Rapid detection of adulteration in powdered camel milk using ATR-IR spectroscopy and chemometrics

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Academic Editor: Prof. Fabrizio Cincotta – University of Messina, Italy

Received: 23 May 2025; Accepted: 7 October 2025; Published: 1 January 2026

ORIGINAL ARTICLE

Abstract

As global interest in camel milk rises because of its nutritional benefits, it is increasingly processed into powder to increase shelf life and transport. However, adulteration with cow milk powder poses a serious food safety issue. This study developed multivariate models using attenuated total reflectance infrared spectroscopy (ATR-IR) to detect adulteration in camel milk powder with cow milk powder. Partial least squares regression (PLSR) was applied after preprocessing, which included Savitzky–Golay (SG) smoothing (first and second derivatives) and multiplicative scatter correction (MSC), to quantify adulteration levels ranging from 0% (control) to 50% (w/w). Preprocessing techniques, including SG smoothing (first and second derivatives) and multiplicative scatter correction (MSC), enhanced model performance. Calibration achieved an R^2 of ≥99.6% and RMSE <0.93, while cross-validation yielded an R^2 of ≥99.3% and RMSE <1.35. External validation confirmed the model's reliability with R^2 >98% and RMSE <1.8. Deviations remained low across all adulteration levels, even at higher concentrations (≈0.76–0.90 for 40%). These results demonstrate that ATR-FTIR spectroscopy combined with PLSR offers a rapid, nondestructive, and reliable method for detecting adulteration in camel milk powder, making it a valuable tool for quality control in the dairy industry.

Keywords: Adulteration; Camel milk powder; Chemometrics; MIR; PLSR

Introduction

Camel milk is attracting growing interest because of its unique nutritional and medicinal benefits (Ait El Alia, Zine-Eddine, Kzaiber, *et al.,* 2023; Cheikh Ismail *et al.*, 2022; Mohan *et al.*, 2020). Traditionally consumed in arid and semi-arid regions, camel milk is now gaining recognition as a valuable dairy product worldwide, with increasing production and consumption (Faye and Konuspayeva, 2024). According to the Food and Agriculture Organization (FAOSTAT, 2024), global camel milk production exceeds 4 million metric tons annually, marking a 41% increase over the past two decades, with major producers such as Kenya, Somalia, and Pakistan at

 ISSN 1120-1770 online, DOI 10.15586/ijfs.v38i1.3188

the forefront (Ait El Alia, Zine-Eddine, *et al.*, 2025). The rising demand is linked to its distinctive composition, which is rich in vitamins (A, B1, B2, B12, C, D, and E) (Faye, Konuspayeva, and Bengoumi 2019), minerals (calcium, potassium, magnesium, zinc, iron, phosphorus, and selenium) (Konuspayeva, Faye, and Bengoumi, 2022), and bioactive compounds such as lactoferrin, immunoglobulins, lysozyme, and insulin-like proteins (Ho, Zou, and Bansal 2022). Research has suggested that camel milk may offer therapeutic benefits, such as anti-diabetic, anti-inflammatory, and immunomodulatory effects, making it highly sought after in health-conscious markets (Hussain *et al.*, 2021; Muthukumaran *et al.*, 2023; Swelum *et al.*, 2021).

The processing of camel milk has advanced to include a variety of derivative products, including fermented beverages, cheeses, and powders (Ait El Alia, Zine-Eddine, Ajbli, *et al.*, 2023; Ho, Zou, and Bansal, 2022; Konuspayeva and Faye 2021). Among these, powdered camel milk has gained significant popularity because of its extended shelf life, ease of transportation, and convenience in international trade (Rakhmatulina *et al.*, 2024). However, as demand increases, so does the risk of adulteration, particularly with cow milk, which is less expensive and more widely available (Bondoc, 2007; Wu *et al.*, 2022). Adulterating camel milk with cow milk raises significant concerns, as it undermines the product's authenticity and economic value, and poses health risks, especially for those with cow milk allergies (Ehlayel *et al.*, 2011).

Detecting milk adulteration is a major challenge in the dairy industry (Bondoc and Șindilar, 2002; Mabood *et al.*, 2017). Multiomics approaches, which include proteomics, metabolomics, lipidomics, and genomics, are gaining traction for their potential in authenticating dairy products (Ait El Alia, Chaji, *et al.*, 2025; Qin *et al.*, 2022). Proteomics focuses on identifying species-specific protein markers that can distinguish milk species, enabling precise adulteration detection (Agregán *et al.*, 2021). Metabolomics investigates small molecular variations, creating biochemical profiles that reveal subtle compositional differences (Suh, 2022). Lipidomics examines the unique lipid profiles of various milk types, making it an effective method for identifying unauthorized mixtures (Liu and Rochfort, 2023). Genomics, meanwhile, analyzes genetic material to confirm the species origin of the milk and detect adulteration at the DNA level (Mafra, Honrado, and Amaral, 2022). By combining these multiomics techniques with advanced statistical and machine learning models, researchers can enhance the accuracy of milk authentication, enabling reliable detection of even trace levels of adulteration and ensuring the integrity of camel milk products (Ait El Alia, El Mrabet, *et al.*, 2025; Su *et al.*, 2024).

Infrared (IR) spectroscopy, particularly attenuated total reflectance-Fourier transform IR (ATR-FTIR) spectroscopy, has emerged as an effective tool for detecting milk adulteration, because of its ability to generate detailed molecular fingerprints of food samples (Balan *et al.*, 2020; Saji *et al.*, 2024). This method allows for the differentiation of pure and adulterated milk based on their distinct spectral features. When combined with chemometric techniques such as principal component analysis (PCA), partial least squares regression (PLSR), and support vector machines (SVM), ATR-FTIR spectroscopy significantly improves the detection and quantification of adulterants (El Mrabet *et al.*, 2024; El Orche *et al.*, 2024 ; Grassi *et al.*, 2022).

This study aims to investigate the potential of ATR-FTIR spectroscopy, coupled with chemometric analysis, to authenticate powdered camel milk and identify adulteration with cow milk. By employing advanced spectroscopic and computational techniques, the research intends to provide a fast and reliable solution for ensuring the authenticity and safety of camel milk products in the global market.

Materials and Methods

Samples

The camel milk powder used in this study (protein: 25.0%, carbohydrates: 42.0%, and fat: 22.0%) was provided by a workshop specializing in the valorization of camel-based products in Laayoune, Morocco, ensuring guaranteed purity. Cow milk powder was purchased from a local supermarket in Beni Mellal, Morocco. Adulteration was performed by mixing camel milk powder with cow milk powder at 19 different concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 26, 30, 34, 38, 40, 44, 48, and 50%), resulting in a total of 57 samples. Each concentration was prepared in triplicate to ensure reliability.

Fourier Transform Mid-Infrared (FT-MIR) Spectral Analysis

FT-MIR spectroscopy was conducted using a Shimadzu IR Xross spectrophotometer (serial No. A229360) in attenuated total reflection (ATR) mode, without prior sample preparation. Spectra were recorded at a resolution of 4 cm^{-1} by directly applying samples onto the ATR cell. After each measurement, the ATR cell was cleaned with an isopropanol solution and dried with soft paper to prevent contamination. The reflectance values obtained were converted into absorbance (A) and subsequently analyzed.

Multivariate Data Analysis

The collected spectral data underwent preprocessing using various techniques to enhance signal clarity and minimize unwanted spectral variations arising from instrumental factors or sample inconsistencies. The SG polynomial smoothing method was applied to compute first and second derivatives, refining the spectral signals (Souhassou *et al.*, 2018). In addition, multiplicative scatter correction (MSC) was utilized to reduce variations unrelated to the chemical composition of the samples, ensuring improved data consistency and comparability (El Mrabet *et al.*, 2025).

Each sample was analyzed in triplicate, and all replicate spectra were individually included in the chemometric model to preserve experimental variability and improve model reliability.

To estimate the extent of adulteration in milk powder samples based on their FT-MIR spectra, PLSR was employed as a predictive modeling approach. PLSR is a statistical method that establishes the relationship between independent variables (X) and a dependent variable (Y) in high-dimensional datasets, particularly when multicollinearity is present among the predictor variables (Bazzoli Lambert-Lacroix, and Martinez 2023). This technique is widely adopted in spectroscopy, analytical chemistry, and other data-driven fields to construct models for predicting dependent variable values from input data (Ali *et al.*, 2025; Shi *et al.*, 2025).

The predictive models were assessed using key statistical metrics, including the root mean square error of calibration (RMSEC), the coefficient of determination (R^2), and the root mean square error of cross-validation (RMSECV). To evaluate the robustness and generalizability of the models, validation was performed using the leave-one-out (LOO) cross-validation method.

For external validation, independent data that were not included in the calibration phase were analyzed using only its IR spectra. The predicted values were subsequently compared with the reference values to assess the model's accuracy and performance.

Software

Data processing and visualization were conducted using Unscrambler X (v10.4) and the R programming language (v4.3.2).

Results and Discussion

ATR-IR spectra of pure and adulterated camel milk powder

The ATR-MIR spectra provide insights into the molecular composition of pure camel milk powder and its adulteration with commercial cow milk powder in varying concentrations from 2% to 50%. Figure 1 (a) shows a set of spectra that represents the raw IR absorption data, while Figure 1 (b) shows the derivative spectra, which enhance subtle differences in composition between the samples.

IR spectroscopy identifies key molecular vibrations that define the composition of milk powders. The spectral region between 3500 and 3200 cm^{-1} corresponds to O-H and N-H stretching vibrations, primarily associated with water content and protein structures, such as casein and whey proteins (Santos, Pereira-Filho, and Rodriguez-Saona 2013). Changes in this region indicate variations in protein concentration as cow milk powder is introduced. The range between 3000 and 2800 cm^{-1} represents C-H stretching vibrations linked to lipids, mainly triglycerides (Mohamed *et al.*, 2021). Differences in this section suggest variations in fat composition between camel and cow milk powders.

The protein-rich region, located between 1700 and 1500 cm^{-1}, contains the amide I and amide II bands, which provide structural information on milk proteins. The amide I band, found near 1650 cm^{-1}, corresponds to C=O stretching, while the amide II band, near 1550 cm^{-1}, arises from N-H bending and C-N stretching (Aernouts *et al.*, 2011; AlYammahi *et al.*, 2023). Variations in these peaks suggest differences in protein composition, likely because of the introduction of cow milk proteins, especially β-Lg. The spectral range between 1200 and 900 cm^{-1} is associated with carbohydrate-related vibrations, including C=O stretching in lactose and phospholipids (Mohamed *et al.*, 2021; Pinto *et al.*, 2021). Differences in this region reflect changes in carbohydrate concentration, particularly lactose, which is higher in cow milk (Swelum *et al.*, 2021). The fingerprint region, extending from 900 to 600 cm^{-1}, represents complex vibrational patterns from lactose and other minor compounds. Any noticeable shifts in this section further confirm compositional differences because of adulteration.

Derivative spectroscopy enhances spectral contrast, making it easier to detect subtle compositional changes. The derivative spectra improve peak resolution and enable a more precise differentiation between pure and adulterated samples. The variations in peak intensities,

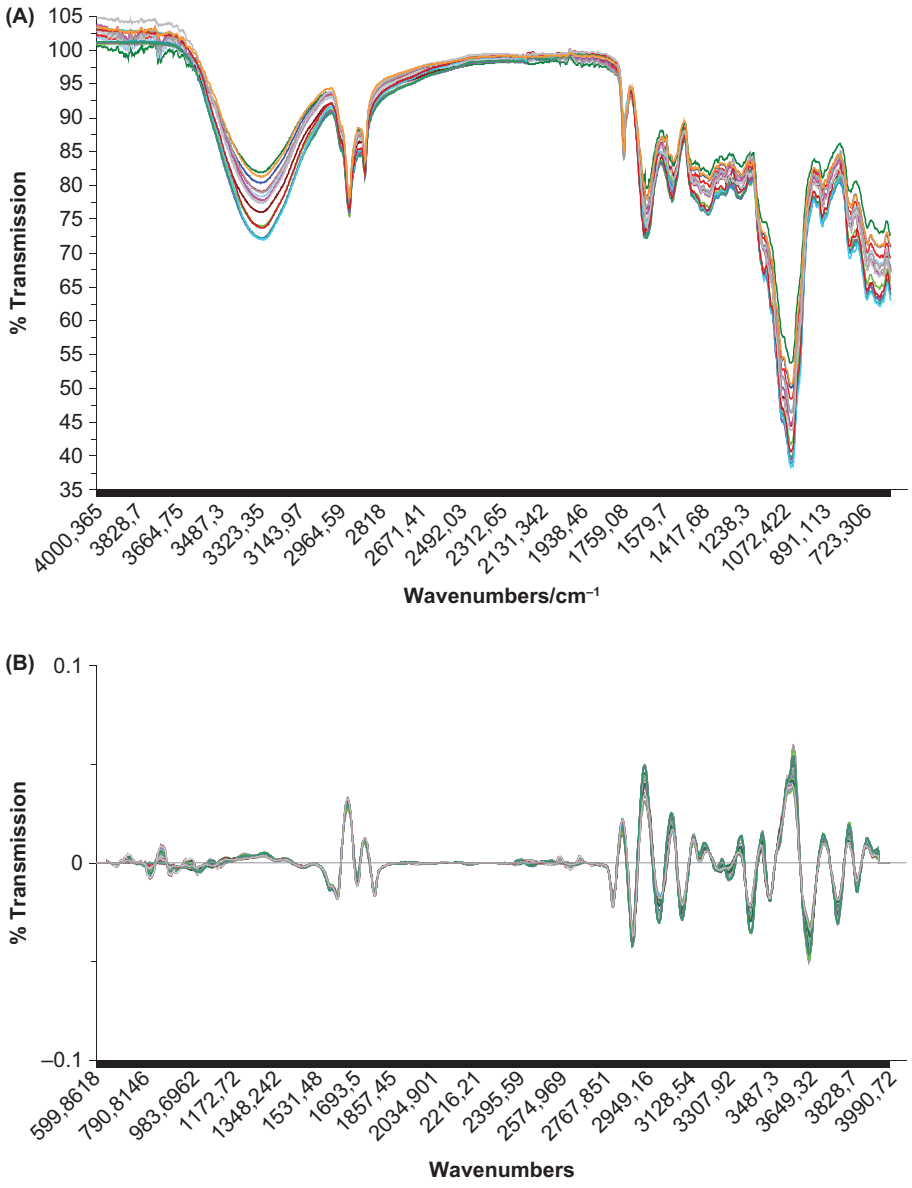

Figure 1. Attenuated total reflectance infrared spectroscopy spectra (700–4000 cm^{-1}) (A) and derivatives (B) of camel milk powder and adulterants.

especially in the protein and carbohydrate regions, suggest alterations in composition as the level of cow milk powder increases. Systematic spectral shifts indicate progressive changes in protein and lactose content. Modifications in the amide I and II bands point to alterations in protein structure, while lipid-related peaks in the 2800–3000 cm^{-1} range exhibit differences in intensity, confirming variations in fat content between camel and cow milk powders.

The ATR-MIR spectra, along with derivative analysis, effectively highlight the compositional changes resulting from the adulteration of camel milk powder with cow milk powder. As the level of adulteration increases, noticeable spectral shifts occur, particularly in the protein, carbohydrate, and lipid regions. These spectral differences serve as valuable indicators for detecting and quantifying milk powder adulteration. ATR-MIR spectroscopy proves to be a reliable analytical tool for assessing milk authenticity and ensuring quality control (Teixeira *et al.*, 2020). Further statistical evaluation could refine adulteration detection methods and improve the accuracy of quality assessment models (Biancolillo *et al.*, 2020).

Quantification of camel's milk powder adulteration

The correlation between the percentage of cow milk powder in the prepared samples and their IR spectra was

analyzed using the PLSR technique. This approach was designed as an effective and straightforward method for detecting adulteration in camel milk powder. To improve the accuracy of the analysis, mathematical preprocessing techniques were employed to minimize additive and multiplicative effects, including measurement noise, background interference, and baseline fluctuations that could impact spectral quality. The applied preprocessing methods consisted of multiplicative scatter correction (MSC, detrending, and the SG derivative).

The effectiveness of the developed models was evaluated using several statistical metrics, including the coefficient of determination for cross-validation (R^2cv) and the root mean square error during calibration (RMSEcal), cross-validation (RMSEcv), and external validation. Table 1 presents the outcomes derived from applying ATR-MIR in combination with PLSR to both raw and preprocessed data.

Table 1. Performance parameters of partial least squares regression.

	R^2cal	RMSEC	R^2cv	RMSECV	LV
Raw data	0.995	1.033	0.991	1.488	7
SG1 10pt	0.997	0.854	0.993	1.256	7
SG1 15pt	0.997	0.810	0.994	1.196	7
SG1 25pt	0.997	0.835	0.993	1.295	7
SG2 10pt	0.996	0.893	0.993	1.320	7
SG2 15pt	0.996	0.921	0.993	1.348	7
SG2 25pt	0.997	0.869	0.994	1.248	7
MSC	0.996	0.920	0.993	1.230	7

The optimal number of latent variables (LVs) was selected based on the minimum RMSECV obtained using the LOO approach. As shown in the scree plot (Figure 2), the RMSECV decreased markedly up to the seventh LV before stabilizing, indicating that seven components provided the best balance between predictive performance and model simplicity, thus minimizing the risk of overfitting.

The blue line represents the calibration error, while the red line corresponds to the cross-validation error. The minimum RMSE observed at seven LVs indicates the optimal model complexity, providing a balance between predictive accuracy and overfitting control.

In assessing the results, models with the lowest RMSEcal and RMSEcv values were prioritized. All developed models exhibited strong predictive capabilities. Across the full mid-IR spectral range, R^2cv values were found to be between 99.1% and 99.4%, while RMSEcv values ranged from 1.196% to 1.488%. The optimal model was selected based on the highest R^2cv and the lowest RMSEcv, ensuring that the number of LVs remained reasonable to prevent overfitting. This best-performing model utilized the MSC preprocessing method, achieving an R^2cv of 99.3% and an RMSEcv of 1.23%. Figure 3 shows the PLSR in the 700–4000 cm^{-1} range of the calibration set pretreated with multiplicative dispersion correction (MSC) for camel milk powder adulterated with cow's milk powder.

Other preprocessing techniques, such as the first and second derivatives of the SG filter at different data points, also provided satisfactory results. Specifically, SG1 at 15 points and SG2 at 25 points achieved R^2cv values of

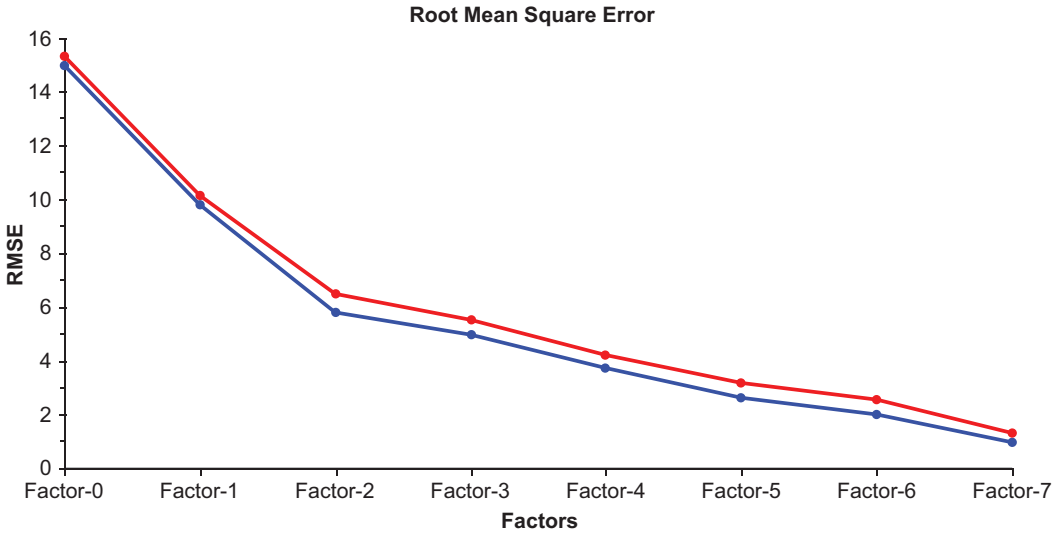

Figure 2. Scree plot illustrating the evolution of the root mean square error (RMSE) as a function of the number of latent variables (LVs) in the PLSR model.

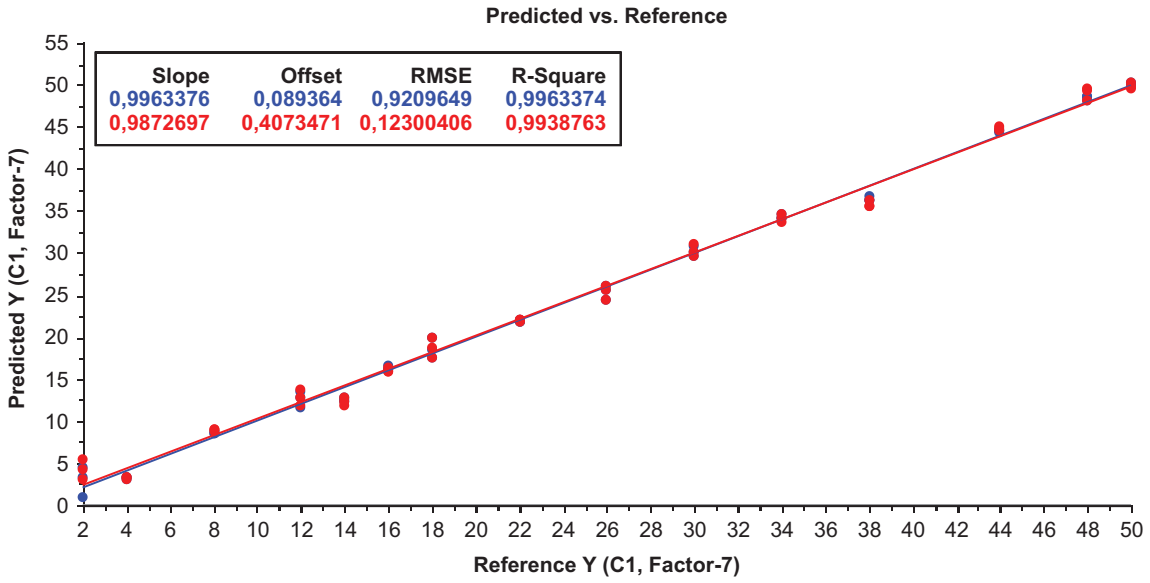

Figure 3. Partial least squares regression in 700–4000 cm^{-1} of the calibration set for camel milk powder adulterated with cow's milk powder.

99.4%, with corresponding RMSEcv values of 1.196% and 1.248%, respectively.

The effectiveness of modeling improves when data obtained through different techniques are processed using SG smoothing and MSC, both of which are widely used. In dairy authentication, mid-IR (MIR) spectra of goat milk adulterated with cow milk at varying concentrations were preprocessed using different techniques, including SG and MSC, resulting in an enhanced PLSR model (Du *et al.*, 2025). In addition, SG1 was applied for preprocessing near-IR hyperspectral imaging data to identify soybean flour in edible insects. When combined with regression methods, a one-dimensional convolutional neural network (1D-CNN) using SG first derivative (SG1) spectra achieved optimal prediction accuracy (Hernanda *et al.*, 2025). Furthermore, these preprocessing algorithms have demonstrated their potential for processing nonspectroscopic data, including hyperspectral imaging (Chen *et al.*, 2025).

Twelve independent samples were utilized for external validation to further assess the effectiveness of the models in quantifying camel milk powder adulteration. These samples, prepared at representative adulteration levels not included in the calibration set, were used to simulate real testing conditions. The test samples were analyzed under the same experimental protocol, and their adulteration levels were predicted using the PLSR models. This approach allowed us to evaluate the robustness and generalization capacity of the developed models.

Table 2 provides a summary of the results obtained from all the developed models, along with Figure 4.

Table 2. Performance of the partial least squares regression models by external validation using attenuated total reflectance infrared spectroscopy.

	R^2p	RMSEP	LV
Raw data	0.979	1.885	7
SG1 10pt	0.984	1.683	7
SG1 15pt	0.983	1.695	7
SG1 25pt	0.984	1.651	7
SG2 10pt	0.989	1.368	7
SG2 15pt	0.984	1.669	7
SG2 25pt	0.981	1.796	7
MSC	0.987	1.486	7

As shown, the R-squared values for PLSR exceeded 98%, while the mean square error remained below 1.7 for most of the preprocessed models. These findings indicate that the PLSR models demonstrated strong predictive performance and can be considered a reliable method for detecting even small amounts of cow milk powder adulteration in camel milk powder.

Table 3 presents the predicted values, deviations, and reference values derived from a preprocessed PLSR model applied to ATR-IR spectral data for detecting cow milk powder adulteration in camel milk powder. The model's performance is assessed by comparing the predicted values with the reference values and analyzing the extent of deviations.

The close agreement between predicted and reference values highlights the model's effectiveness in quantifying

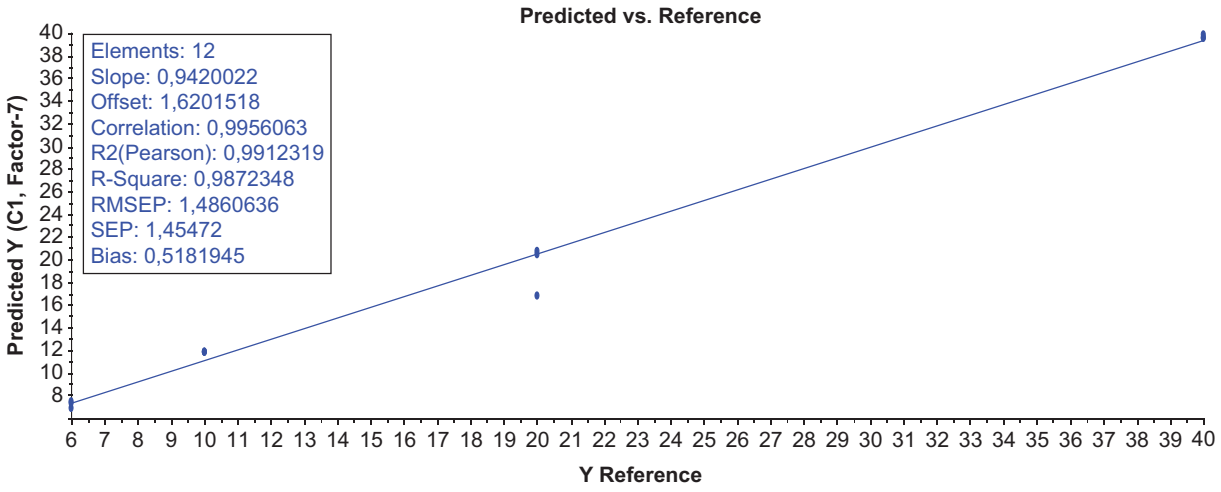

Figure 4. Partial least squares regression model for cow milk powder adulteration in camel milk powder using MSC pretreatment during external validation.

Table 3. Predicted versus reference values and deviations in the partial least squares regression model for the detection of adulteration.

Predicted value	Deviation	Reference value
11.874	1.133	10
11.874	1.133	10
11.874	1.133	10
20.725	1.100	20
20.377	0.961	20
16.797	1.017	20
39.914	0.899	40
39.563	0.758	40
39.563	0.758	40
7.437	1.830	6
7.354	1.766	6
6.867	1.669	6

adulteration levels. At lower adulteration concentrations (e.g., 10% and 20%), the predicted values remain within a narrow range of the actual values, with relatively small deviations (≈1.13 for 10% and ≈1.02 for 20%). This suggests that the model successfully captures spectral differences associated with minor compositional changes in the milk powder samples, highlighting its high sensitivity.

For higher adulteration levels (40%), the model continues to demonstrate strong predictive performance, with predicted values closely matching the reference values and deviations remaining low (≈0.76–0.90). This indicates that the PLSR model effectively distinguishes between pure camel milk powder and highly adulterated samples, likely because of the enhanced spectral contrast observed in ATR-IR spectra at higher levels of adulteration.

The integration of MIR spectroscopy with chemometric techniques has demonstrated significant efficacy in verifying the authenticity and ensuring the quality control of dairy products (Barrera Morelli *et al.*, 2025; Hayes *et al.*, 2023;). Recently, Ait El Alia *et al.*, (2024) applied MIR spectroscopy in conjunction with chemometric modeling to detect the adulteration of raw camel milk with cow milk, providing an analytical alternative to conventional sensory evaluation methods. Their study successfully distinguished between authentic and adulterated camel milk samples using PCA and hierarchical cluster analysis (HCA). Furthermore, PLSR and principal component regression (PCR) calibration models exhibited robust predictive capabilities in quantifying adulteration levels.

In the authentication of camel milk powder, our study demonstrated the potential of ATR-IR spectroscopy combined with PLSR as a rapid and nondestructive approach, outperforming proteomic and genomic methodologies. Li *et al.*, (2021) utilized ultra-performance liquid chromatography (UPLC) to detect adulteration in camel milk powder, employing bovine β-lactoglobulin (β-Lg) as a marker protein. Their findings revealed a strong linear calibration curve (R^2 = 0.998) when analyzing adulterated samples, with a detection threshold as low as 5%.

In addition, ultra-high-performance liquid chromatography coupled with quadrupole/exactive high-resolution mass spectrometry (UHPLC-Q/Exactive-HRMS) has been implemented to identify animal-derived components in camel milk and its processed derivatives. This method yielded highly linear standard calibration curves (R^2 > 0.99), facilitating precise quantification of adulteration levels. The limits of detection (LOD) for bovine and caprine milk in liquid camel milk were determined to be 0.35% and 0.49%, respectively, while in camel milk

powder, these values slightly increased to 0.68% and 0.73% (Gu *et al.*, 2024).

Furthermore, polymerase chain reaction (PCR) and real-time PCR have been employed for the molecular detection of adulteration in camel milk powder, specifically targeting Cytochrome b (CYTB) as a DNA marker. The real-time PCR assay demonstrated strong linearity, with R^2 values of 0.982 and 0.992 correlating ovine or bovine content with the Ct ratio (specific gene/internal reference gene). Method validation using simulated adulterated samples confirmed accuracy, with recovery rates ranging from 80% to 110% and a coefficient of variation below 7% (Wu *et al.*, 2022).

Conclusion

This research highlights the potential of MIR spectroscopy for detecting and measuring the adulteration of camel milk powder with commercially available cow milk powder. Differentiating one dairy matrix from another presents challenges, yet the use of preprocessed MIR spectra with MSC and partial least squares (PLS) analysis demonstrated high accuracy in adulteration detection. The strong results obtained can be attributed to the development of robust chemometric models that effectively capture sample variability, as reflected in their figures of merit. Therefore, this method offers a fast, nondestructive, and reliable approach for evaluating the quality of camel milk powder and identifying possible adulteration with cow milk powder.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2601).

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Author Contributions

O. Ait El Alia did writing–original draft, methodology, investigation, formal analysis, and data curation. A. El Orche was concerned with formal analysis, writing–review and editing, supervision, and investigation. M. Kaddouri was involved in writing– original draft, methodology, and investigation. S. Boukrouh was responsible for writing–review and editing, and investigation. A. El Mrabet looked into investigation and formal analysis. M. Bouhrim did writing–review and editing, and investigation. M. Al-zharani was responsible for supervision and data curation. F. A. Nasr and A. Ahmed Qurtam did project administration, writing–review and editing, and funding acquisition. K. Boutoial did supervision and investigation.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2601)

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