Proceedings of Women in Academia, Research and Management for Work-life Initiatives for Sustainable Health & Empowering Safety (WARM-WISHES 2026)

Application of Advanced Analytical Techniques for Chemical and Microbial Characterization of Cheese: Emerging Trends in Dairy Quality Assessment

Prachi Pandey
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India

Garima Awasthi*
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India

***Corresponding author email:** gawasthi@lko.amity.edu

Received: 19 Apr 2026 | Received Revised Version: 24 Apr 2026 | Accepted: 12 May 2026 | Published: 27 May 2026

DOI: 10.37547/tajas/warm-16

Abstract

Cheese are complex, diverse matrices of fermented milk with dynamic rheology. Microbial succession coupled with biochemistry impacts the flavor, texture, and quality attributes such as safety, nutrition, and authenticity. Routine evaluation of cheese relies on conventional methods are insufficient for monitoring the multitude of changes that occur due to ripening. The intersection of advanced omic technologies, molecular cell biology, and instrumental analytical chemistry has revolutionized research in dairy science and quality assurance system in modern dairy industry. The objective of this paper is to provide a comprehensive overview of innovative analytical methods of cheese evaluation and the chemical and microbial characterization of cheese. This method includes HPLC, GC-MS, HRMS, FTIR, NMR, PCR, biosensors, metagenomics, and NGS. With these technologies, the detection of dairy pathogens, as well as metabolomic profiling of peptides, detection of volatile dairy flavor compounds, and the authentication of dairy products is achieved with great sensitivity and reproducibility. The development of flavor and ripening is further analysed by the combined approaches of chemical characterization and microbial genomics. Other technologies that will likely serve to create the future boundaries of quality assurance in the dairy industry are the use of AI and analytics, blockchain for end-to-end traceability, and precision fermentation. The integration methods of analysis developed in the dairy industry stand to greatly improve food safety, proactive compliance with regulatory standards and the competitiveness of the dairy industry on a global scale.

Keywords: Cheese, GC-MS, HPLC, metagenomics, biosensors, PCR, dairy, flavor profiling, food authenticity, quality control, microbiology.

Cite This Article: Pandey, P., & Awasthi*, G. (2026). Application of Advanced Analytical Techniques for Chemical and Microbial Characterization of Cheese: Emerging Trends in Dairy Quality Assessment. The American Journal of Applied Sciences, 168–177. https://doi.org/10.37547/tajas/warm-16

1. Introduction

Over the past thirty years, the global dairy industry has grown significantly in milk production, herd productivity, and technology in dairy farming. In countries like the United States, better breeding methods, automation, precision feeding, and the merging of dairy farms into larger, more efficient herds have all raised milk yield per cow. This growth has coincided with a steady increase in the consumption of fermented dairy products, such as cheese, yogurt, butter, and sour cream. This rise is due in part to consumers becoming more aware of their nutritional and probiotic benefits. Recent reports from the United States Department of Agriculture show that per capita cheese consumption in the United States surpassed 42 pounds per person in 2023, marking one of the highest levels recorded to date. At the same time, the growing global demand for specialty and functional cheeses has sped up the use of new processing and analytical technologies in the dairy industry. (USDA, 2024a).

Cheese is one of the most significant fermented dairy products worldwide due to its nutritive value, sensorial variety, its extensive utilization in cooking and its economic significance. Cheesemaking is the co-agulation of milk proteins either with calf rennet, or with suitable coagulating agents like animal, vegetables, or microbial rennet, acidifiers like lemon juice, vinegar or by addition of culture, the removal of the whey and controlled microbial maturation. Made from milk, either whole milk or nonfat milk or both; buttermilk; milk proteins, or the combination of milk and buttermilk. Various biochemical processes like proteolysis, lipolysis, glycolysis, and amino acid metabolism which take place during cheesemaking and ripening processes result in characteristic flavor, texture, aroma, and appearance profile typical for each cheese type. Ripening is also dependent on starter cultures, non-starter lactic acid bacteria, yeasts, moulds, pH, salt concentration, temperature, and moisture (Zheng et al, 2021, Atasever, & Mazlum, 2024).

There has been a large rise in worldwide cheese production in the last few decades, due to increasing demand from consumers for both fermented and functional dairy products. The FAO reports that worldwide production of milk has seen continued increases due to progress in technology and the expansion of industrial dairy processing systems, and cheese production went from 5.9 million tons in 1963 to 14.9 million tons in 1993 (Food and Agriculture Organization, 2024). Reports from both the USDA and OECD-FAO have claimed that the total production of cheese exceeded twenty-two million metric tons during 2023-2024 due to accelerated growth in the dairy industry and rising consumer demand for fermented dairy products across the globe (USDA, 2024b). The increasing preference for artisanal, probiotic and specialty cheeses by today's consumers due to their high sensorial and nutritive qualities, has stimulated the demand for modern analytical tools that can guarantee their authenticity, consistency, and microbial safety. As dairy industry transitions to "Industry 4.0," there is an urgent demand for high-resolution, non-destructive, and fast analytical instruments. Consequently, advanced analytical techniques such as High Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC–MS), Fourier Transform Infrared (FTIR) spectroscopy, High-Resolution Mass Spectrometry (HRMS), Near-Infrared (NIR) Spectroscopy, Nuclear Magnetic Resonance (NMR), Polymerase Chain Reaction (PCR), biosensors, metagenomics, and next-generation sequencing are increasingly being employed for comprehensive cheese characterization, mapping the "flavour-ome" and ensuring product authenticity (Food Chemistry, 2024). These technologies provide greater sensitivity, specificity, and analytical throughput while enabling rapid detection of pathogens, volatile flavour compounds, bioactive peptides, and adulteration markers.

The simultaneous integration of multi-omics (metagenomics and metabolomics), allows researchers to link specific microbial successions with chemical metabolite production, and help them to predict ripening outcomes, long before the product reaches the consumer (Liu et al., 2026). Integration of omics technologies with artificial intelligence and chemometric analysis has further accelerated the development of predictive quality monitoring systems in the dairy industry. The complexity of these systems becomes essential to avoiding food fraud and the early detection of new diseases as well as quality standards in a global market. Dairy producers face more pressure to move to a more sustainable way of producing milk because different interest groups within the dairy supply chain are in competition with each other (El-tahlawy et al., 2025). These types of approaches allow better real time monitoring of ripening development, microbial succession, and metabolic production, to improve food safety and optimize industrial processes.

According to Fig.1 consumer trends, nowadays food consumers desire dairy products that are cheap and environmentally friendly, safe, healthy and tasty. However, upstream industries responsible for providing starter cultures, enzymes and flavorings often experience volatile pricing and irregular supply that can negatively impact on the quality, constancy, and cost of dairy foods.

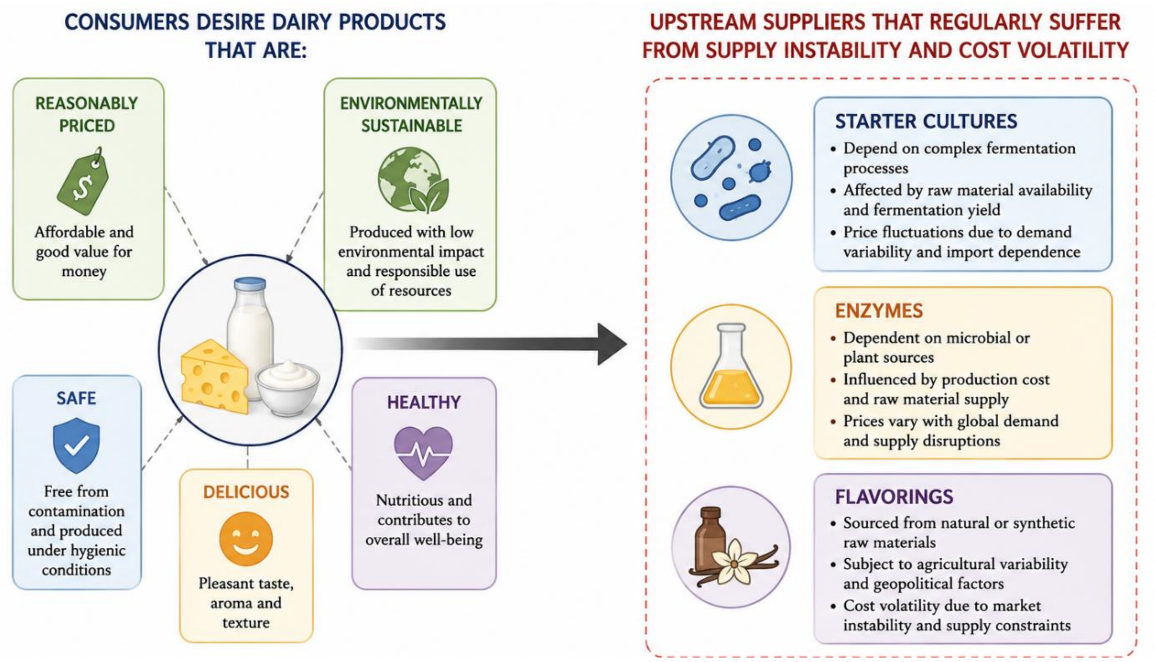

Figure 1: Reliable, sustainable, and affordable supply by partners upstream guarantee that consumer access to healthy, tasty, good-priced and eco-friendly products

2. Advanced Chemical Characterization Techniques

The chemical characterization of cheese is essential for identifying cheese composition, ripening, origin, as well as sensory properties. Various highly specific and reproducible methods based on state-of-the-art analytical techniques have been developed for determination of peptides, organic acids volatile substances, lipids, and bioactive substances. (Bertrand et al., 2011).

2.1 High-Performance Liquid Chromatography (HPLC)

Dairy science research utilizes HPLC for routine analysis for organic acids, amino acids, peptides, carbohydrates, and adulterants. The method employed with HPLC is sensitive, reproduceable, and quantifiable. Organic acids (i.e., Lactic acid, citric acid, acetic acid, propionic acid) are important as the quality and flavor development of cheese ripening, while playing a key role in microbiological stability. Like organic acids, peptides are an important molecule during cheese ripening, influencing both flavor production and texture and nutritional quality of cheese. The analysis of both organic acids and peptides can help elucidate the biochemical events that occurred during ripening, information concerning the bioactive properties and quality of cheese. Their analysis is therefore important for ensuring consistency, functionality, and quality control in dairy products (Tekin, and Hayaloglu 2023).

The extraction techniques utilized for HPLC analysis of organic acids and peptides in cheese samples, particularly for adulteration detection such as whey addition. These extraction and purification techniques include protein precipitation, supernatant filtering, and chromatographic separation. These methods ensure precise readings of markers like casein or organic acids such as lactic acid. The amount of water, fat, protein, pH, titratable acidity, presence of chlorides and neutralizers, and determination of total solids and non-fat solids are the fundamental physical-chemical parameters are quantified of received raw milk in many dairy plants. Nevertheless, these analyses do not identify the presence of cheese whey in milk at the time it arrives at the plant. Therefore, a dependable, quantitative, quick, and easily accessible method for quality control in dairy facilities are required (Ames et al., 2001).

Organic Acids Extraction

Researchers have established techniques for the extraction of organic acids such as citric acid and lactic acid. The cheese is shredded and combined with 25 g of 0.01 N sulfuric acid buffer, that was tempered at 70°C in water bath. The sample was grinded using homogenized at 12,000 rpm, and centrifuged at 3,000 rpm for 15 minutes at 5°C. After removing the fat layer and passing the filtrate through Whatman 1 paper, 0.5 mL was centrifuged once more at 11,000 rpm for 10 minutes using centrifugal filters (Millipore). The supernatant was added to an HPLC with UV/RI detection for organic acids (Amamcharla & Metzger, 2011). Few other researchers used the technique and collected Parmesan, Mozzarella, Swiss, and Cheddar cheese from market and successfully quantified organic acids using HPLC analysis, they reported up to 1.89% lactic acid in Parmesan (Ahmed et al., 2023).

Peptide Extraction

Adulteration of raw milk with cheese whey is a significant concern in the dairy industry due to its impact on product quality and consumer safety. The detection of cheese whey adulteration in milk is one of the major applications of HPLC. The researchers in 2022 demonstrated the use of casein glycomacropeptide (cGMP) as a biomarker for detecting whey addition through molecular exclusion chromatography analysis. Milk proteins were precipitated using 24% (w/v) trichloroacetic acid (TCA), and samples were analyzed with a Shodex KW-802.5 molecular exclusion column, where a characteristic peak appeared at a retention time of 10.8 minutes, and the peak was different for the different percentages of cheese whey, as higher the concentration, the higher the peak. The procedure proved very linear (R=0.9984) in its estimation of whey concentration in milk and MALDI-TOF spectroscopy and immunochromatography additionally confirmed the existence of cGMP monomer in whey- adulterated samples throughout chymosin enzymatic coagulation. This study presents a simple and economical chromatographic method applicable to routine dairy quality analysis (Vera-Bravo et al., 2022).

The study of biochemistry of cheese ripening also owes much to the HPLC based peptide profiling. The breakdown of casein, by proteolysis, gives rise to various bioactive peptides which possess antioxidants, antimicrobial and antihypertensive activities (Basilicata et al., 2018). As the period of ripening extends, the diversity of peptides is enhanced, and beneficial organoleptic properties and functional nutrition values can be improved.

2.2 Gas Chromatography–Mass Spectrometry (GC–MS)

GC-MS is a very powerful analytical technique for the analysis of volatile flavor compounds headspace solid-space microextraction (HS-SPME) is a frequently used sample preparation technique for GC-MS analysis of cheese volatiles due to its ability to extract aroma compounds with reduced amount of matrix interference (Curioni & Bosset, 2002). Normally, the grated or homogenized cheese sample (0.5-1 g) was put into a sealed headspace vial (20 mL) in the common HS-SPME processes and thermostated with a defined temperature, then the volatile was extracted (Poveda et al., 2008). Typical fibers like 50/30 m DVB/CAR/PDMS is typically exposed to the sample headspace and then heated and injected into the GC injector for volatile analysis (Curioni & Bosset, 2002). Many compounds (aldehydes, ketones, sulfur compounds, alcohol, esters, and free fatty acids) that are associated with the characteristics aroma of ripening cheese have been identified using GC-MS with HS-SPME (Qian & Reineccius, 2002; Sablé & Cottenceau, 1999). These volatile compound profile can discriminate ripening stage and specific aroma pattern in Cheddar or Grana Padano cheeses (Pillonel et al., 2003). GC-MS of fatty acid methyl esters (FAMEs) has been routinely used to characterizing cheese lipids because it could clearly separate and identify short-chain, medium-chain, and long-chain fatty acids in cheese, and the branched-chain, odd numbered and cyclopropane fatty acids (Collins et al., 2003; Marseglia et al., 2013). Cyclopropane fatty acids (CPFAs) have been suggested as markers of authenticity in protected dairy products and both GC-MS methods employing FAME derivatization and EI-MS has shown authentic Parmigiano Reggiano cheese is characterized by CPFA levels below roughly 60 mg/kg cheese fat. (Caligiani et al., 2016). The GC-MS method is widely used for investigating biochemical and metabolic transformations occurring during the ripening process, as both starter and (NSLAB) covert lactose to lactic acid then to volatile flavor flavor compounds via lipolysis and proteolysis (Collins et al., 2003; McSweeney, 2004). With a passage of time the concentration of fatty acid increases and they are associated with flavor intensity and mouth feel and are the precursor to bioactive lipids such as conjugated linoleic acid isomers (CLA) (Collins et al., 2003). GS-MS of the fatty acid composition is widely used for authentication of PDO cheeses and characteristic lipids such as the trans-C18:1 fatty acid, attributed to grass-fed milk, are employed to verify authentic samples against adulterated product with vegetable fat added (Caligiani et al., 2016; Riccio et al., 2025).

The use of advanced chemometric tools, such as PCA and PLS-DA, in conjunction with GC-MS has become an efficient method for dairy products classification and authentication

Furthermore, it has been used to authenticate milk products on the basis of fatty acids and volatile marker patterns related to feed, origin and milk source (Luykx & Van, 2008; Pillonel et al., 2003).

Advanced chemometric tools such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) are frequently integrated with GC–MS data for classification and authentication of dairy products, significantly improving discrimination between authentic and adulterated samples (Pillonel et al., 2003; Ampuero & Bosset, 2003). Mass spectral libraries like NIST and Wiley were successfully used to enable fully automatic identification of GC-MS peaks and to provide reliable compound annotation with the help of multivariate models (Qian & Reineccius, 2002). GC-MS generated volatile profiling data closely correlate to sensory perceptions and specific volatile compounds like methional, for instance is responsible for “boiled potato” off-flavor (Curioni & Bosset, 2002). GC-MS has been applied widely to analyze biochemical pathways during the ripening of cheese, such as lipolysis and proteolysis, where microbial enzymatic activities lead to formation of aldehydes, ketones, acids, and sulfur compounds responsible for the cheese flavor profile (McSweeney, 2004; Smit et al., 2005). Furthermore, it has been used to authenticate milk products based on fatty acids and volatile marker patterns related to feed, origin and milk source (Luykx & Van, 2008; Pillonel et al., 2003).

2.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

It has also proven useful as a non-destructive analytical tool for dairy metabolomics and for determining authenticity of products. It is suitable for simultaneous monitoring of a range of metabolites within a complex matrix such as cheese and does not require a large sample size or complex sample preparation procedures. NMR has been used in metabolomic studies to analyze composition of fats, organic acids and cyclopropane fatty acids related to dairy authentication. (Eltemur et al., 2025).

Compared with conventional chromatographic methods, NMR offers rapid analysis, minimal sample destruction, and high reproducibility. However, relatively high instrumentation costs and lower sensitivity for trace-level compounds remain important limitations.

2.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is increasingly used in dairy quality control for rapid and non-destructive evaluation of cheese composition. FTIR enables estimation of fat, protein, moisture, and lactose content while also supporting adulteration detection. Integration of FTIR with chemometric analysis allows rapid discrimination of cheese varieties and geographical origin. The study helps to investigate the adulteration of goat milk with skim milk powder (SMP) as a primary approach for future research on halloumi cheese adulteration. The SMP from different geographical origins were collected and different concentrations of SMP (0.5%, 1%, and 3%) were added to Cyprus goat milk and heated at 90°C before FTIR analysis and chemometric classification. The findings indicated that the level of adulteration played a greater role in sample discrimination than the origin of the milk powder. These results suggest that milk powder origin is unlikely to significantly influence the detection of adulteration in future halloumi cheese studies (Tarapoulouzi et al., 2026).

Both near infrared and mid-infrared spectroscopic techniques are applicable to monitoring in industrial processes since they deliver rapid information with minimal sample preparation, they are also appropriate to inline monitoring for modern processes.

3. Advanced Microbial Characterization Techniques

The characteristic of microbiota is of immense importance to secure the safety, maturation and shelf-life stability of cheese. Molecular techniques enable rapid and highly sensitive methods to detect pathogens and useful microorganisms in the dairy environments. The characterization of the microbial flora in cheese uses both the culture dependent microbiological techniques and the molecular techniques and the molecular techniques to identify the pathogenic and useful microorganisms like the lactic acid bacteria that include Lactobacillus spp and Streptococcus spp to assure the safety and the quality of the dairy products in the environment which can be easily contaminated by pathogens that are Listeria monocytogenes, Salmonella spp, etc. (Quigley et al., 2012; Fusco et al., 2015; Nero et al., 26). (Quigley et al., 2012; Fusco et al., 2015; Nero et al., 2026). Conventional plating counting methods and PCR or QPCR based assays were commonly applied for rapid profiling and detection of pathogen. (O'Sullivan et al., 2013; Law et al., 2015).

3.1 Polymerase Chain Reaction (PCR) and Quantitative PCR

PCR- based techniques are commonly used for quick detection of foodborne pathogens such as *Listeria monocytogenes, Salmonella spp*. And pathogenic Escherichia coli in cheese matrices. The target DNA sequence is amplified using PCR, thereby allows detection of low number of microorganisms. The sensitivity of PCR is about 10- 10 CFU/mL or CFU/g after enrichment of food samples, even for complex dairy matrices, which are useful for foodborne pathogens at a very low level (Brautsch et al., 2016; Ding et al., 2017). In PCR technique, the target DNA is amplified using three different temperature steps called thermal cycle: denaturation at 94–95°C, primer annealing at 55–60°C, and primer extension using Taq polymerase at 72°C (Mullis & Faloona, 1987). In addition, for identification of specific foodborne pathogens, a pair of specific primer pairs for virulence genes such as for pathogenic *Escherichia coli* (stx1 and stx2), for *Listeria monocytogenes* (hlvA or inlA) and for *Salmonella spp*.(invA or ttr) were targeted in multiplex PCR approach (Paton & Paton, 1998; Kim et al., 2016). Amplified products can be observed either by agarose gel electrophoresis with conventional PCR or measuring the fluorescent signals with qPCR and ddPCR systems (Porcellato et al., 2016). The different amplicons generated from different pairs of primer pairs in a single reaction system with differentiate multiple foodborne pathogens in multiplex PCR method (Kim et al., 2016).

Compared to conventional microbiological techniques, real-time qPCR provides the advantages of better sensitivity and specificity with reduced time of testing (Kim et al., 2014). Multiplex PCR technique provides ability to simultaneously identify different foodborne pathogens in single PCR reaction, and it plays a significant role in food safety control and regulatory surveillance in industrial dairy manufacturing.

3.2 Metagenomics and Next-Generation Sequencing

Metagenomics and next generation sequencing are promising approaches to study microbial ecology of fermented foods. Compared with culture-dependent methods, metagenomics allows characterizing total microbial community structure, including viable and non-culturable organisms (Tsouggou et al., 2026).

High-throughput sequencing approaches allow detailed analysis of starter cultures, non-starter lactic acid bacteria, yeasts, moulds, and spoilage microorganisms during cheese ripening. Integration of metagenomics with metabolomics has significantly improved understanding of microbial contributions to flavour development and biochemical transformation pathways (Tilocca et al., 2020). Metagenomics uses next generation sequencing to study the total microbial DNA present in cheese and to identify the microbial composition, functional genes and diversity within the cheese and to identify the microbial identification, functional genes and diversity within the cheese without the limitations imposed by the culture methods. The use of culture-independent technique of 16S rRNA gene sequencing, preferably V3-V4 region, is an accepted standard for studying bacterial diversity while shotgun metagenomics can differentiate between strains of bacteria and study functional genes (De Filippis et al., 2014). This technique has already been utilized to study ripening of cheese; these include NSLAB. Lactobacillus casei, surface bacteria such as Psychrobacter and Debaryomyces and starter cultures such as *Lactococcus lactis* and *Streptococcus thermophilus* during ripening (Wolfe et al., 2014; Alessandria et al., 2016).

These technologies also support microbial traceability, geographical authentication, and quality prediction in artisanal and industrial cheese production systems.

3.3 Biosensors for Cheese Analysis

Biosensors, an electronic device which converts chemical, biochemical, and biological signals into electrical signals. Biosensors are reliable, rapid and inexpensive devices, that combine biological recognition elements with physicochemical transducers for portable analysis of toxins, lactose, urea, glucose and detect pathogenic microorganisms present in dairy products. Many different biosensors are available currently and they are applicable for the detection of pathogen and ripening monitoring in cheese. These biosensors are electrochemical biosensors, optical biosensors, and quartz crystal microbalance systems (Jangra, 2022).

Electrochemical biosensors based on aptamers and antibodies provide highly sensitive detection of *Listeria monocytogenes, Salmonella*, and *Escherichia coli* within short analysis times. Optical biosensors such as surface plasmon resonance systems allow real-time monitoring of microbial interactions and metabolite production (Savas, 2025).

Integration of biosensors with smartphone applications and artificial intelligence platforms has further improved portability and industrial applicability. These systems have strong potential for onsite dairy quality monitoring and real-time hazard detection (Banicod et al., 2025).

4. Integration of Chemical and Microbial Analyses

Combined chemical and microbial analyses provide a comprehensive understanding of cheese ripening dynamics and flavour development, where microbial metabolism contributes significantly to the formation of volatile compounds, peptides, and organic acids, while physicochemical parameters influence microbial succession and enzymatic activities during ripening (McSweeney, 2004; Wolfe et al., 2014). It has been established that integrating multiple omics techniques, such as metabolomics, metagenomics, proteomics and chemometrics can provide a valuable approach to characterizing and evaluating the quality of dairy products (Ercolini, 2013; De Filippis et al., 2016). By correlating the composition of the microbial community with the production of flavor compounds and the sensory properties of cheese (Wolfe et al., 2014). In addition, utilizing AI supported analytical systems and predictive models could enhance process optimization, product standardization and quality assurance in modern dairy industries (Xu et al., 2022).

5. Advantages and Limitations

These are more advanced analytical techniques have demonstrated increased sensitivity, specificity, speed, and reproducibility when compared to traditional microbiology and chemistry based analytical methods (Law et al., 2015). Molecular methods, such as PCR and qPCR are beneficial due to reduced deduction time of pathogenic microorganisms; spectroscopic methods have been implemented for quick non-destructive quality testing of products (Fusco et al., 2015; Rodriguez-Saona & Allendorf, 2011). Conversely, limitations to routine use in industry, particularly for smaller dairies and in developing countries are cost matrix interference, complicated sample preparation, and the need for trained personnel (Ercolini, 2013). Thus, rapid screening paired with confirmatory molecular methods are often suggested for industrial food quality screening (Cocolin et al., 2011; Law et al., 2015).

6. Future Perspectives

These AI, ML, and industry 4.0 technologies will shape up future dairy quality evaluation systems. The use of AI assisted chemometric data in conjunction with FTIR, GC-MS, and metagenomics is hoped for further optimize modeling prediction of ripeness, spoilage and detection of adulteration. In food safety monitoring and traceability in supply chain smart biosensors and blockchain based traceability are anticipated to strengthen surveillance of food safety. Engineered strains of microbes; recombinant enzyme and precision fermentation would revolutionize the manufacturing of functional dairy products and cheese. In coming decades, the integration of omics with real-time digital monitoring would lead to data driven manufacturing and hazard prediction.

7. Conclusion

The modernization of cheese characterization and dairy quality management is greatly facilitated by advanced analytical technologies such as HPLC, GC-MS, NMR, PCR, biosensors, and metagenomics. Each offers highly sensitive and accurate methods for microbial ecologies and flavor profiles, authenticity and food safety. Combining chemical profiling, microbial genomics and AI assisted analysis further contributes to elucidating cheese ripening biochemistry and predicting its quality. The adoption of these advanced analytical platforms across the dairy industry has a direct impact on compliance regulations, minimizing financial loss from recalls and competing in a global marketplace. Cost-effective, fast, portable analytical tools are likely to foster a new generation of dairy quality assurance programs.

Author Declaration Statements

Declaration: The authors hereby declare that the manuscript submitted for consideration is an original work and has not been published or submitted elsewhere for publication. The authors take full responsibility for the integrity, accuracy, and ethical compliance of the work presented in the manuscript.

Conflict of Interest: All authors confirm that:

- Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. **– Yes / Not Applicable√**
- All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged. **– Yes / Not Applicable√**
- Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable. **– Yes / Not Applicable√**

References

1. Ahmed, M. E., Hammam, A. R., Ali, A. E. F., Alsaleem, K. A., Elfaruk, M. S., Kamel, D. G., & Moneeb, A. H. (2023). Measurement of carbohydrates and organic acids in varieties of cheese using high-performance liquid chromatography. *Food Science & Nutrition, 11*(5), 2081–2085.
2. Alessandria, V., Ferrocino, I., De Filippis, F., Fontana, M., Rantsiou, K., Ercolini, D., & Cocolin, L. (2016). Microbiota of an Italian Grana-like cheese during manufacture and ripening unraveled by 16S rRNA-based approaches. *Applied and Environmental Microbiology, 82*(13), 3988–3995.
3. Amamcharla, J. K., & Metzger, L. E. (2011). Development of a rapid method for the measurement of lactose in milk using a blood glucose biosensor. *Journal of Dairy Science, 94*(10), 4800–4809.
4. Ampuero, S., & Bosset, J. O. (2003). The electronic nose applied to dairy products: A review. *Sensors and Actuators B: Chemical, 94*(1), 1–12.
5. Ames, J. M., Guy, R. C., & Kipping, G. J. (2001). Effect of pH, temperature, and moisture on the formation of volatile compounds in glycine/glucose model systems. *Journal of Agricultural and Food Chemistry, 49*(9), 4315–4323.
6. Atasever, M., & Mazlum, H. (2024). Biochemical processes during cheese ripening. *Veterinary Sciences and Practices, 19*(3), 174–182.
7. Banicod, R. J. S., Tabassum, N., Jo, D. M., Javaid, A., Kim, Y. M., & Khan, F. (2025). Integration of artificial intelligence in biosensors for enhanced detection of foodborne pathogens. *Biosensors, 15*(10), 690.
8. Basilicata, M. G., Pepe, G., Sommella, E., Ostacolo, C., Manfra, M., & Campiglia, P. (2018). Peptidome profiles and bioactivity elucidation of buffalo-milk dairy products after gastrointestinal digestion. *Food Research International, 105*, 1003–1010.
9. Bertrand, E., Machado-Maturana, E., Chevarin, C., Portanguen, S., Mercier, F., Tournayre, R., & Berdagué, J. (2011). Heat-induced volatiles and odor-active compounds in a model cheese. *International Dairy Journal, 21*, 806–814.
10. Brautsch, A., Wang, Y., Graber, H. U., et al. (2016). Fast detection and quantification of four dairy propionic acid bacteria in milk samples using real-time quantitative polymerase chain reaction. *International Dairy Journal, 61*, 37–43.
11. Caligiani, A., Nocetti, M., Lolli, V., Marseglia, A., Palla, G., & Sforza, S. (2016). Development of a quantitative GC–MS method for the detection of cyclopropane fatty acids in cheese as new molecular markers for Parmigiano Reggiano authentication. *Journal of Agricultural and Food Chemistry, 64*(20), 4158–4164.
12. Cocolin, L., Dolci, P., & Rantsiou, K. (2011). Biodiversity and dynamics of meat fermentations: The contribution of molecular methods for a better comprehension of a complex ecosystem. *Meat Science, 89*(3), 296–302.
13. Collins, Y. F., McSweeney, P. L. H., & Wilkinson, M. G. (2003). Lipolysis and free fatty acid catabolism in cheese: A review of current knowledge. *International Dairy Journal, 13*(11), 841–866.

14. Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal, 12*(12), 959–984.

15. De Filippis, F., La Storia, A., & Ercolini, D. (2016). Metagenomics insights into food fermentations. *Microbial Biotechnology, 10*(1), 91–102.

16. De Filippis, F., Parente, E., & Ercolini, D. (2014). Metagenomics insights into food fermentations. *Microbial Biotechnology, 10*(1), 91–102.

17. Ding, S., Chen, J., Ye, X., et al. (2017). A multiplex RT-PCR assay for *S. aureus, L. monocytogenes,* and *Salmonella* spp. detection in raw milk with pre-enrichment. *Frontiers in Microbiology, 8*, 989.

18. El-tahlawy, A. S., Alawam, A. S., Rudayn, H. A., Allam, A. A., Mahmoud, R., Abd El-Raheem, H., & Alahmad, W. (2025). Advanced analytical and digital approaches for proactive detection of food fraud as an emerging contaminant threat. *Talanta Open, 12*, 100499.

19. Eltemur, D., Morozova, K., Ceccon, A., Riccio, G. M., Robatscher, P., Oberhuber, M., & Scampicchio, M. (2025). 1H NMR detection of cyclopropane fatty acids in yoghurt as molecular markers for haymilk authentication. *Journal of Food Composition and Analysis*, 108623.

20. Ercolini, D. (2013). High-throughput sequencing and metagenomics: Moving forward in the culture-independent analysis of food microbial ecology. *Applied and Environmental Microbiology, 79*(10), 3148–3155.

21. Food and Agriculture Organization. (2024). *FAOSTAT statistical database: Dairy production and products*. FAOSTAT.

22. Fusco, V., Quero, G. M., Cho, G. S., Kabisch, J., Meske, D., Neve, H., Bockelmann, W., & Franz, C. M. A. P. (2015). Culture-dependent and culture-independent nucleic acid-based methods used in the microbial safety assessment of milk and dairy products. *Comprehensive Reviews in Food Science and Food Safety, 14*(4), 493–537.

23. Jangra, S. (2022). Development of biosensor-based technology for the detection of pathogenic microorganisms and biomolecules in dairy products. In *Advances in Dairy Microbial Products* (pp. 377–384). Woodhead Publishing.

24. Kim, S., Kim, J., Kim, M., et al. (2016). Multiplex PCR for simultaneous identification of *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* in food. *3 Biotech, 6*, 205.

25. Kim, T. G., Jeong, S. Y., & Cho, K. S. (2014). Comparison of droplet digital PCR and quantitative real-time PCR for examining population dynamics of bacteria in soil. *Applied Microbiology and Biotechnology, 98*, 6105–6113.

26. Law, J. W. F., Ab Mutalib, N. S., Chan, K. G., & Lee, L. H. (2015). Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. *Frontiers in Microbiology, 5*, 770.

27. Liu, C., Hao, J., Lu, H., Ma, P., He, Y., Xue, Y., ... & Wang, S. (2026). Metagenomic and metabolomic insights into microbial-metabolite interactions underlying flavor formation in Hebei-style soybean paste fermentation. *Food Research International*, 118665.

28. Luykx, D. M. A. M., & van Ruth, S. M. (2008). An overview of analytical methods for determining the geographical origin of food products. *Food Chemistry, 107*(2), 897–911.

29. Marseglia, A., Caligiani, A., Comino, L., Righi, F., Quarantelli, A., & Palla, G. (2013). Cyclopropyl and ω-cyclohexyl fatty acids as quality markers of cow milk and cheese. *Food Chemistry, 140*(4), 711–716.

30. McSweeney, P. L. (2004). Biochemistry of cheese ripening. *International Journal of Dairy Technology, 57*(2–3), 127–144.

31. Mullis, K., & Faloona, F. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology, 155*, 335–350.

32. Nero, L. A., Andretta, M., Almeida, T. T., Ferreira, L. R., Camargo, A. C., Yamatogi, R. S., ... & Call, D. R. (2021). Lactic microbiota of the minas artisanal cheese produced in the Serro region, Minas Gerais, Brazil. *LWT, 148*, 111698.

33. O'Sullivan, D. J., Giblin, L., McSweeney, P. L., Sheehan, J. J., & Cotter, P. D. (2013). Nucleic acid-based approaches to investigate microbial-related cheese quality defects. *Frontiers in Microbiology, 4*, 1.

34. Paton, A. W., & Paton, J. C. (1998). Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA,* and *hlyA*. *Journal of Clinical Microbiology, 36*(2), 598–602.

35. Pillonel, L., Ampuero, S., Tabacchi, R., & Bosset, J. (2003). Analytical methods for the determination of the geographic origin of Emmental cheese: Volatile compounds by GC/MS-FID and electronic nose. *European Food Research and Technology, 216*(2), 179–183.

36. Porcellato, D., Narvhus, J., & Skeie, S. B. (2016). Detection and quantification of *Bacillus cereus* group in milk by droplet digital PCR. *Journal of Microbiological Methods, 127*, 1–6.

37. Poveda, J. M., Cabezas, L., & McSweeney, P. L. H. (2008). Preliminary observations on the use of headspace solid-phase microextraction for studying the aroma of Manchego cheese. *International Dairy Journal, 18*(5), 551–557.

38. Qian, M., & Reineccius, G. (2002). Identification of aroma compounds in Parmigiano Reggiano cheese by gas chromatography/olfactometry. *Journal of Dairy Science, 85*(6), 1362–1369.

39. Quigley, L., O'Sullivan, O., Beresford, T. P., Ross, R. P., Fitzgerald, G. F., & Cotter, P. D. (2012). High-throughput sequencing for detection of subpopulations of bacteria not previously associated with artisanal cheeses. *Applied and Environmental Microbiology, 78*(16), 5717–5723.

40. Riccio, G. M., Eltemur, D., Fava, F., Martini-Lösch, D., Peratoner, G., Venir, E., ... & Ceccon, A. (2025). Differentiating cyclopropane fatty acids to support milk authenticity through GC–MS and NMR spectroscopy. *Food Chemistry: X*, 103033.

41. Rodriguez-Saona, L. E., & Allendorf, M. E. (2011). Use of FTIR for rapid authentication and detection of adulteration of food. *Annual Review of Food Science and Technology, 2*, 467–483.

42. Sablé, S., & Cottenceau, G. (1999). Current knowledge of soft cheeses flavor and related compounds. *Journal of Agricultural and Food Chemistry, 47*(12), 4825–4836.

43. Savas, S. (2025). For the detection and diagnosis of common pathogens. In *Current Developments in Biosensors and Emerging Smart Technologies* (Vol. 12, p. 267).

44. Smit, G., Smit, B. A., & Engels, W. J. M. (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese. *FEMS Microbiology Reviews, 29*(3), 591–610.

45. Tarapoulouzi, M., Kyriacou, S., Ioannidis, I., Pashalidis, I., & Theocharis, C. R. (2026). Fourier-transformed infrared (FTIR) spectroscopy for detecting milk powder addition in Cyprus goat milk: A preliminary study for future adulteration detection in halloumi cheese. *Quality Assurance and Safety of Crops & Foods, 18*(1), 74–81.

46. Tekin, A., & Hayaloglu, A. A. (2023). Understanding the mechanism of ripening biochemistry and flavour development in brine ripened cheeses. *International Dairy Journal, 137*, 105508.

47. Tilocca, B., Costanzo, N., Morittu, V. M., Spina, A. A., Soggiu, A., Britti, D., Roncada, P., & Piras, C. (2020). Milk microbiota: Characterization methods and role in cheese production. *Journal of Proteomics, 210*, 103534.

48. Tsouggou, N., Korozi, E., Pemaj, V., Drosinos, E. H., Kapolos, J., Papadelli, M., ... & Papadimitriou, K. (2026). Advances in shotgun metagenomics for cheese microbiology: From microbial dynamics to functional insights. *Foods, 15*(2), 259.

49. United States Department of Agriculture. (2024a). *Dairy data: Per capita consumption of dairy products*. Economic Research Service.

50. United States Department of Agriculture. (2024b). *Dairy: World markets and trade*. Foreign Agricultural Service.

51. Vera-Bravo, R., Hernández, A. V., Peña, S., Alarcón, C., Loaiza, A. E., & Celis, C. A. (2022). Cheese whey milk adulteration determination using casein glycomacropeptide as an indicator by HPLC. *Foods, 11*(20), 3201.

52. Wolfe, B. E., Button, J. E., Santarelli, M., & Dutton, R. J. (2014). Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell, 158*(2), 422–433.

53. Xu, Y., Chai, Y., & Wang, Y. (2022). Artificial intelligence and machine learning applications in dairy food processing and quality assessment: A review. *Trends in Food Science & Technology, 122*, 256–273.

54. Zheng, X., Shi,X., & Wang, B. (2021). A review on the general cheese processing technology, flavor biochemical pathways and the influence of yeasts in cheese. *Frontiers in Microbiology, 12*, 703284.