Indian J Anim Health (2026), 65(1)- Special Issue: 21-32
DOI: https://doi.org/10.36062/ijah.2026.spl.07326

Review Article

Recent trends in rapid diagnostic techniques for milk quality and safety assessment

Prince[1], S. K. Shivanna[1*] and K. Gandhi[1]

[1]Dairy Chemistry Division, ICAR-National Dairy Research Institute (NDRI), Karnal - 132 001, Haryana, India

Abstract

Milk is highly nutritious and widely consumed food, but its complex composition and perishable nature make it highly susceptible to adulteration and contamination. Ensuring milk quality and safety is therefore a major concern for public health, regulatory authorities, and the dairy industry. Conventional analytical methods, although accurate, are often time-consuming, labour-intensive, and require sophisticated instrumentation, limiting their applicability for real-time and on-site monitoring. In recent years, rapid diagnostic techniques have emerged as effective alternatives for the detection of milk adulterants, contaminants, and quality parameters. This review highlights recent trends in rapid diagnostic techniques for milk quality and safety, including paper-based assays, biosensors, spectroscopic methods such as Fourier Transform Infrared (FTIR), and Raman spectroscopy and molecular and immunological approaches. Spectroscopic techniques, in particular, offer rapid, non-destructive, and highly sensitive analysis of milk by detecting molecular-level changes. Additionally, advancement in chemometrics and machine learning have significantly improved the accuracy and applicability of these techniques. The review also discusses the advantages, limitations and future potential of emerging technologies such as lab-on-a-chip systems, smartphone-based diagnostics, and artificial intelligence-driven tools. Overall, rapid diagnostic techniques are expected to play a crucial role in ensuring milk quality and enhancing food safety.

Keywords: Adulteration, Biosensors, Food safety, Milk, Rapid diagnostics, Spectroscopy

Highlights

- Overview of conventional and advanced milk diagnostic techniques
- Rapid and portable tools for real-time milk analysis
- AI, IoT, and nanotechnology enhancing detection and monitoring.
- Challenges and future scope for cost-effective field diagnostics

INTRODUCTION

Milk is one of the most essential components of the human diet, providing a rich source of proteins, fats, lactose, vitamins, and minerals required for growth and development. Due to its high nutritional value and widespread consumption, maintaining milk quality and safety is of paramount importance (Hooda et al., 2025). However, milk is highly perishable and susceptible to microbial spoilage, chemical contamination, and deliberate adulteration, posing significant challenges for the dairy industry. Milk adulteration has emerged as a major global concern, often driven by economic incentives and supply–demand imbalances (Aslam et al., 2023). Common adulterants such as water, starch, urea, detergents, hydrogen peroxide, and synthetic milk components are frequently added to increase volume or mask poor quality (Malik et al., 2022; Lanjewar et al., 2024; Dalal et al., 2025; Verma, 2025). Additionally, contaminants including aflatoxin M1, antibiotic residues, pesticides, and heavy metals pose serious health risks to consumers. The complex matrix of milk further complicates detection, as these adulterants and contaminants may not always produce visible or sensory changes (Ahranjani et al., 2025).

Traditional analytical techniques, including chromatography, microbial assays, and conventional wet chemistry methods, have been widely employed for milk quality assessment. Although these methods are accurate and reliable, they are often time-consuming, require skilled personnel, and depend on sophisticated laboratory infrastructure, limiting their applicability for routine monitoring and rapid decision-making, particularly in field conditions and decentralized dairy systems. In response, there has been growing interest in rapid diagnostic techniques that offer quick, cost-effective, and reliable results with minimal sample preparation. Spectroscopic methods such as Fourier Transform Infrared (FTIR) and Raman spectroscopy have gained prominence due to their ability to provide rapid and non-destructive analysis (Li et al., 2023). FTIR enables detection of adulteration through characteristic absorption patterns, while Raman spectroscopy offers molecular-level

*Corresponding Author, E-mail: sonuksgowda@gmail.com

insights based on vibrational signatures (Ahranjani et al., 2025). When coupled with chemometrics tools such as principal component analysis (PCA) and partial least squares (PLS), these techniques significantly enhance detection accuracy and reliability (Rocchetti & Callaghan, 2021; Zhang et al., 2022).

In addition to spectroscopic approaches, biosensors and paper-based diagnostic devices have emerged as promising tools for rapid milk testing. Paper-based assays provide low-cost, portable, and user-friendly solutions, whereas biosensors offer high specificity and sensitivity through biological recognition elements such as enzymes, antibodies, and nucleic acids. Recent advancements in nanotechnology, artificial intelligence, and digital image analysis have further transformed milk diagnostics, enabling improved pattern recognition and predictive modelling (Agiomavriti et al., 2024). Smartphone-based platforms and lab-on-a-chip devices are facilitating real-time, on-site analysis with minimal technical expertise (Khan et al., 2025). Despite these advancements, challenges such as lack of standardization, need for validation, high initial costs, and variability in milk composition due to factors like breed, feed, and lactation stage persist. In this context, the present review aims to provide a comprehensive overview of recent trends in rapid diagnostic techniques for milk quality and safety, focusing on their principles, applications, advantages, limitations, and future prospects.

Classification of rapid diagnostic techniques

Rapid diagnostic techniques for milk quality and safety can be broadly classified based on their underlying principles and application areas. Such classification helps in understanding the scope, functionality, and suitability of different methods for specific analytical needs. Different types of adulteration detection techniques and their classification are shown in Fig. 1.

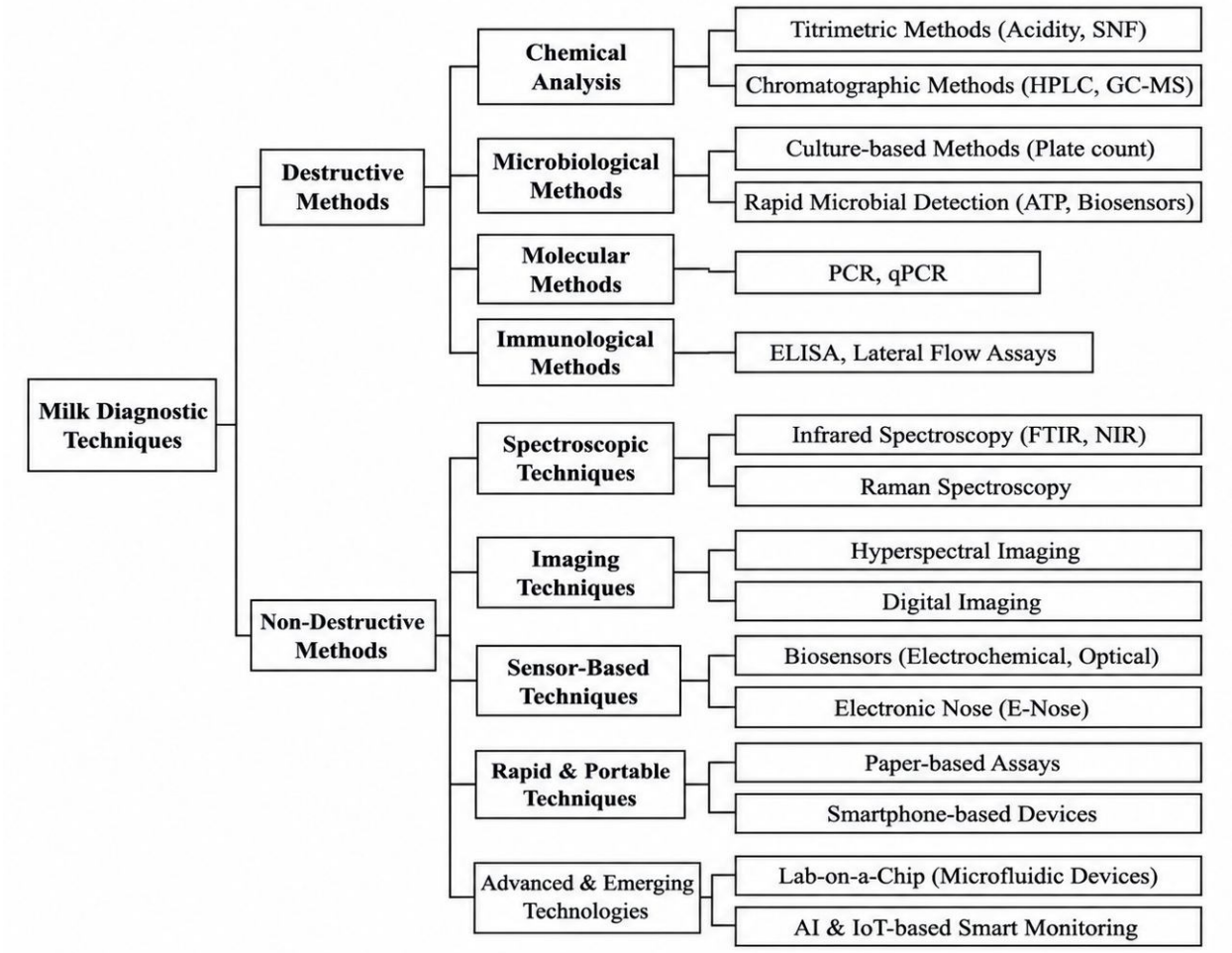


Fig. 1. Classification of milk diagnostic techniques

Table 1. Detection techniques for various milk adulterants

Product	Target adulterant	Technique	Reference
Milk	Urea	Mid-IR	Santos et al., 2013
Goat milk	β-lactoglobulin of cow milk	HPLC-MS	Chen et al., 2004
Milk	Sugars	Immunosensor	Das et al., 2016
Milk powder	Vegetable oil	MS	Garcia et al., 2012
Milk	Whey	NMR	Santos et al., 2016
Milk powder	Whey powder	Spectroscopy	Bilge et al., 2016
Milk	Soybean	MS	Du et al., 2018
Camel milk	Goat Milk	NIR	Mabood et al., 2017
Milk	Melamine	FTIR	Hansen and Holroyd, 2019
Milk powder	Melamine	Chemometrics	Moncayo et al., 2017

Classification based on principle: Rapid diagnostic techniques can be categorized into three major categories, including chemical-based, biological-based, and physical or spectroscopic methods (Aslam et al., 2023).

Chemical-based methods rely on specific chemical reactions between target analytes and reagents, resulting in measurable changes such as colour development or precipitation. These methods are commonly used in simple field-level tests for detecting adulterants like starch, urea, and hydrogen peroxide. Although they are easy to perform and cost-effective, their sensitivity and specificity may be limited.

Biological-based methods involve the use of biological recognition elements such as enzymes, antibodies, or nucleic acids. These include biosensors, immunoassays, and DNA-based detection techniques. Such methods offer high specificity due to selective binding between the analyte and the bioreceptor. They are widely used for detecting contaminants such as antibiotic residues, aflatoxin M1, and pathogenic microorganisms.

Physical or spectroscopic methods are based on the interaction of electromagnetic radiation with milk components. Techniques such as Fourier Transform Infrared (FTIR), Raman spectroscopy, and Near Infrared (NIR) spectroscopy fall under this category. These methods provide rapid, non-destructive, and highly sensitive analysis by detecting molecular-level changes in milk composition.

Classification based on application: Based on their applications, rapid diagnostic techniques can be divided into three major categories (Hooda et al., 2025), including adulterant detection, contaminant detection and microbial detection.

Adulterant detection techniques are designed to identify substances intentionally added to milk such as water, starch, detergents, and synthetic compounds, including urea, melamine and formalin. These methods are crucial for ensuring authenticity and preventing economic fraud. Different techniques used to detect various adulterants are tabulated in Table 1.

Contaminant detection techniques focus on identifying harmful chemical residues and toxins, including aflatoxin M1, pesticide residues, heavy metals, and veterinary drug residues. These contaminants pose serious health risks and are strictly regulated in many countries.

Microbial detection techniques are used to identify spoilage and pathogenic microorganisms in milk. Rapid detection of microbes is essential for ensuring product safety and shelf-life stability. Techniques such as PCR-based assays and biosensors are increasingly being used for this purpose.

Integrated and hybrid approaches: Recent trends indicate a shift toward integrated diagnostic systems that combine multiple principles to enhance detection efficiency. For example, spectroscopic techniques are often coupled with chemometrics analysis, while paper-based devices are integrated with smartphone-based image processing (Deshmukh et al., 2026). Such hybrid approaches improve sensitivity, accuracy, and field applicability, making them highly promising for modern dairy diagnostics.

Paper-based and colorimetric diagnostic methods

Paper-based and colorimetric diagnostic methods have gained significant attention in recent years due to their simplicity, affordability, and suitability for on-site applications. These techniques are particularly valuable in resource-limited and decentralized dairy systems, where access to advanced laboratory infrastructure is limited.

Principle of paper-based assays: Paper-based diagnostic methods primarily rely on the principle of

capillary action, which enables the passive movement of liquid sample through porous paper matrices without the need for external pumping systems. The paper substrate, typically composed of cellulose fibres, acts as a platform for carrying reagents and facilitating chemical reactions.

Colorimetric detection is based on specific chemical interactions between target analytes and reagents impregnated on the paper. These reactions result in visible colour changes, which can be qualitatively observed by the naked eye or quantitatively analysed using digital image processing tools (Deshmukh et al., 2026). The intensity of hue of the colour formed is often proportional to the concentration of the analyte.

Types of paper-based devices: Paper-based diagnostic platforms can be broadly categorized into the following types:

a) Simple test strips: These are the most basic forms of paper-based assays, consisting of reagent-coated strips that produce a colour change upon contact with the sample. They are widely used for detecting common adulterants such as starch, urea, detergents, and hydrogen peroxide (Deshmukh et al., 2026).

b) Lateral Flow Assays (LFAs): LFAs are more advanced paper-based devices that utilize immune chromatographic principles. They consist of multiple zones, including sample pads, conjugate pads, and detection zones (Shi et al., 2022). LFAs are commonly used for detecting specific analytes such as aflatoxin M1, antibiotic residues, and pathogens, offering higher specificity compared to simple test strips.

c) Microfluidic Paper-Based Analytical Devices (µPADs): µPADs represent a significant advancement in paper-based diagnostics. These devices use patterned hydrophilic channels and hydrophobic barriers to control fluid flow and enable multiplexed detection. µPADs allow simultaneous analysis of multiple parameters, making them highly suitable for comprehensive milk quality assessment (Pou et al., 2026).

Advantages: Paper-based diagnostic methods offer several advantages that make them highly suitable for routine milk quality monitoring. They are low-cost and affordable, ensuring accessibility for widespread use, and their portability allows for on-site and real-time testing. These methods are easy to use, requiring minimal technical expertise, and provide rapid results, typically within minutes. Additionally, they involve minimal sample preparation, further enhancing their practicality and efficiency in field applications.

Recent innovations and future directions: Recent advancements have significantly enhanced the capabilities of paper-based diagnostic systems. Integration with smartphone-based imaging and digital image analysis software has enabled quantitative estimation of analytes by analysing colour intensity (Deshmukh et al., 2026). This has transformed traditional qualitative assays into more reliable semi-quantitative or quantitative tools. Additionally, the incorporation of nanomaterials, such as gold nanoparticles and carbon-based materials, has improved the sensitivity and detection limits of paper-based devices (Das et al., 2026). Development of multiplexed µPADs allows simultaneous detection of multiple adulterants, increasing efficiency and reducing analysis time. Emerging trends also include the use of machine learning algorithms for interpreting colorimetric data, further enhancing accuracy and reproducibility. These innovations are expected to expand the applicability of paper-based diagnostics in the dairy sector.

Biosensors in milk diagnostics

Biosensors have emerged as powerful analytical tools for rapid and sensitive detection of contaminants and quality parameters in milk. Their ability to provide real-time, selective, and accurate detection makes them highly suitable for modern dairy diagnostics. In recent years, significant advancements in biotechnology, nanotechnology, and materials science have greatly enhanced the performance and applicability of biosensors in milk quality and safety assessment (Lee et al., 2025).

Principle and components of biosensors: A biosensor is an analytical device that converts a biological response into a measurable signal. It typically consists of three main components: a bioreceptor, a transducer, and a signal processor with a display system. The bioreceptor is a biological recognition element, such as enzymes, antibodies, nucleic acids, or cells, that specifically interacts with the target analyte. The transducer converts this biorecognition event into a measurable signal, which may be electrical, optical, or thermal in nature. The signal processor and display system then amplify and process the signal to produce a readable output. The specificity of biosensors is primarily determined by the bioreceptor, whereas their sensitivity depends on the efficiency of the transduction mechanism (Fernandez et al., 2024).

Types of biosensors: Biosensors used in milk diagnostics can be broadly classified based on the type of bioreceptor or transduction mechanism.

Enzyme-based biosensors: These biosensors utilize enzymes as bioreceptors to catalyse specific reactions with target analytes. They are commonly used for detecting compounds such as urea and glucose in milk. The enzymatic reaction produces measurable changes in parameters such as pH, current, or absorbance.

Immunosensors: Immunosensors are based on antigen–antibody interactions and are widely used for detecting contaminants such as aflatoxin M1, antibiotic residues, and hormones. These sensors offer high specificity and are often employed in formats such as enzyme-linked immunosorbent assays (ELISA) and lateral flow assays.

Optical and electrochemical biosensors: Based on the transduction mechanism, biosensors can be broadly categorized into optical and electrochemical types. Optical biosensors detect changes in properties such as light absorption, fluorescence, or refractive index, whereas electrochemical biosensors measure variations in electrical parameters like current, voltage, or impedance. Among these, electrochemical biosensors are particularly popular due to their high sensitivity, rapid response, and ease of miniaturization, making them highly suitable for practical applications (Das et al., 2016).

Nanotechnology-based biosensors: The integration of nanotechnology has significantly improved the performance of biosensors. Nanomaterials such as gold nanoparticles, carbon nanotubes, graphene, and quantum dots are widely used to enhance signal amplification, increase surface area for bioreceptor immobilization, and improve detection limits. For example, gold nanoparticles are commonly used in colorimetric and lateral flow assays, providing visible signal enhancement. Similarly, graphene-based biosensors offer excellent electrical conductivity, enabling highly sensitive electrochemical detection (Lee et al., 2025).

Advantages of biosensors: Biosensors offer several advantages over conventional analytical methods, making them highly effective for milk quality assessment. They provide high specificity and sensitivity due to selective biorecognition mechanisms, enabling accurate detection of target analytes. These devices allow rapid detection, often delivering results within minutes, and require minimal sample preparation, which simplifies the analytical process. Additionally, biosensors have the potential for real-time and on-site monitoring, enhancing their applicability in field conditions. Their capability for miniaturization and automation further supports their integration into portable and user-friendly systems. Collectively, these features make biosensors highly suitable for routine milk quality monitoring and field applications.

Spectroscopic techniques in milk diagnostics

Spectroscopic techniques have become indispensable tools in modern dairy diagnostics due to their ability to provide rapid, non-destructive, and highly sensitive analysis of milk. These techniques are based on the interaction of electromagnetic radiation with matter, producing characteristic spectral fingerprints that reflect the molecular composition of the sample. In recent years, spectroscopic methods such as Fourier Transform Infrared (FTIR), Raman spectroscopy, and Near Infrared (NIR) spectroscopy have gained widespread attention for the detection of milk adulteration, contaminants, and compositional variations (Cenitti et al., 2023).

Fourier Transform Infrared (FTIR) spectroscopy: Fourier Transform Infrared (FTIR) spectroscopy is one of the most widely used techniques in dairy analysis, operating in the mid-infrared region to measure the absorption of infrared radiation by molecular bonds within a sample. The technique is based on the principle that different chemical bonds absorb infrared radiation at specific wavelengths corresponding to their vibrational modes. Functional groups present in milk components, such as C–H, O–H, and C=O, generate characteristic absorption bands, producing a unique spectral fingerprint for the sample.

FTIR has diverse applications in milk diagnostics, including the determination of major milk constituents such as fat, protein, and lactose, detection of adulterants like water, urea, and synthetic milk components, identification of chemical contaminants, and monitoring of processing and quality changes (Ahranjani et al., 2025). Due to its speed and reliability, it is extensively employed in the dairy industry for routine quality control. The technique offers several advantages, including rapid and non-destructive analysis, minimal sample preparation, the ability to perform simultaneous multi-component analysis, and high reproducibility. However, it also has certain limitations, such as the requirement for robust

calibration models, potential sensitivity issues due to overlapping spectral bands, and relatively high initial instrument costs.

Raman spectroscopy: Raman spectroscopy is another powerful vibrational spectroscopic technique that provides complementary information to Fourier Transform Infrared (FTIR) spectroscopy. It is based on the principle of inelastic scattering of light, known as Raman scattering, which occurs when monochromatic light interacts with molecular vibrations, resulting in spectral shifts that provide detailed information about molecular structure and composition. In milk diagnostics, Raman spectroscopy has been widely applied for the detection of chemical adulterants such as melamine and urea, analysis of milk composition, identification of structural changes in proteins and lipids, and detection of contaminants at low concentrations (Taylan et al., 2020). It is particularly useful for detecting non-polar molecules and compounds that may be less responsive in FTIR analysis (Dalal et al., 2025). The technique offers several advantages, including minimal interference from water, high specificity for molecular identification, suitability for detecting low-level adulterants, and being a non-destructive and rapid analytical method. However, it also has certain limitations, such as fluorescence interference in some samples, high instrumentation cost, and the requirement for skilled expertise for accurate spectral interpretation.

Near Infrared (NIR) spectroscopy: Near-infrared (NIR) spectroscopy operates in the near-infrared region and is widely used for rapid compositional analysis in the dairy industry. It is based on the measurement of absorption of near-infrared radiation associated with overtones and combination bands of molecular vibrations, particularly those involving C–H, N–H, and O–H bonds. In milk diagnostics, NIR spectroscopy is extensively applied for the rapid estimation of key components such as fat, protein, and moisture content, as well as for the detection of adulteration and monitoring of milk processing and storage conditions (Ceniti et al., 2023). Its speed and ease of operation make it especially suitable for industrial applications. The technique offers several advantages, including being fast and non-destructive, suitable for online and inline analysis, and requiring minimal or no sample preparation. However, it also has certain limitations, such as lower sensitivity compared to mid-infrared methods, the need for robust calibration models, and a limited ability to detect trace-level contaminants (Hebling et al., 2022).

Role of chemometrics in spectroscopic analysis: The complexity of spectral data obtained from milk necessitates the use of advanced statistical and mathematical tools collectively known as chemometrics, which enhance the accuracy, reliability, and interpretability of spectroscopic measurements. Common chemometrics techniques include Principal Component Analysis (PCA), which is used for pattern recognition and classification of milk samples; Partial Least Squares (PLS) regression, which is employed for the quantitative prediction of milk components; and discriminant analysis, which helps differentiate between pure and adulterated samples. By integrating these approaches, chemometrics enables the development of robust predictive models capable of accurately detecting adulteration and quantifying milk constituents even in complex matrices (Xu et al., 2013).

Recent advances and emerging trends: Recent advancements in spectroscopic techniques have primarily focused on enhancing sensitivity, portability, and data analysis capabilities. The development of portable and handheld spectrometers has enabled on-site analysis of milk, thereby reducing reliance on laboratory-based testing and facilitating rapid decision-making in field and industrial settings. In addition, the integration of spectroscopic methods with machine learning and artificial intelligence has significantly improved their diagnostic potential, as these approaches can efficiently process large spectral datasets, identify hidden patterns, and enhance prediction accuracy (Agiomavriti et al., 2024). Another emerging trend is the application of hyperspectral imaging, which combines spectroscopy with imaging to deliver both spatial and spectral information, allowing detailed microstructural analysis of milk and dairy products. Furthermore, the combined use of multiple spectroscopic techniques, such as FTIR and Raman spectroscopy, is being increasingly explored to improve detection accuracy and to overcome the inherent limitations of individual methods.

Molecular and immunological techniques

Molecular and immunological techniques have become essential tools in the rapid and sensitive detection of biological and chemical contaminants in milk. These techniques are highly specific and are widely used for detecting pathogens, toxins, and residues that may not be easily identified using

conventional or simple rapid methods. In recent years, advancements in molecular biology and immunochemistry have significantly improved the speed, sensitivity, and reliability of these diagnostic approaches.

Polymerase Chain Reaction (PCR)-based techniques: Polymerase Chain Reaction (PCR) is one of the most widely used molecular techniques for the detection of microbial contaminants in milk, owing to its high sensitivity and specificity. It involves the amplification of specific DNA sequences, enabling the detection of even minute quantities of pathogenic microorganisms. The technique operates on the principle of selective amplification of target DNA using specific primers, DNA polymerase, and a series of temperature-controlled cycles known as thermal cycling. The amplified DNA products are subsequently detected through methods such as gel electrophoresis or fluorescence-based analysis, allowing confirmation of the presence of target organisms (Ahranjani et al., 2025).

In milk diagnostics, PCR has been extensively applied for the detection of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*, as well as for the identification of spoilage microorganisms and the detection of antibiotic resistance genes (Ahranjani et al., 2025). Recent advancements in PCR technology have further enhanced its applicability, particularly with the development of real-time PCR (qPCR), which enables rapid and quantitative detection (Ahranjani et al., 2025), and multiplex PCR, which allows the simultaneous identification of multiple pathogens in a single reaction. These innovations have significantly improved the efficiency and throughput of microbial analysis in dairy systems.

PCR offers several advantages, including high sensitivity and specificity, rapid detection compared to conventional culture-based methods, and the ability to detect non-culturable organisms. However, it also has certain limitations, such as the requirement for specialized equipment and trained personnel, susceptibility to contamination that may result in false-positive outcomes, and the inability to distinguish between live and dead cells without additional processing steps.

Enzyme-Linked Immunosorbent Assay (ELISA): Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used immunological technique for the detection of specific antigens or antibodies in milk, offering high sensitivity and specificity for various analytes. The method is based on the principle of specific binding between an antigen and its corresponding antibody. This interaction is detected using enzyme-linked secondary antibodies, which, upon the addition of a suitable substrate, produce a measurable colour change that can be quantified spectrophotometrically.

In milk diagnostics, ELISA has been extensively applied for the detection of contaminants such as aflatoxin M1, antibiotic residues, hormones, and allergens (Malik et al., 2022). Its ability to selectively identify target compounds in complex matrices makes it particularly valuable for ensuring milk safety and quality. Additionally, ELISA is well-suited for screening large numbers of samples, making it a practical choice for routine analysis in dairy laboratories.

The technique offers several advantages, including high specificity and sensitivity, relatively simple operation, and cost-effectiveness as compared to many advanced analytical methods. However, it also has certain limitations, such as being relatively time-consuming compared to some rapid diagnostic tests, requiring multiple steps and reagents, and the possibility of cross-reactivity, especially when dealing with complex sample matrices like milk.

Lateral Flow Immunoassays (LFAs): Lateral flow immunoassays (LFAs) are rapid and user-friendly diagnostic tools that combine the specificity of immunological reactions with the simplicity of paper-based devices. These assays operate on the principle of immunochromatography, in which the sample migrates along a membrane by capillary action. During this process, target analytes bind to labelled antibodies, resulting in the formation of visible lines that indicate the presence or absence of the analyte, enabling quick visual interpretation without the need for complex instrumentation.

In milk diagnostics, LFAs are widely used for the detection of contaminants such as aflatoxin M1, screening of antibiotic residues, and identification of pathogenic microorganisms (Aslam et al., 2023). Their simplicity and rapid response make them particularly suitable for on-site testing and routine screening in dairy supply chains. The technique offers several advantages, including rapid results typically obtained within minutes, ease of use and interpretation, and portability, making it ideal for field applications.

Other emerging molecular and immunological methods: In addition to PCR and ELISA, several advanced techniques are being explored for milk

diagnostics to enhance speed, sensitivity, and field applicability. Loop-mediated isothermal amplification (LAMP) is a rapid DNA amplification method that operates at a constant temperature, eliminating the need for thermal cycling and making it faster and more suitable for on-site applications than conventional PCR. Aptamer-based assays utilize synthetic nucleic acid molecules, known as aptamers, as recognition elements, offering high specificity, stability, and reproducibility compared to traditional antibody-based systems. Furthermore, immunosensors integrate immunological recognition with sensor technology to enable rapid and sensitive detection of analytes such as toxins, residues, and other contaminants in milk (Lee et al., 2025), thereby expanding the scope of real-time and portable diagnostic solutions.

Challenges and future perspectives: Despite their advantages, these techniques face challenges such as high cost, requirement for skilled personnel, and dependence on laboratory infrastructure. Efforts are ongoing to develop portable, cost-effective, and user-friendly versions of these technologies. Recent trends include the integration of molecular and immunological techniques with microfluidics, nanotechnology, and digital detection platforms, which are expected to enhance their applicability in field conditions. The development of point-of-care diagnostic devices is also gaining momentum, enabling rapid and decentralized milk testing.

Emerging and advanced technologies in milk diagnostics

The field of milk diagnostics is rapidly evolving with the integration of advanced technologies such as artificial intelligence (AI), smartphone-based detection systems, lab-on-chip devices, and Internet of Things (IoT)-enabled monitoring (Agiomavriti et al., 2024). These innovations aim to overcome the limitations of conventional and laboratory-based techniques by providing rapid, real-time, portable, and highly accurate diagnostic solutions. The convergence of these technologies is transforming milk quality assessment from a centralized laboratory process to a decentralized, field-deployable system.

Artificial intelligence and machine learning: Artificial Intelligence (AI) and Machine Learning (ML) have emerged as powerful tools for analyzing complex datasets generated from modern diagnostic techniques, particularly those involving spectroscopy and imaging systems. In milk diagnostics, these technologies are widely applied for the analysis of spectral data obtained from FTIR, Raman, and NIR techniques, enabling precise interpretation of compositional and structural information. They are also used for the detection and classification of milk adulteration patterns, the prediction of milk quality parameters based on historical datasets, and the identification of anomalies and contamination trends within the dairy supply chain. Commonly employed ML algorithms, such as Support Vector Machines (SVM), Artificial Neural Networks (ANN), and Random Forest models, are highly effective for classification and predictive modelling tasks. These approaches offer significant advantages, including high accuracy, strong predictive capabilities, and the ability to handle large and complex datasets, while also improving continuously through learning. However, their application is not without limitations, as they require extensive datasets for proper training, are susceptible to issues such as overfitting and model bias, and depend heavily on computational resources for efficient implementation.

Smartphone-based detection systems: Smartphone-based diagnostics are gaining significant popularity in milk analysis due to their portability, accessibility, and seamless integration with advanced sensing technologies. These systems utilize smartphone cameras, built-in sensors, and dedicated mobile applications to analyze milk samples efficiently. Typically, they operate on the principle of image analysis, where colour changes in paper-based strips or sensor platforms are captured using the smartphone camera and subsequently quantified through specialized applications. The captured data is processed using embedded algorithms to generate quantitative or semi-quantitative results. In milk diagnostics, such systems are widely applied for the detection of adulterants using paper-based strips, quantification of colorimetric reactions, and on-site testing of various quality parameters. Their major advantages include portability, ease of use, cost-effectiveness, and the ability to enable real-time data sharing and storage, making them highly suitable for field-level applications. However, these systems also face certain limitations, including variability in results due to changing lighting conditions, relatively lower sensitivity compared to conventional laboratory instruments, and dependence on proper device calibration for accurate measurements.

Lab-on-chip and microfluidic devices: Lab-on-chip (LOC) technology represents a significant

advancement in milk diagnostics by integrating multiple laboratory functions onto a single micro-scale chip, enabling rapid, automated, and efficient analysis of milk samples. These devices operate on the principle of microfluidics, where tiny volumes of fluids are precisely manipulated through microchannels, allowing controlled reactions and detection within a compact and highly organized system. In the context of milk diagnostics, LOC technology is applied for the detection of pathogens and toxins, analysis of milk composition, and integration with biosensors for real-time monitoring of quality parameters. The technology offers several advantages, including minimal consumption of samples and reagents, rapid and automated processing, and the potential for multiplex detection of multiple analytes simultaneously. However, despite its promising capabilities, LOC technology faces certain limitations, such as the complexity involved in device fabrication, high initial development costs, and limited commercialization and adoption within the dairy sector.

Internet of Things (IoT) in milk quality monitoring: The Internet of Things (IoT) plays a transformative role in modern milk diagnostics by enabling the seamless connection of sensors and devices for real-time monitoring and data exchange throughout the dairy supply chain. It facilitates continuous monitoring of critical milk storage parameters such as temperature, pH, and other environmental conditions, ensuring quality preservation from farm to consumer. IoT systems are also used to track milk quality during transportation and can be integrated with smart sensors for automated and continuous quality assessment. The key advantages of IoT in milk diagnostics include real-time monitoring with instant alerts, improved traceability and transparency, and enhanced efficiency in supply chain management. However, its implementation is associated with certain challenges, including concerns related to data security and privacy, the need for reliable infrastructure and connectivity, and the high initial cost of setup and deployment.

Integration of nanotechnology: Nanotechnology has significantly enhanced the sensitivity and specificity of diagnostic tools used in milk analysis, offering advanced solutions for detecting contaminants and ensuring quality. Nanomaterials such as gold nanoparticles, quantum dots, and carbon nanotubes are widely utilized in the development of highly efficient sensing platforms due to their unique physicochemical properties. In milk diagnostics, these materials are applied to enhance colorimetric detection in paper-based assays, enabling clearer and more precise visual responses, as well as in the development of highly sensitive biosensors capable of detecting analytes at very low concentrations. They are particularly valuable for the detection of trace-level contaminants, including toxins and adulterants, which are often difficult to identify using conventional methods. Nanotechnology-based sensors provide rapid response times, improved detection limits, and high reliability, making them highly suitable for advanced and real-time milk diagnostic applications.

Challenges and limitations of current diagnostic techniques

Despite significant advancements in diagnostic technologies for milk quality and safety, several challenges and limitations continue to hinder their widespread adoption and effectiveness. These challenges are associated with technical constraints, economic factors, operational complexities, and variability in milk composition. A critical understanding of these limitations is essential for improving existing methods and guiding future research.

Sensitivity and specificity issues: Many rapid diagnostic techniques, particularly paper-based assays and lateral flow devices, face limitations in terms of sensitivity and specificity. While they provide quick results, their ability to detect contaminants at trace levels is often inferior to advanced laboratory-based techniques such as chromatography and molecular methods. Cross-reactivity with structurally similar compounds can lead to false-positive or false-negative results, especially in complex matrices like milk. This is a major concern in the detection of toxins, antibiotic residues, and adulterants.

Matrix complexity of milk: Milk is a highly complex biological fluid composed of proteins, fats, carbohydrates, minerals, and various bioactive compounds, and this inherent complexity often interferes with analytical measurements, affecting the accuracy and reliability of diagnostic techniques. In spectroscopic methods, overlapping spectral signals from different components such as lipids, caseins, and lactose, can make precise interpretation challenging. Similarly, immunological assays may experience matrix interference from milk components such as fats, endogenous enzymes, and naturally occurring antibodies, which can reduce assay sensitivity and

specificity. Molecular techniques, although highly sensitive, often require extensive sample preparation to eliminate interfering substances and ensure accurate detection. These challenges complicate method development and highlight the need for robust calibration, validation, and standardization protocols to achieve reliable and reproducible results in milk diagnostics.

Need for calibration and standardization: Advanced diagnostic techniques such as FTIR, NIR, and Raman spectroscopy depend heavily on robust calibration models for accurate prediction and analysis of milk components. The development of these models requires large, high-quality datasets along with standardized reference methods to ensure reliability. However, significant variability in milk composition, arising from factors such as the breed of animals, feed composition, and stage of lactation, poses a major challenge in creating universal calibration models applicable across different conditions (Ahranjani et al., 2025). This natural variability can lead to inconsistencies in analytical results if not properly accounted for. Furthermore, the lack of standardization across laboratories in terms of methodologies, instrumentation, and calibration protocols further limits the reproducibility and comparability of results, emphasizing the need for harmonized approaches in milk diagnostics.

Cost and infrastructure requirements: High-end diagnostic techniques, including molecular methods, spectroscopic tools, and advanced sensor-based systems, often require expensive instrumentation and well-equipped laboratory infrastructure, which significantly limits their accessibility, particularly in developing regions and small-scale dairy operations. Beyond the initial investment in equipment, additional costs associated with maintenance, consumables, and the need for skilled manpower further increase the financial burden. These cumulative expenses make routine implementation of such advanced diagnostic methods challenging, highlighting the need for cost-effective, simplified, and scalable solutions that can be adopted across diverse dairy settings.

Requirement of skilled personnel: Many advanced diagnostic methods require trained personnel for operation, data interpretation, and maintenance. Techniques such as PCR, Raman spectroscopy, and chemometrics analysis involve technical expertise that may not be readily available in all settings. Improper handling or interpretation can lead to inaccurate results, reducing the reliability of these techniques in routine applications.

Limited field applicability: Although significant progress has been made in the development of portable and rapid diagnostic tools for milk analysis, many advanced techniques remain largely confined to laboratory environments. Their limited field applicability is influenced by factors such as environmental conditions, including temperature and humidity fluctuations, which can affect performance and reliability. Additionally, power requirements and the fragility of sensitive equipment further restrict their use in on-site or rural settings. Even smartphone-based and other portable diagnostic devices, despite their advantages, may encounter challenges related to calibration, consistency of results, and long-term durability under real-world operating conditions (Aslam et al., 2023). These limitations highlight the need for developing robust, field-ready diagnostic solutions capable of performing reliably under diverse environmental and operational constraints.

Regulatory and validation challenges: The adoption of new diagnostic technologies requires validation and approval by regulatory authorities. Lack of standardized protocols and validation frameworks can delay the implementation of novel methods. Additionally, differences in regulatory standards across countries create challenges for global acceptance and harmonization of diagnostic techniques.

Data management and integration issues: With the increasing adoption of digital technologies in milk diagnostics, large volumes of data are continuously generated from various analytical and monitoring systems. Effectively managing, analyzing, and integrating this data into meaningful decision-making processes remains a significant challenge. Key issues such as data storage, interoperability between different platforms and devices, and cybersecurity concerns must be carefully addressed to fully harness the potential of advanced diagnostic technologies. Ensuring secure, standardized, and efficient data handling frameworks is essential for improving reliability, enabling real-time insights, and supporting informed decision-making across the dairy supply chain.

Future perspectives

The future of milk diagnostics is expected to be shaped by the integration of multidisciplinary

innovations, particularly the convergence of nanotechnology, biosensors, and microfluidics with artificial intelligence-driven data analysis. The development of portable, user-friendly, and highly sensitive point-of-care devices will be a major focus, enabling rapid decision-making at farm and processing levels. Additionally, the incorporation of digital technologies such as cloud computing and blockchain will enhance traceability, data transparency, and regulatory compliance. Continued research and collaboration among academia, industry, and policymakers will be crucial in developing robust, cost-effective diagnostic platforms capable of meeting the evolving demands of milk quality and safety monitoring.

Conclusion

The advancement of diagnostic techniques for milk quality and safety has significantly strengthened the ability to detect adulterants, contaminants, and biological hazards with improved accuracy and efficiency. While conventional methods remain reliable and serve as reference standards, their limitations in terms of time and labour have driven the development of rapid and advanced analytical approaches. Techniques such as spectroscopic analysis, molecular diagnostics, and immunological assays have enhanced sensitivity and specificity, enabling early detection and better-quality control across the dairy value chain. Rapid diagnostic tools and emerging technologies have transformed milk testing by enabling real-time, on-site analysis, thus improving responsiveness and reducing dependence on centralized laboratories. Despite these advancements, challenges such as high implementation costs, the need for technical expertise, and lack of standardization continue to hinder widespread adoption. Overcoming these barriers through innovation, cost reduction, and policy support is essential to ensure that these technologies can be effectively utilized across diverse and resource-limited settings, ultimately safeguarding consumer health and strengthening the dairy industry.

Conflict of interest: No conflict of interest among the authors.

Author's contributions: P: Writing - original draft preparation; SKS, KG: Writing - review and editing.

Data availability statement: Data will be available from the corresponding author upon reasonable request.

Use of artificial intelligence tools: No AI tools were used for the preparation of the manuscript.

REFERENCES

Agiomavriti, A. A., Nikolopoulou, M. P., Bartzanas, T., Chorianopoulos, N., Demestichas, K., & Gelasakis, A. I. (2024). Spectroscopy-based methods and supervised machine learning applications for milk chemical analysis in dairy ruminants. *Chemosensors, 12*(12), 263. https://doi.org/10.3390/chemosensors12120263

Ahranjani, J. P., Dehghan, K., Esfandiari, Z., & Joolaei Ahranjani, P. (2025). A systematic review of spectroscopic techniques for detecting milk adulteration. *Critical Reviews in Analytical Chemistry*, 1-32. https://doi.org/10.1080/10408347.2025.2477535

Aslam, R., Sharma, S. R., Kaur, J., Panayampadan, A. S., & Dar, O. I. (2023). A systematic account of food adulteration and recent trends in the non-destructive analysis of food fraud detection. *Journal of Food Measurement and Characterization, 17*(3), 3094–3114. https://doi.org/10.1007/s11694-023-01846-3

Bilge, G., Sezer, B., Eseller, K. E., Berberoglu, H., Topcu, A., & Boyaci, I. H. (2016). Determination of whey adulteration in milk powder by using laser induced breakdown spectroscopy. *Food Chemistry, 212*, 183–188. https://doi.org/10.1016/j.foodchem.2016.05.169

Ceniti, C., Spina, A. A., Piras, C., Oppedisano, F., Tilocca, B., Roncada, P., & Morittu, V. M. (2023). Recent advances in the determination of milk adulterants and contaminants by mid-infrared spectroscopy. *Foods, 12*(15), 2917. https://doi.org/10.3390/foods12152917

Chen, R. K., Chang, L. W., Chung, Y. Y., Lee, M. H., & Ling, Y. C. (2004). Quantification of cow milk adulteration in goat milk using high-performance liquid chromatography with electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry, 18*(10), 1167–1171. https://doi.org/10.1002/rcm.1460

Dalal, A., Krishna, H., Khan, K. M., Kumar, N., Chourasia, C., Sahu, K., & Majumder, S. K. (2025). Detection of urea adulteration in packaged milk samples using spatially-offset Raman spectroscopy. *International Dairy Journal, 168*, 106307. https://doi.org/10.1016/j.idairyj.2025.106307

Das, S., Goswami, B., & Biswas, K. (2016). Milk adulteration and detection: A review. *Sensor Letters, 14*(1), 4–18. https://doi.org/10.1166/sl.2016.3580

Deshmukh, S. V., Gandhi, K., Sharma, R., Shivanna, S. K., Dhanda, P., & Pahsyntiew, J. (2026). Development of a paper strip assay for the simultaneous detection of nitrites and nitrates in milk. *Journal of Food Measurement and Characterization*, 1–17. https://doi.org/10.1007/s11694-026-04373-z

Du, L., Lu, W., Cai, Z. J., Bao, L., Hartmann, C., Gao, B., & Yu, L. L. (2018). Rapid detection of milk adulteration using intact protein flow injection mass spectrometric fingerprints combined with chemometrics. *Food Chemistry, 240*, 573–578. https://doi.org/10.1016/j.foodchem.2017.07.107

Fernández González, A., Badía-Laíño, R., Costa-Fernández, J. M., & Soldado, A. (2024). Progress and challenge of sensors

for dairy food safety monitoring. *Sensors, 24*(5), 1383. https://doi.org/10.3390/s24051383

Garcia, J. S., Sanvido, G. B., Saraiva, S. A., Zacca, J. J., Cosso, R. G., & Eberlin, M. N. (2012). Bovine milk powder adulteration with vegetable oils or fats revealed by MALDI-QTOF MS. *Food Chemistry, 131*(2), 722–726. https://doi.org/10.1016/j.foodchem.2011.09.062

Hansen, P. W., & Holroyd, S. E. (2019). Development and application of Fourier transform infrared spectroscopy for detection of milk adulteration in practice. *International Journal of Dairy Technology, 72*(3), 321–331. https://doi.org/10.1111/1471-0307.12592

Hebling e Tavares, J. P., da Silva Medeiros, M. L., & Barbin, D. F. (2022). Near-infrared techniques for fraud detection in dairy products: A review. *Journal of Food Science, 87*(5), 1943–1960. https://doi.org/10.1111/1750-3841.16143

Hooda, A., Vikranta, U., & Duary, R. K. (2025). Principles of food dairy safety: Challenges and opportunities. In S. Chandra Deka, C. Nickhil, & A. K. Haghi (Eds.), *Engineering solutions for sustainable food and dairy production* (Food Engineering Series). Springer. https://doi.org/10.1007/978-3-031-75834-8_2

Khan, M. U., Zhen, L., Tian, J., Farid, A., Chen, Z., & Liu, H. (2025). From detection to prevention: A cyber-secure AI ecosystem for mitigating milk adulteration and dairy fraud. *Trends in Food Science & Technology*, 105503. https://doi.org/10.1016/j.tifs.2025.105503

Lanjewar, M. G., Parab, J. S., & Kamat, R. K. (2024). Machine learning based technique to predict the water adulterant in milk using portable near infrared spectroscopy. *Journal of Food Composition and Analysis, 131*, 106270. https://doi.org/10.1016/j.jfca.2024.106270

Lee, I., So, H., Ho, K. K., Li, Y., & Jun, S. (2025). Flow-based dielectrophoretic biosensor for detection of bacteriophage MS2 as a foodborne virus surrogate. *Biosensors, 15*(6), 353. https://doi.org/10.3390/bios15060353

Li, W., Huang, W., Fan, D., Gao, X., Zhang, X., Meng, Y., & Liu, T. C. Y. (2023). Rapid quantification of goat milk adulteration with cow milk using Raman spectroscopy and chemometrics. *Analytical Methods, 15*(4), 455–461. https://doi.org/10.1039/D2AY01697D

Mabood, F., Jabeen, F., Ahmed, M., Hussain, J., Al Mashaykhi, S. A., Al Rubaiey, Z. M., & Manzoor, S. (2017). Development of new NIR-spectroscopy method combined with multivariate analysis for detection of adulteration in camel milk with goat milk. *Food Chemistry, 221*, 746–750. https://doi.org/10.1016/j.foodchem.2016.11.109

Malik, A., Baig, M. D., Tiwari, V. K., & Khatkar, B. S. (2022). Detection and quantification of anionic detergent in milk using rhodamine 6G dye and toluene solvent. *Indian Journal of Dairy Science, 75*(2), 119–124. https://doi.org/10.33785/IJDS.2022.v75i02.003

Moncayo, S., Manzoor, S., Rosales, J. D., Anzano, J., & Caceres, J. O. (2017). Qualitative and quantitative analysis of milk for the detection of adulteration by laser induced breakdown spectroscopy (LIBS). *Food Chemistry, 232*, 322–328. https://doi.org/10.1016/j.foodchem.2017.04.017

Pou, K. J., Packirisamy, M., & Raghavan, V. (2026). Development of a microfluidic paper-based analytical device (μPAD) for detection of urea, nitrite, and starch adulteration in milk with smartphone app-assisted detection. *Innovative Food Science & Emerging Technologies*, 104555. https://doi.org/10.1016/j.ifset.2026.104555

Rocchetti, G., & O'Callaghan, T. F. (2021). Application of metabolomics to assess milk quality and traceability. *Current Opinion in Food Science, 40*, 168–178. https://doi.org/10.1016/j.cofs.2021.04.005

Santos, P. M., Pereira-Filho, E. R., & Colnago, L. A. (2016). Detection and quantification of milk adulteration using time domain nuclear magnetic resonance (TD-NMR). *Microchemical Journal, 124*, 15–19. https://doi.org/10.1016/j.microc.2015.07.013

Santos, P. M., Pereira-Filho, E. R., & Rodriguez-Saona, L. E. (2013). Rapid detection and quantification of milk adulteration using infrared microspectroscopy and chemometrics analysis. *Food Chemistry, 138*(1), 19–24. https://doi.org/10.1016/j.foodchem.2012.10.024

Shi, Q., Tao, C., & Kong, D. (2022). Multiplex SERS-based lateral flow assay for one-step simultaneous detection of neomycin and lincomycin in milk. *European Food Research and Technology, 248*(8), 2157–2165. https://doi.org/10.1007/s00217-022-04038-3

Taylan, O., Cebi, N., Yilmaz, M. T., Sagdic, O., & Bakhsh, A. A. (2020). Detection of lard in butter using Raman spectroscopy combined with chemometrics. *Food Chemistry, 332*, 127344. https://doi.org/10.1016/j.foodchem.2020.127344

Verma, R. K. (2025). Detection of melamine in milk using molecular imprinting polymerization and metalloporphyrin-based fiber optic probe. *Optical Fiber Technology, 93*, 104281. https://doi.org/10.1016/j.yofte.2025.104281

Xu, L., Yan, S. M., Cai, C. B., Wang, Z. J., & Yu, X. P. (2013). The feasibility of using near-infrared spectroscopy and chemometrics for untargeted detection of protein adulteration in yogurt: Removing unwanted variations in pure yogurt. *Journal of Analytical Methods in Chemistry, 2013*, 201873. https://doi.org/10.1155/2013/201873

Zhang, T., Wu, X., Wu, B., Dai, C., & Fu, H. (2022). Rapid authentication of the geographical origin of milk using portable near-infrared spectrometer and fuzzy uncorrelated discriminant transformation. *Journal of Food Process Engineering, 45*(8), e14040. https://doi.org/10.1111/jfpe.14040

Received - 04.05.2026, Accepted - 15.05.2026, Published - 25.05.2026 (Online), 01.06.2026 (Print)
Section Editor: Dr. Panjagari Narender Raju, Editorial Board Member