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TARGET-CENTRIC APPROACHES FOR CAMEL MILK ADULTERATION DETECTION: A REVIEW OF PROTEIN, METABOLITE AND GENE BASED ANALYTICAL STRATEGIES

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ABSTRACT

Milk fraud is a critical challenge for food safety and the dairy industry. To ensure product authenticity, various detection technologies have been developed and implemented. These methods cover key analytical targets such as proteins, lipids, small molecule metabolites, aromatic compounds and DNA and incorporate detection techniques such as chromatography, spectroscopy, immunology and biosensors. This review article presents an in depth analysis of advances in the field of camel milk authenticity verification studies. This analysis focuses on the evolution of methods for detecting adulteration, taking into account various target components. In addition, the article explores the technological development of detection techniques, highlighting their applicability in a variety of scenarios. The main objective of this study was to provide technical support to promote the sustainable development of the camel milk industry.

Key words: Adulteration detection, biosensors, camel milk, chromatography, ELISA, infrared and raman spectroscopy, isothermal amplification, mass spectrometry, nucleic acid sensing technology, omics techniques, PCR

Dairy products such as milk from cows, goats, sheep and camels serve as vital food sources for humans and various animals. They are rich in essential nutrients including calcium, vitamins and fats (White and Gleason, 2023).

Camel milk is recognised for its significant nutritional and medicinal value. Its components are often compared to human breast milk (Ho *et al*, 2022). In addition, the composition of camel milk, rich in functional proteins and enzymes such as lactoferrin, peptidoglycans, antibodies, lysozyme and lactoperoxidase, gives it remarkable immune and pathogen-resistant properties. Research has shown that casein hydrolysates derived from camel milk have notable inhibitory properties on dipeptidyl peptidase-IV (DPP-IV), suggesting a hypoglycemic action (Su *et al*, 2024). Indeed, the consumption of camel milk is recognised for its benefits to human health, particularly in the prevention of serious diseases (Shakeel *et al*, 2022).

In China, the main camel breeds contribute to the annual national production of camel milk, which amounts to 2,700 tons (China Dataset, 2024). These breeds include the Alax Bactrian camel, the Urad Gobi red camel, the Sunite Bactrian camel, the Xinjiang Tarim Bactrian camel, the Xinjiang Junggar Bactrian camel and the Qinghai Bactrian camel. These breeds have a milk production that can vary between 730 and 1,095 kilograms per lactation period, with an average duration of 14 to 16 months (Xiao *et al*, 2022).

The increase in demand for camel milk has led to a significant increase in the risk of adulteration, jeopardising product quality and consumer safety. Common methods used to prepare these products include adding water or incorporating animal milk of unknown origin, such as cow's milk. While these processes artificially increase the quantity of the products, they significantly reduce their nutritional value. The adulteration of cow's milk

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with contaminants is particularly important because the beta-lactoglobulin present in milk can induce allergic reactions in consumers (Ceniti *et al,* 2023) and pose health risks, including gastrointestinal disorders (Spink *et al,* 2017). These practices not only compromise the authenticity of camel milk but also undermine consumers' right to information. It is therefore imperative to implement an efficient system for detecting adulteration with camel milk.

Milk fraud is a critical challenge for food safety and the dairy industry. To ensure product authenticity, various detection technologies have been developed and implemented. These methods cover key analytical targets such as proteins, lipids, small molecule metabolites, aromatic compounds and DNA and incorporate detection techniques such as chromatography, spectroscopy, immunology and biosensors. This review article presents an in-depth analysis of advances in the field of camel milk authenticity verification studies. This analysis focuses on the evolution of methods for detecting adulteration, taking into account various target components. In addition, the article explores the technological development of detection techniques, highlighting their applicability in a variety of scenarios. The main objective of this study was to provide technical support to promote the sustainable development of the camel milk industry.

Analysis of Adulteration Detection Methods

A. Protein-based method

The protein composition and types of milk from different animal sources exhibit significant differences and these specific proteins can serve as target molecules for species identification. The primary markers for detecting adulteration in dairy products include κ-casein, αs1-casein, α-lactalbumin and β-lactoglobulin. The utilisation of detection technologies founded upon protein targets (e.g., immunochemical analysis, electrophoresis, chromatography and mass spectrometry) has emerged as a pivotal method for the analysis of the species composition of raw milk. This preeminence can be attributed to their exceptional specificity and sensitivity (Jia *et al,* 2025).

1. ELISA

Bovine β-lactoglobulin constitutes 7–12% of total milk protein and 43.6–50.0% of whey protein. Given the absence of this protein in camel milk, it is widely used as a marker for bovine milk adulteration in camel milk. Chi *et al* (2024) developed an enzyme-linked immunosorbent assay (ELISA)-based detection method using bovine β-lactoglobulin (β-Lg), successfully achieving quantitative analysis of trace amounts of cow's milk adulteration in camel milk. This method demonstrates high sensitivity (linear detection range of 1%-100%) and is minimally affected by temperature and pH fluctuations (Chi *et al,* 2024).

Nanoantibodies (Nb), defined as the recombinant variable regions of camelid heavy-chain antibodies, possess an approximate molecular weight of 15 kD, which is approximately one-tenth that of traditional IgG antibodies. This characteristic, coupled with their ultra-small molecular size, enables efficient penetration of biological barriers and binding to hidden epitopes, thereby significantly enhancing detection sensitivity. Rodríguez-Camejo *et al* (2023) developed a highly sensitive sandwich enzyme-linked immunosorbent assay (ELISA) method utilising this characteristic, achieving a quantification limit of 40 picograms per millilitre (pg/mL). This method utilises direct enzyme/fluorescein labeling, thereby obviating the necessity for secondary antibodies and markedly reducing detection time (Rodríguez-Camejo *et al,* 2023). It is imperative to acknowledge that the aforementioned methodologies are contingent upon intact protein conformation epitopes. However, commercially available camel milk products (e.g., powdered milk and liquid milk) undergo high-temperature and high-pressure processing, which has the potential to destroy key epitopes and lead to false-negative results. For instance, Villa *et al* (2022) reported that an indirect enzyme-linked immunosorbent assay (ELISA) method employing rabbit anti-β-lactoglobulin antibodies was wholly incapable of quantifying β-lactoglobulin content in high-pressure sterilised products.

Subsequent studies by Khuda *et al* (2012) have corroborated the notion that processing methodologies exert a substantial influence on the recovery rate and variability of results for casein and β-lactoglobulin in the detected substances. Molecular dynamics simulation technology offers an innovative solution to this problem by virtue of its ability to precisely simulate the effects of temperature and pressure on the conformation of target substances during processing (Zhang *et al,* 2024). Consequently, researchers employed a novel approach that integrated molecular dynamics simulations to screen for stable epitopes of β-lactoglobulin. This strategy was complemented by the utilisation of an indirect/direct competitive ELISA to assess the epitope-binding capacity of antibodies. The established

enzyme-linked immunosorbent assay (ELISA) method exhibited a limit of detection (LOD) and limit of quantification (LOQ) of 0.25 milligrams per kilogram (mg/kg) and 1.07 mg/kg, respectively. This method successfully detected β-lactoglobulin in 23 commercially available processed products. In a similar way, Feng *et al* (2025) screened for a specific epitope of bovine αs1-casein E207 using bioinformatics and developed a quantitative detection method for buffalo milk adulterated with cow's milk. To this end, monoclonal antibodies were prepared using 10 short peptides as antigens and a competitive enzyme-linked immunosorbent assay (ELISA) system was established. This method yielded an IC50 value of 3.8 μg/mL, with detection limits of 1.6 mg/kg and 15 mg/kg in buffalo milk and milk powder, respectively. The recovery rates exhibited a range from 96.2% to 106%, with relative standard deviations ranging from 7.8% to 12.2% (Feng *et al,* 2025). It is important to acknowledge that ELISA-based detection methods have certain advantages, including high specificity and low cost. However, their reliance on intact protein conformation can result in a high rate of false positives and false negatives, which significantly restricts the technology's potential for widespread application.

2. Chromatography and mass spectrometry method

Chromatography/mass spectrometry (GC/MS) is the core analytical method for detecting adulteration in dairy products. The instrument utilises a chromatographic column to achieve precise separation of target compounds. This separation is based on the differences in distribution between the stationary phase and the mobile phase. The efficacy of this technique is attributable to its incorporation of a pre-separation step, a feature that confers upon it a notable advantage over the ELISA method with respect to quantitative accuracy for single substances. This pre-separation step enables the precise quantification of adulteration levels, a crucial aspect of analytical chemistry. For instance, Li *et al* (2024a) developed a method based on ultra-performance liquid chromatography (UPLC). They used bovine β-lactoglobulin (β-Lg) as a marker to qualitatively and quantitatively detect cow's milk adulteration in camel milk. The method demonstrated adequate linearity within the range of 25–1,000 mg/L, exhibiting an RSD of 0.45% for 30% adulterated samples.

The composition of animal milk systems is intricate and their high fat/carbohydrate content can induce substantial matrix effects, thereby interfering with the accuracy of detection results. More critically, the process often causes denaturation of target proteins (e.g., β-lactoglobulin), significantly reducing the accuracy of intact protein detection. To address this, researchers employed an enzymatic hydrolysis strategy to cleave large-molecule proteins into small peptide fragments. They then combined this with mass spectrometry to screen for highly specific and stable characteristic peptides as markers. This combination significantly enhanced detection sensitivity and interference resistance. For instance, Zhang *et al* (2022) employed characteristic peptides derived from αS2-casein, β-casein and κ-casein as markers to quantitatively analyse eight types of animal milk. The limit of quantification (LOQ) for target peptides was determined to be 5–30 μg/L, with recovery rates ranging from 95.2% to 104.5%, as reported by Zhang *et al* (2022). Gu *et al* (2024) identified 22 characteristic peptides from eight species using UHPLC-Q/Exactive-HRMS combined with BLAST alignment, with detection limits of 0.35%–0.49% for liquid milk and 0.68%–0.73% for powdered milk (Gu *et al,* 2024). Furthermore, Hong *et al* (2022) were the first to apply desorption electrospray ionisation mass spectrometry (DESI-MS) to analyse biomarkers in five types of milk sources (cow's milk, goat's milk, camel's milk, soy milk and oat milk). The method was able to achieve 100% cross-validation accuracy and excellent sensitivity for adulteration levels ranging from 0.1% to 5% v/v by identifying specific markers based on mass spectrometry feature differences and combining them with a linear discriminant analysis (LDA) model. This method does not necessitate complex pretreatment, thereby ensuring high classification accuracy while enhancing detection efficiency. It provides a rapid solution for milk adulteration control (Hong *et al,* 2022). Despite the evident advantages of chromatography and mass spectrometry technology in the realm of food adulteration detection, its implementation remains encumbered by substantial limitations. The substantial financial demands associated with the acquisition and maintenance of chromatography and mass spectrometry instruments serve as a considerable economic hindrance. Concurrently, the stringent operational requirements, which demand a high degree of expertise from the operators, act as a significant impediment to the technology's broader dissemination and utilisation. The implementation of this technology in the domain of adulteration detection is significantly constrained by two major restrictions.

3. Biosensors method

In recent years, sensing detection technology has emerged as a key development direction in the field of adulteration detection due to its significant advantages, including label-free operation, simple sample pretreatment, rapid response and high sensitivity. The instrument's detection limit is reported to be at the ng/mL level, with results typically obtained within minutes. Furthermore, the technology's portable and low-power devices are propelling the transition of detection operations from laboratories to on-site rapid quantitative applications. In specific applications, Gao *et al* (2022) developed a sensor based on a porous silicon Bragg reflector. The sensor is capable of detecting β-lactoglobulin by meticulously monitoring alterations in fluorescence and refractive index, which are induced by the reaction of the target biomolecule before and after detection. The device's analytical sensitivity is demonstrated by its capacity to detect β-lactoglobulin in camel milk, with a minimum detection limit of 0.12 nanograms per millilitre. This feature, along with its affordability and high-throughput capabilities, positions the device as a promising tool for analytical chemistry research and applications (Gao *et al,* 2022). In pursuit of higher sensitivity, Li *et al* (2022) constructed a label-free photoelectrochemical immunosensor based on quantum dots (QDs) for detecting β-lactoglobulin. The QDs effectively enhanced the photoelectrical response, enabling the sensor to achieve a detection limit of 0.88 pg·mL^{-1} (Li *et al,* 2022). Meng *et al* (2024) successfully prepared a high-performance molecularly imprinted polymer (MIP) sensor for detecting β-lactoglobulin. The sensor was created using trypsin as a template removal agent. The electrochemical sensor has been demonstrated to detect β-lactoglobulin in a concentration range of 4 to 100 nanograms per millilitre (ng/mL), with a detection limit as low as 3.58 ng/mL (Meng *et al* (2024). It is noteworthy that fluorescence sensing modes generally exhibit superiority over electrochemical detection with regard to sensitivity and accuracy. In order to surmount the constraints imposed by matrix interference in the domain of fluorescence detection and to further capitalise on its advantages, Li *et al* (2024) pioneered a photothermal detection strategy founded upon fluorescence sensing. This strategy culminated in the development of a fluorescence-photothermal dual-mode immunosensor based on nanobodies. This design effectively eliminated matrix interference on the fluorescent signal, with performance characteristics of a detection limit of 0.034 nanograms per millilitre (ng/mL) in fluorescent mode and 0.075 ng/mL in photothermal mode (Li *et al,* 2024). The advantages of this sensing detection technology extend beyond the detection of β-lactoglobulin, as the detection targets can be flexibly switched. For instance, it can be utilised to detect proteins that are unique to cow's milk but absent in camel milk, such as κ-casein. Kourti *et al* (2024) developed an immunosensor chip with a distinctive dual-channel configuration, capable of distinguishing between κ-casein and bovine serum albumin in cow's milk. This innovation enabled the chip to significantly reduce background interference, a significant advancement in the field. This method does not necessitate sample pretreatment and can detect milk adulteration as low as 0.05% by volume within 12 minutes. It exhibits over 10 times the sensitivity of existing ELISA techniques (Kourti *et al,* 2024). In an effort to expedite on-site screening, Sharma *et al* (2021) developed a competitive lateral flow immunoassay. This assay was based on the principle of lateral flow immunoanalysis, utilising non-immunoglobulin antigens and carbon nanoparticles to rapidly detect buffalo milk adulteration in cow's milk. In practice, polyclonal antibodies specific to buffalo milk proteins are conjugated to carbon nanoparticles and coated onto a binding pad; the test line is immobilised with buffalo milk casein at a concentration of 1.6 µg/cm; and the control line is goat anti-rabbit antibody immobilised at 0.5 µg/cm. During the testing procedure, the sample is to be mixed with borate buffer solution and added dropwise. The underlying principle of this method is as follows: in the absence of the target buffalo milk protein in the sample, the test line will appear black or gray; if the target protein is present, the test line signal will weaken or disappear due to competitive binding. This method achieves a sensitivity of 5% adulteration rate, with detection taking only 5 minutes, making it suitable for milk collection stations and heat-treated milk samples. In sum, this method provides a powerful tool for rapid on-site screening (Sharma *et al,* 2021).

B. Based on characteristic metabolites method

Characteristic metabolites refer to compounds other than proteins and nucleic acids found in biological organisms, such as carbohydrates, lipids and other small molecules. Due to the variation in the composition of these metabolites across different animal milk sources, they can function as distinctive indicators for detecting adulteration in dairy products. Among these, spectroscopic analysis is a representative technique for adulteration

detection based on characteristic metabolites. This technology is characterised by its ease of operation and its ability to produce rapid detection results, thereby markedly enhancing detection efficiency. Spectroscopic techniques frequently employed in adulteration detection include infrared spectroscopy, Raman spectroscopy and Omics techniques.

1. Infrared spectroscopy

The foundation of near-infrared (NIR) spectroscopy is the absorption characteristics of substances within the near-infrared region at designated wavelengths. Through the analysis of these absorption spectra, the chemical composition of the substance can be ascertained. This technology utilises the principle of light detection, typically without the necessity of complex sample pretreatment, to discern the absorption, reflection, emission, or scattering response of light by the sample. This enables rapid screening, non-destructive testing and comprehensive characterisation of the sample. For instance, Yuan *et al* (2022) employed a near-infrared spectrometer to obtain spectral data from 43 samples of camel milk mixed with hydrolysed protein and established a detection model based on 33 of these data points. The model demonstrated adequate linearity within the concentration range of 0.01–2 g hydrolysed protein/100 mL camel milk, exhibiting a correlation coefficient R^2 of 0.95 (Yuan *et al,* 2022). Given the inherent complexity of near-infrared spectral data, multivariate statistical analysis of the dataset is typically required and chemometric tools are employed for this purpose (Wang *et al,* 2019). Common methods employed in adulteration detection include principal component analysis (PCA), partial least squares (PLS) analysis and multivariate curve resolution-alternating least squares (MCR-ALS) analysis. For instance, Pereira *et al* (2020) employed a combination of near-infrared spectroscopy (NIR) with partial least squares discriminant analysis (PLS-DA) and the projection search algorithm (iSPA-PLS) to achieve both qualitative and quantitative detection of milk adulteration. This method achieved a detection limit as low as 1.0154 g/100 g (approximately 1%) for milk, with an accuracy rate of up to 100% (Pereira *et al,* 2020). Another study combined near-infrared (NIR) spectroscopy with chemometric methods to successfully perform adulteration quantitative analysis on infant formula containing urea, melamine and starch. The study established a quantitative model based on partial least squares regression (PLS) combined with feature wavelength selection, demonstrating excellent predictive performance with a prediction set coefficient of determination R^2 greater than 0.987 (Zhao *et al,* 2022). However, the primary constraint of PLS is its requirement for a substantial sample size to ensure precise calibration and the necessity to circumvent multicollinearity issues among components.

Support vector machines (SVM), a supervised learning algorithm, can be trained with fewer samples compared to other machine learning classifiers, a quality that lends to its wide applicability. For instance, Buoio *et al* (2024) proposed a hybrid method combining near-infrared spectroscopy (NIR) with variable clustering-support vector machines (VC-SVM) for milk type discrimination, achieving 100% classification specificity.

The advent of artificial intelligence technology has precipitated a paradigm shift in the realm of instrument detection, wherein the integration of multi-source data and the implementation of information fusion technology have emerged as pivotal strategies. The integration of diverse data sources facilitates a more comprehensive and accurate analysis, thereby significantly enhancing the accuracy of adulteration detection. In recent years, significant advancements in artificial intelligence and digital algorithms, particularly when combined with spectroscopic techniques, have revitalised this field. For instance, Colak *et al* (2025) employed a combination of Fourier transform infrared (FTIR) spectroscopy and artificial intelligence (AI) algorithms to detect adulterated milk in buffalo milk. A comparative analysis was conducted of the physicochemical and microbiological characteristics, as well as the spectral data, of 13 concentration mixtures ranging from 0.2% to 10%. In this analysis, six artificial intelligence (AI) algorithms and chemometric methods, including SIMCA and DD-SIMCA, were evaluated. Subsequent to the screening of key spectral features utilising particle swarm optimisation (PSO), the model founded on the ensemble bagged tree algorithm accomplished an accuracy rate of 90.38%. The findings suggest that the integration of FTIR with AI facilitates the effective detection of adulteration, offering a rapid and convenient approach for adulteration detection in the food testing domain (Colak *et al,* 2025).

Although, near-infrared spectroscopy (NIR) has been widely applied in adulteration detection in fields such as food and pharmaceuticals due to its rapid and non-destructive advantages, its spectra primarily reflect the harmonic and sum vibrations of molecules,

resulting in relatively broad and overlapping absorption peaks, which to some extent limit its resolution capabilities. Conversely, mid-infrared spectroscopy (MIRS) is predominantly characterised by the fundamental vibrations of molecules, manifesting as rich and sharp absorption bands with pronounced absorption intensity. This approach has been demonstrated to yield a more substantial and distinctive array of frequency and intensity data. Furthermore, the characteristic vibration peaks of typical functional groups are predominantly concentrated in this region. The unique absorption characteristics of MIRS offer significant advantages in material identification and quantitative analysis. For instance, Feng *et al* (2019) employed mid-infrared spectroscopy to detect milk powder adulterated with rice flour or soybean flour. By establishing a regression model using partial least squares, the researchers were able to simultaneously detect the content of adulterants, with sensitivity and specificity both exceeding 90% (Feng *et al,* 2019). Another study successfully identified the quality characteristics of camel milk compared to other animal milks using mid-infrared spectroscopy. By comparing conventional nutritional components, β-casein and trace element content, the study confirmed the significant uniqueness of camel milk (Li *et al,* 2024b). Chu employed mid-infrared spectroscopy (MIRS) in conjunction with contemporary statistical machine learning methodologies to accurately detect adulteration in cow's milk or water in buffalo milk (BM), goat milk (GM) and camel milk (CM) (adulteration ratios ranging from 5% to 50%). In comparison with conventional methodologies such as partial least squares (PLS), support vector machines (SVM), projection pursuit regression (PPR) and Bayesian regularised neural networks (BRNN), machine learning exhibited superior performance, markedly enhancing the detection accuracy of MIRS for adulteration in high-value dairy products (Chu *et al,* 2023). In addition to upgrading models, studies have centered on integrating mid-infrared spectroscopy with fluorescence spectroscopy, in conjunction with multivariate analysis, to detect three types of animal milk adulteration in camel milk. This approach has enabled the detection of adulteration concentrations as low as 0.5% v/v (Boukria *et al,* 2023). This foundation is being built upon with advancements in computational power and machine learning methods, which are enabling the increased application of multivariate models to enhance the performance of infrared models. For instance, Yao *et al* (2023) integrated conventional statistical methodologies with state-of-the-art machine learning techniques, thereby markedly enhancing the precision and dependability of Fourier Transform Mid-Infrared Spectroscopy (FT-MIR) in the identification of adulterants in camel milk, achieving a minimum detection threshold of 3.27 g of cow's milk per 100 g of camel milk. (Yao *et al,* 2023).

2. Raman spectroscopy

Raman spectroscopy is a molecular vibration spectroscopy technique based on the Raman scattering effect. Its popularity in the field of analysis stems from its high sensitivity, minimal sample requirements, fast analysis speed and ease of operation. A notable advantage of this method over infrared spectroscopy is its low sensitivity to moisture. In the context of analysing liquid milk with elevated water content, infrared spectroscopy frequently obscures signals from other compounds due to the pronounced O-H stretching vibration absorption exhibited by water molecules. Conversely, Raman spectroscopy demonstrates a negligible response to water molecules and minimal interference from water media, rendering it especially well-suited for direct analysis of liquid samples. This advantage has been effectively applied in the domain of liquid milk testing. For instance, Ni *et al* (2023) utilised a combination of Raman spectroscopy with lactose index screening (LIS) and support vector machine (SVM) technology to achieve rapid and accurate identification of pasteurised milk and UHT milk, with a classification accuracy rate of 100% (Ni *et al,* 2023). Furthermore, Raman spectroscopy has exhibited remarkable efficacy in the analysis of powdered samples. Zhang *et al* (2025) employed Raman spectroscopy in conjunction with the Multi-Class Classifier model to effectively differentiate between various types of animal-derived powdered milk, with the model demonstrating an accuracy exceeding 93% for each type of powdered milk. In addressing the more challenging cross-species domain shift issue in milk powder adulteration detection, Ruan *et al* (2024) innovatively introduced meta-learning methods. Their study demonstrated that in a detection task involving 11 adulteration ratios, the identification rate for pure camel milk powder reached 98.92%. It is noteworthy that the meta-learning method attained an overall accuracy of 84.4%, signifying a substantial enhancement of 24.67% in performance when compared to SVM (Ruan *et al,* 2024). In order to enhance detection sensitivity and satisfy the requirements of trace substance analysis, surface-enhanced Raman spectroscopy (SERS)

technology was introduced. This technology utilises the "hot spot" effect generated by metal nanoparticles to significantly amplify the intensity of Raman signals. For instance, Li *et al* (2017) developed a method based on silver nanoparticle (Ag NP) monolayer thin film SERS substrates for the rapid detection of melamine in milk. SERS methods based on gold nanoparticles (AuNPs) have been employed for the determination of urea in milk, with a correlation coefficient R^2 of 0.9873 for the quantitative model (Hussain *et al,* 2019).

3. Omics techniques

Omics technologies provide powerful tools for in-depth analysis of the complex compositional differences between various animal milks and enable more precise detection of adulteration. This technology encompasses multiple levels, revealing the molecular fingerprints of dairy products from various dimensions. At the metabolomics level, the technology focuses on analysing fundamental differences. The analysis of raw camel milk (RCM) and heat-treated camel milk (HCM) was performed using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS). The present study utilised ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS) to analyse camel milk powder (CMP). This analytical approach enabled the identification of 119 significantly different metabolites across key categories, including carbohydrates, glycosaminoglycans, adenosine and phospholipids. This provides significant scientific evidence for the authenticity verification and process impact assessment of camel milk products (Li *et al,* 2022). Building on this, the field of lipidomics seeks to establish species-specific fingerprint profiles: In their seminal study, Wang *et al* (2025) pioneered the application of supercritical fluid chromatography-quadrupole time-of-flight mass spectrometry (SFC-Q-TOF-MS) to the systematic separation and identification of triglyceride (TAG) components in milk derived from six different livestock species. The breeds of livestock present include the Bactrian camel, Holstein cow, goat, Mongolian horse, yak and water buffalo. The triglyceride fingerprinting models constructed through chemometric analysis exhibited a high degree of similarity with species taxonomy and demonstrated excellent accuracy in both internal and external validation. This work presents an innovative lipidomics approach for authenticating the species origin of dairy products (Wang *et al,* 2025). In order to enhance analytical throughput and dimensionality, multi-component simultaneous analysis techniques have been demonstrated to be advantageous. Piras *et al* developed a droplet homogenisation technique based on atmospheric pressure MALDI-Q-TOF mass spectrometry, innovatively achieving simultaneous quantitative analysis of metabolites, lipids and proteins in milk. When employed in conjunction with machine learning software, this method has been shown to achieve 100% accuracy in the precise identification of cow, sheep, goat and camel milk species, thereby significantly enhancing the efficiency of multidimensional analysis (Piras *et al,* 2021). Concurrently, the exploration of novel non-protein detection markers has emerged as a pivotal research direction aimed at enhancing the specificity and sensitivity of detection. This pursuit is driven by the recognition of limitations inherent to traditional protein markers, including thermal stability and species cross-reactivity. In response, researchers are actively pursuing the identification of new detection targets, thereby expanding the repertoire of available analytical methods. In contrast, Li *et al* (2023) adopted a different approach, establishing a detection method for goat milk adulterated with cow milk based on the non-protein marker N-acetylglucosamine. The study revealed that the concentration of this biomarker in milk (146.7 mg/L) is 32 times higher than in goat milk. The method demonstrated good linearity within a 1–100% adulteration range, with a detection limit as low as 0.3%, by optimising pretreatment (cold acetone removal of milk proteins) and detection methods (silylation derivatisation-GC-MS detection, HPLC-MS/MS quantification). The method features simple pretreatment (approximately 10 minutes per sample) and rapid and precise advantages, effectively demonstrating the feasibility and efficiency of non-protein biomarkers for milk adulteration quantification (Li *et al,* 2023). In conclusion, flavouromics offers a distinctive supplementary dimension for the tracking of milk products. Zhou *et al* (2025) combined headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) and headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) technologies to systematically analyse the flavour characteristics of commercially available yak, donkey, camel, goat and cow milk powders in China. The study identified 141 volatile compounds and, by integrating multivariate statistical models such as PCA and OPLS-DA, successfully achieved traceability differentiation among these five types of milk powder, offering a new perspective for dairy

product identification based on flavour fingerprints (Zhou *et al,* 2025).

C. Gene-based methods

In comparison with metabolite-based methods, somatic cells present in animal milk exhibit the presence of nucleic acids (predominantly DNA), thereby offering a distinctive target for the detection of adulteration. It is imperative to note that DNA exhibits remarkable structural stability under the conditions prevalent during dairy processing, including heating and pressure, thereby establishing its reliability as a detection indicator. Gene-based adulteration identification technology is a method of analysing gene fragments from a target species to determine their relative or absolute abundance. This technology is capable of achieving highly sensitive and specific identification of adulteration in animal milk from different species. Representative technologies currently employed for nucleic acid amplification and detection include polymerase chain reaction (PCR), isothermal amplification technology and nucleic acid sensing technology.

1. PCR technology

Since its introduction in 1985, the first-generation PCR technology has become an important detection method by amplifying DNA templates using DNA polymerase and oligonucleotide primers under periodic temperature cycles. Conventional PCR methods principally depend on agarose gel electrophoresis for the detection of amplified products. For instance, Wu *et al* (2022) employed this method to detect adulteration of camel milk with cow's milk and sheep's milk, achieving a minimum detection limit of 1%. While this method boasts advantages such as sensitivity, accuracy and low cost, its operational process is cumbersome, involving multiple steps such as gel preparation and electrophoresis and it cannot monitor the amplification process in real time.

The advent of real-time fluorescent quantitative PCR (RT-qPCR) technology in 1992 provided a solution to this challenge. This technology incorporates fluorescent reporter groups into the PCR reaction system, thereby enabling real-time monitoring of the amplification process. When utilised in conjunction with reference genes or standards, it facilitates a streamlined, one-step quantitative assessment of target gene content or adulterant concentration within the specimen. For instance, Wajahat *et al* employed a qPCR method reliant upon SYBR Green fluorescent dye to detect sheep milk and cow milk adulteration in camel milk, thereby achieving a substantial enhancement in sensitivity with a detection limit of 0.1% (Wajahat *et al,* 2022). Furthermore, the integration of multiplex-specific primers within a singular reaction system enables the efficient simultaneous amplification of multiple target genes by qPCR. The triple real-time fluorescent quantitative PCR method developed by Sarkar and associates is a paradigmatic application. This method is predicated on specific fragments of the cytochrome b gene from cattle, sheep and camels, thereby enabling the simultaneous detection of cow's milk and sheep's milk components in camel milk, with detection limits of 2×10^{-2} ng/µL and 2×10^{-2} ng/µL, respectively (Sarkar *et al,* 2024). In the context of PCR testing, researchers frequently select either mitochondrial genes or nuclear genes as targets. In addition to mitochondrial genes, nuclear genes are also frequently utilised. For instance, Wang *et al* (2020) developed a novel quantitative PCR detection system for camel milk adulteration based on a single-copy nuclear gene. This system effectively addresses the quantitative bias that is often observed in traditional mitochondrial genes due to their multi-copy nature. It accomplishes this by leveraging a single-copy housekeeping gene that functions as both a camel species-specific gene and an internal control gene. A linear model was established between camel milk content and the ratio of Ct values (specific gene Ct/internal control gene Ct), with an R^2 value of 0.9614. The validation of the simulated adulterated sample demonstrated recovery rates ranging from 90% to 120%, exhibiting a coefficient of variation of less than 10%, thereby demonstrating a harmonious convergence of accuracy and precision. The normalisation PCR system, which is based on single-copy nuclear genes, provides a reliable technical foundation for determining the content of camel milk and assessing penalties for adulteration (Wang *et al,* 2020). Nevertheless, conventional quantitative polymerase chain reaction (qPCR) remains constrained in its capacity for absolute gene quantification. To address this issue, digital PCR (dPCR) technology has emerged as a highly sensitive nucleic acid molecular absolute quantification technique. dPCR does not rely on internal reference genes; rather, it achieves absolute quantification by directly counting the fluorescent signals of the target gene. For instance, Li *et al* (2023) employed dPCR technology to detect adulteration in camel milk. This technology subdivides the PCR reaction system into tens of thousands of autonomous micro-reaction

units (e.g., droplets), enabling the calculation of the absolute copy number of the target gene based on the number of units that undergo positive amplification. Despite the fact that dPCR offers extremely high sensitivity, specificity and accuracy and does not rely on Ct values for quantification, it also has some limitations. These limitations include low levels of automation and integration, requiring sophisticated and complex instruments. As a result, dPCR is less suitable for high-throughput testing of large-scale samples.

2. Isothermal amplification technology

Despite the notable benefits offered by thermal cycling amplification techniques, such as polymerase chain reaction (PCR), in terms of nucleic acid precision detection, these methods are often hampered by their reliance on costly thermal cycling instruments, thus constraining their overall applicability. In order to overcome this limitation, researchers have developed isothermal amplification techniques. This technology facilitates the specific amplification of target nucleic acid sequences at a constant temperature, expeditiously producing large numbers of copies to meet detection requirements. Among the array of techniques utilised, loop-mediated isothermal amplification (LAMP) stands out as a prominent and frequently employed method. The reaction is known to occur at a constant temperature of approximately 60°C and is characterised by a brief duration of completion. For instance, Yu *et al* (2021) employed LAMP technology to successfully detect five animal milk-derived genes, including those from camel milk, with a minimum detection limit of 0.05 ng/μL. In simulated adulteration experiments, this method demonstrated the capacity to detect as little as 2.5% (v/v) of cow's milk components in camel milk (Yu *et al,* 2021). However, conventional LAMP detection methodologies frequently necessitate the opening of the reaction tube to introduce dye or undertake endpoint detection, a process that can readily result in aerosol contamination of the amplification products. In order to address this issue, researchers integrated LAMP amplification with lateral flow strip (LFS) detection functionality into a closed, fan-shaped, self-driven microfluidic (SDM) system. This system necessitates only a constant-temperature heating plate (e.g., equipped with a portable power source) and does not require an external power supply to complete detection within one hour. This capability enables contamination-free, visual interpretation of cow's milk adulteration as low as 1% in camel milk.

In addition to LAMP, Recombinase Polymerase Amplification (RPA) is another significant isothermal technology. In contrast to LAMP, which generally necessitates 4–6 primers and functions at elevated temperatures (~60°C), RPA exhibits more accommodating temperature requirements (37–42°C). The core mechanism of this system involves the use of recombinase and polymerase to rapidly amplify target gene sequences at constant temperatures. The shortest reaction time is controllable within 30 minutes. For instance, Zhou and associates have developed a specific RPA method for camel, cow and sheep milk. This approach exhibited excellent specificity, with the capability of detecting as low as 10 pg/μL genomic DNA and high sensitivity when testing milk powder samples. The entire detection process was completed within 30 minutes (Zhou *et al,* 2024). In order to further enhance the accuracy and usability of RPA, researchers are continuously upgrading the technology. A significant advancement in the field is the integration of the CRISPR/Cas12a detection system with RPA technology by Huang and associates this combined method enables direct visualization of milk adulteration results under UV light, thereby significantly simplifying the operational process (Huang *et al,* 2023).

In summary, isothermal amplification technology can be efficiently performed at ambient temperature without reliance on sophisticated temperature control equipment. This approach not only leads to a substantial reduction in testing costs but also genuinely fosters the advancement of nucleic acid testing towards portability and on-site testing.

3. Nucleic acid sensing technology

In order to address the pressing need for simple, low-cost, rapid and on-site nucleic acid testing, nucleic acid sensing technology (also known as gene sensing technology) has emerged. This technology utilises specific nucleic acid sequences (ssDNA probes) as sensitive elements, converting the hybridisation between the probe and target nucleic acids (such as species-specific genes in dairy products) into detectable optical, electrical, or acoustic signals via a transducer. This enables direct or indirect detection of the target.

Fluorescence sensing has become the most widely used nucleic acid sensing method due to its stability. For instance, the daPCR-CHA-FB detection method developed by Thanakiatkrai and associates (which integrates digital PCR-assisted hybridisation chain reaction with fluorescence

signal readout) can detect bovine DNA in dairy products within two hours. This method does not necessitate the purification of DNA or the use of costly equipment and it has a detection limit as low as 0.01 nanograms of DNA (Thanakiatkrai *et al*, 2024). Nevertheless, to achieve true portability and visualisation, paper-based colourimetric sensing demonstrates unique advantages. Researchers developed a paper-based sensing platform based on gold nanoparticle-streptavidin complex colourimetric detection for the visual detection of bovine DNA which was demonstrated to detect adulteration levels as low as 0.01% (v/v) in a binary mixture system of dairy products, with results that are discernible to the naked eye (Bougadi and Kalogianni, 2020). A significant benefit of nucleic acid sensing is its ability to recognise molecules with high sensitivity, allowing for amplification-free detection. Researchers ingeniously utilised the chain displacement and polymerisation functions of the Bst enzyme to promote efficient binding between the probe and target DNA, developing a nucleic acid amplification-free lateral flow strip detection method. This method has been shown to significantly simplify the operational process (by virtue of the fact that it does not require an amplification step), effectively avoid amplification aerosol contamination and improve detection accuracy (Wang *et al,* 2024). In addition to the utilisation of fluorescence and colourimetric methodologies, surface-enhanced Raman spectroscopy (SERS) has been employed for nucleic acid sensing, a process distinguished by its exceptional sensitivity. Researchers employ nanometal particles (e.g., core-shell structured Ag@Au nanoparticles) as SERS substrates, thereby achieving significant enhancement of target DNA signals and effective suppression of background noise from complex matrices (e.g., milk). Researchers combined the trans-cleavage activity of CRISPR/Cas12a (for signal amplification) with SERS detection based on Ag@Au nanoparticles to achieve ultra-sensitive detection of trace DNA in milk, with a detection limit as low as 224 Amol on a portable Raman spectrometer (Pan *et al,* 2022).

Conclusion

Due to its high value, camel milk is currently one of the dairy products most affected by adulteration and mislabeling issues. Various analytical techniques have been developed to detect adulteration in camel milk. The most appropriate technique should be selected based on the source of the adulterants and specific requirements. This paper reviews existing research and categorised camel milk adulteration detection methods into three main strategies: protein-based, metabolite-based and gene-targeted. Widely applied techniques include chromatography, spectroscopy and advanced sensing detection technologies. While these techniques offer high sensitivity and accuracy, significant challenges remain when applied to camel milk adulteration detection. Processing-induced denaturation of target components, degradation of small molecules and interference from complex food matrices can severely impact the reliability of detection results.

Looking ahead, camel milk adulteration detection methods are evolving toward integration and simplification. Advancements in materials science and artificial intelligence algorithms are expected to make existing detection technologies more cost-effective and efficient, providing stronger technical support for ensuring the quality of camel milk.

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