 3. Performance statistics of the developed FT-NIR calibration models for proximate analysis of milk powder

Parameter	Preprocessing	PLS Rank	Calibration		Cross-validation		Bias	RPD
			R^2	RMSEE (%)	R^2	RMSECV (%)		
Fat	SNV + First Derivative	10	0.9949	0.530	0.9933	0.609	0.00975	12.20
Moisture	SNV + First Derivative	9	0.9303	0.266	0.9055	0.309	−0.00261	3.25
Ash Content	SNV + First Derivative	10	0.9245	0.391	0.8996	0.448	−0.00468	3.16
Protein	SNV + First Derivative	7	0.9382	0.396	0.9297	0.442	−0.00185	3.77

Among the evaluated analytes, the fat calibration model achieved the highest predictive performance, with calibration and cross-validation coefficients of determination exceeding 99%, accompanied by the lowest prediction errors and the highest RPD value (12.20). These results indicate an excellent agreement between FT-NIR predictions and the corresponding reference measurements. The superior performance of the fat model is consistent with the strong absorption of C–H overtone and combination bands in the near-infrared region, which provide highly informative spectral features for chemometric modeling.

Protein and moisture models also demonstrated good predictive performance, with cross-validation coefficients exceeding 90% and RPD values greater than 3. Although both constituents possess characteristic N–H and O–H absorption bands within the NIR region, their predictive performance was slightly lower than that observed for fat. This difference is likely associated with narrower concentration ranges and increased overlap among absorption bands of multiple milk constituents, resulting in more complex spectral–chemical relationships.(X. Wang, Yu, et al., 2026)

Compared with the other proximate parameters, ash exhibited the lowest predictive performance. Unlike organic compounds, minerals do not directly absorb near-infrared radiation because they lack NIR-active X–H bonds. Consequently, ash prediction relies on indirect correlations with organic constituents rather than direct spectral information, which explains the comparatively lower coefficient of determination observed in this study. Nevertheless, the obtained RPD value remained above 3, indicating that the calibration model is still suitable for quantitative analysis.

Besides the high coefficients of determination, all calibration models exhibited relatively low RMSEE and RMSECV values, together with bias values close to zero. Furthermore, only minor differences were observed between calibration and cross-validation statistics, indicating that the selected models retained their predictive capability during internal validation and showed no strong indication of overfitting. According to the classification proposed by Williams and Sobering, calibration models with RPD values greater than 3 are considered

suitable for quantitative applications, whereas RPD values above 8 indicate excellent predictive performance. Therefore, all developed models are considered appropriate for routine quantitative determination of milk powder proximate composition, with the fat model demonstrating outstanding analytical performance.

Overall, these findings demonstrate that the calibration workflow recommended in ISO 21543|IDF 201:2020 is capable of producing robust FT-NIR calibration models for routine proximate analysis of milk powder when representative calibration samples and appropriate spectral preprocessing are employed.

CONCLUSION

This study successfully developed FT-NIR calibration models for determining fat, moisture, ash, and protein contents in milk powder using the Partial Least Squares (PLS) algorithm in accordance with the recommendations of ISO 21543|IDF 201:2020. The use of representative calibration datasets, appropriate spectral preprocessing techniques, and internal cross-validation resulted in robust calibration models with strong predictive performance for all evaluated proximate parameters. All developed models demonstrated high coefficients of determination, low prediction errors, minimal bias, and satisfactory Residual Predictive Deviation (RPD) values, indicating their suitability for routine quantitative analysis of milk powder. These findings confirmed that FT-NIR, when supported by properly developed calibration models, can serve as a rapid, reliable, and non-destructive secondary analytical method for proximate analysis in dairy quality control laboratories.

Future studies should expand calibration databases by incorporating a broader range of milk powder products and increasing sample diversity to enhance the applicability domain of the developed models. External validation involving multiple laboratories is also recommended to further evaluate model transferability and support wider implementation of FT-NIR spectroscopy in laboratories operating under ISO/IEC 17025 requirements.

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