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Milk authenticity tests using FTIR coupled with advanced chemometrics

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Abstract:

Milk is a key component of the human diet, and its authentication is essential to ensure quality, safety, and consumer trust. This study explores the application of Fourier Transform Infrared Spectroscopy (FTIR) in combination with multivariate statistical analysis for the classification of milk samples on species level. A total of 178 milk samples derived from cow, goat, and sheep were analyzed in both liquid and freeze-dried forms using the Thermo Scientific Nicolet 6700 FTIR spectrometer. Prior to analysis, all samples were verified as unadulterated using ELISA testing. Spectral data were preprocessed and analyzed using the MixOmics package in R. Partial Least Squares Discriminant Analysis (PLS-DA) was employed to build predictive models, with data split into training (70%) and testing (30%) sets. Model optimization was achieved through repeated five-fold cross-validation. The classification results demonstrated a high level of accuracy, achieving complete discrimination among the three milk types. These findings underscore the potential of FTIR spectroscopy coupled with multivariate analysis as a rapid and reliable tool for food authentication and quality control.

• Introduction

Milk is an essential food, extremely important for human nutrition and health and a vital component for rural economies. Milk has a vital role in supporting nutritional needs at every stage of life by providing a wide range of nutrients, such as proteins, lipids, carbohydrates, vitamins, and minerals. This study explores the application of Fourier Transform Infrared Spectroscopy (FTIR) in combination with multivariate statistical analysis for the classification of milk samples on species level.

• Material and method

A total of 178 raw milk samples (44 goat, 61 cow, 73 sheep) were collected from farms across Thessaly, Greece, between April and July 2024 during morning milking. Each 50 mL sample was refrigerated, homogenized, and split into two aliquots—one kept raw, the other freeze-dried (BIOBASE BK-FD10P). Samples were stored at −18 °C and thawed gradually before analysis. Organoleptic properties (color, odor) and pH (Eutech/Oakton pH meter) were assessed; freeze-dried samples were checked for uniformity. ATR-FTIR spectra were obtained using a Nicolet 6700 spectrometer (DTGS detector, 4000–400 cm^{-1}, 32 scans/sample, triplicates). Spectra were preprocessed (baseline correction, normalization) and exported to CSV for visualization and analysis.Partial Least Squares Discriminant Analysis (PLS-DA) was performed using R (mixOmics). Data were split into training (70%) and test (30%) sets. Model performance was evaluated with 10-fold cross-validation, and Variable Importance in Projection (VIP) scores identified key spectral features for milk type classification (Figure 1).

Figure 1: Work Flow

• Results and discussions

Raw Sample PLS-DA

PLS-DA models were trained using repeated 10-fold cross-validation. The optimal number of latent variables was 14, minimizing the balanced error rate. The final model, evaluated on the test set, achieved 97.1% accuracy (95% CI: 84.7%–99.9%). Sensitivity and specificity were 100% for cow and goat samples, and 92.9% and 100% for sheep, respectively (Table 1).

	Confusion Matrix			Performance metrics		
	Cow	Goat	Sheep	Sensitivity	Specificity	Balanced Accuracy
Predicted as Cow Milk	12	0	0	1.00	0.96	0.98
Predicted as Goat Milk	0	8	0	1.00	1.00	1.00
Predicted as Sheep Milk	1	0	13	0.93	1.00	0.96
Overall Accuracy: 0.97, 95% CI: (0.85, 1.00)						

Table 1. Classification Performance Metrics and Confusion Matrix for PLS-DA Model of Raw milk samples on the test set.

Freeze-Dried Sample PLS-DA

For freeze-dried milk, the PLS-DA model required more latent variables (14–16) due to the richer spectral detail. The optimal model with 14 LVs achieved 97.1% test accuracy, correctly classifying all cow and sheep samples, with one goat sample misclassified. Higher VIP scores highlighted key spectral regions contributing to milk type discrimination (Figure 2).

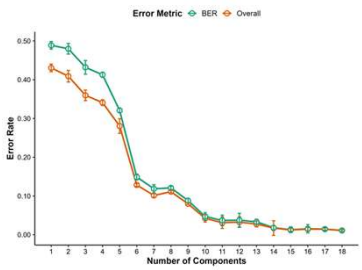

Figure 2: Classification error (BER and overall) of PLS-DA models for freeze-dried milk samples. Optimal performance was reached at 14–16 latent variables.

• Conclusions

This study demonstrates that Fourier-transform infrared (FTIR) spectroscopy, when combined with advanced chemometric techniques, offers a highly effective, non-destructive, and rapid method for discriminating milk samples based on their species and consequently foe detecting milk species fraud. Future research should focus on scaling the approach, validating it under industrial conditions, exploring processed matrices, and integrating more advanced data analysis techniques to enhance both precision and applicability

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