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Metabolomics in ruminant food: Bridging nutritional quality and safety evaluation

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Review

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Abstract

Ruminant-derived foods, predominantly milk and meat, are globally recognized as staples of a high-quality diet. Despite their widespread popularity, there is a notable deficiency in comprehensive standards addressing the nutritional values and safety of these products. This gap significantly limits both the supply of and demand for premium quality milk and meat. This review endeavors to highlight the benefits of utilizing metabolomics for the evaluation of quality and safety in milk and meat products from ruminants. It identifies critical metabolites, genetic signals, and metabolic pathways related to the synthesis of ruminant-derived milk and meat, proposing their potential as nutritional or regulatory targets and biomarkers. These biomarkers are instrumental in predicting and assessing the quality and safety of dairy and meat products, offering guidance for quality-based pricing and food safety inspections in the market. This review offers a critical overview of current metabolomics-based platforms and tools for interpreting the quality and safety of ruminant foods. The core metabolic biomarkers and biological biosynthetic processes of milk and meat enhance our understanding of the interplay between conventional food production from animals and new synthetic biological technologies.

Introduction

Understanding the landscape of animal-based food components is essential to inform potential nutritional material flow and assess protection on human health and food security (Tyndall et al. 2022). The production of these substances through biological transformation is a critical aspect of One Health, particularly regarding their production processes in animals and their consumption by humans (Bizzaro et al. 2022). Milk or meat food component analysis of proteins, fats, carbohydrates, solids and/or ash is widely assessed by traditional identifying methods or standard. In addition, milk and meat processing, storage, origin, breed, feed regime, gender, age, and other factors have a strong effect on the metabolome of the milk and meat of ruminants. Many factors (nutrition, feed sources, genders, management, weather, processing, handling conditions, adulteration, and related concerns) impact the integrity and security of products (Suh 2022). Potential biomarkers and metabolic mechanisms associated with the meat and milk synthesis need more research, which could lay a strong foundation of the food quality standards and then improve the consumers' options and producers' initiative.

Metabolomics, also known as metabonomics, focuses on studying small molecules technology and compounds identification and quantification with the high-throughput techniques (<1500 Da) (German et al. 2005; Plumb et al. 2023), encompasses a variety of both internal and external chemical compounds, including fatty acids (FAs), peptides, amino acids (AAs), carbohydrates, nucleic acids, vitamins, organic acids, alkaloids, polyphenols, minerals and just about any other chemicals that can be utilized, produced or consumed by specific cells or organisms. Metabolomics has contributed significantly to livestock research and industry. This includes breakthroughs in animal health, breeding, and production, showcasing its pivotal role in advancing agricultural practices. Till now, many reviews are focusing on the metabolomics technology summary (Afshari et al. 2020; Akhtar et al. 2021; Munekata et al. 2021) or metabolome database development related to livestock or food, such as a recent comprehensive Livestock Metabolome Database (LMDB, available at http://www.lmdb.ca) was released for targeted metabolomic studies (Goldansaz et al. 2017) and a bovine and bovine rumen fluid metabolome (Foroutan et al. 2020; Saleem et al. 2013). Lipids, encompassing a range of FAs and lipid-soluble bioactive compounds, are of paramount importance in enhancing the sensory qualities of foods, such as flavor and texture, while also extending shelf life. These effects are pivotal in food processing and shape consumer preferences (Muroya 2023; Wittwer et al. 2023; Yu et al. 2024). Lipids found in bovine milk and meat possess a multitude of biological functions, having significant impacts on human health and the physical properties of

food products. The emergence of metabolomics technology underscores the complex interplay between diet and health, highlighting the critical role of these lipids in both nutritional science and food technology (Wishart 2008; Zhang et al. 2023), which bring in a development of recognizing the milk and food quality through the metabolome. The application of metabolomics with selected markers is potentially useful in evaluating the genuineness of unidentified food specimens (Zhong et al. 2022). Ruminant food metabolomics plays a key role in food chemistry, food quality, and the identification of biomarkers linked to economically valuable traits. Thus, to build the database of the ruminant foods of milk and meat metabolome (for the most common ruminant species, namely dairy cow, beef cattle, camel, buffalo, yak, reindeer, sheep, and goats, etc.) is necessary for producers (food industry and animal farmers), consumer preference, and researchers.

Correlation analyses between metabolites and compositional traits of ruminant foods (milk or meat) provide insights into the underlying biological mechanisms and aid in developing fingerprints and biomarkers for identifying food properties (Caboni et al. 2019; Munekata et al. 2021). The core metabolic biomarkers and biological biosynthetic processes of milk and meat enhance our understanding of the interplay between conventional food production from animals and new synthetic biological technologies. In this review, we summarized the recent progress and applications of metabolomics in determining ruminant food products quality to acquire a detailed overview of the metabolite profile and its fluctuations, as well the biomarkers and indicators to reflect the food origin, adulterate, trait, quality, flavor, taste, safety, etc. The milk or meat metabolome not only directly represents food quality and safety but also serves as an indicator of the animal's metabolic properties and health status (Lu et al. 2013; Ye et al. 2023). Thus, this review aimed to clarify the following: (1) What are the preferred metabolomics technologies in ruminant foodomics? (2) What are the most obvious weaknesses and advantages in ruminant metabolomics relative to other fields of metabolomics research? (3) What are the known or measured metabolites and biomarkers for the seven major ruminant food (cow, sheep & goat milk, camel milk, yak milk, beef, sheep & goat meat, yak meat)? (4) What is the relationship between ruminant food metabolome and animal science, as well the connection between ruminant food metabolome and human health and nutrition? and (5) What role does metabolomics play in the production of ruminant food alternatives?

Metabolomics techniques

Techniques and data acquisition

A variety of metabolomics technologies are available, such as nuclear magnetic resonance (NMR), gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), capillary electrophoresis–mass spectrometry (CE-MS), high-performance liquid chromatography with ultraviolet detection (HPLC-UV), and inductively coupled plasma mass spectrometry (ICP-MS), each with distinct advantages and disadvantages. Various analytical platforms, including NMR, HPLC-UV, LC-MS, GC-MS, ICP-MS, and CE-MS, are commonly used in metabolomics studies (Table 1). To date, one of the most exhaustive metabolomic analyses conducted involved the utilization of 5 distinct platforms in a study focusing on the metabolome of bovine ruminal fluid (Saleem et al. 2013). However, for the food-metabolomics study, the GC-MS-, LC-MS-, and NMR-based platform are the frequenters.

GC-MS is a cornerstone of metabolomic research, valued for its efficiency, reproducibility, reliability, selectivity, and robustness. It is distinguished by its exceptional sensitivity and highly consistent fragmentation patterns, making it highly effective for the precise analysis of complex metabolite profiles (Ren et al. 2018; Zeki et al. 2020). Specifically, GC-MS based on volatile organic compounds (VOCs) is always used to detect the volatile metabolites to indicate the meat flavor, which can also be treated as volatilomics (Pavlidis et al. 2019). With the development of new techniques, nowadays, GC × GC-TOF-MS has gained widespread application in the analysis of complex food matrices, thanks to its enhanced resolution and sensitivity (Li et al. 2021). Ion mobility spectrometry (IMS) is a potent analytical tool, and when combined with gas chromatography (GC-IMS), it serves as a rapid approach for profiling VOCs in food (Li et al. 2021). Headspace solid-phase microextraction paired with gas chromatography–mass spectrometry (HS-SPME/GC-MS) emerges as a promising approach for distinguishing between different meat species (Pavlidis et al. 2019). To enhance the volatility and thermal stability of these analytes, strategies such as methoximation and trimethylsilylation are frequently employed in large-scale metabolomics research, facilitating their analysis via GC-MS (Atapattu and Temerdashev 2023; Fiehn 2016). The most advantage of the GC-MS is its high sensitivity in identifying the volatile metabolites that are crucial factors for food flavor evaluation.

LC-MS has become a predominant tool in identifying food metabolites, owing to its high-resolution molecular mass determination and detailed fragmentation patterns observed in MS/MS spectra, thereby facilitating the analysis of complex mixtures with unparalleled precision (Lu et al. 2008). In LC-MS-based metabolomic studies, samples can be directly analyzed with minimal preparation, often requiring only filtration.

HPLC-UV is an analytical technique combining high-pressure liquid chromatography with UV light detection (Amarnath et al. 2003). In metabolomics, HPLC-UV is utilized to separate, identify, and quantify metabolites in complex biological samples based on their absorption of UV light (Amarnath et al. 2003). This method is especially useful for analyzing compounds with known UV absorbance characteristics, making it valuable for targeted metabolite analysis and contributing to the comprehensive profiling of metabolomes in dairy and meat products (Herzallah 2009; Korchazhkina et al. 2006; Zergiebel et al. 2023).

NMR spectroscopy is a high-performance tool for the analysis of metabolome and organic compounds, which has been successfully utilized in milk and meat (Klein et al. 2012; Zhu et al. 2020). NMR stands out in the realm of metabolomics for offering a wider spectrum of profiling data, coupled with the benefits of straightforward sample preparation and relatively swift analysis. NMR spectroscopy offers a distinct advantage over LC-MS and GC-MS: a direct and quantitative correlation exists between molar concentration and the intensity of NMR resonances (Tenori et al. 2018). On the other hand, compared with other MS-based analytical techniques, NMR requires simple sample pretreatment, which is time saving and NMR analysis is environmentally friendlier due to its reduced consumption of organic solvents. However, low resolution and sensitivity hinder its utilization in identifying novel compounds and in foodomics. The 1H-NMR metabolomic approach has been successfully applied to study the potential biomarkers of different diet (Madrid-Gambin et al. 2018), geographical origin (Jung et al. 2010), and in detection of the adulteration in

Table 1. Metabolomics techniques used in metabolomics studies

Technique	Application	Advantages	Disadvantages	Specific uses
GC-MS	Detecting volatile organic compounds (VOCs)	High efficiency, reproducibility, reliable, selective, strong sensitivity, highly repeatable fragmentation	Requires complex sample processing for non-volatiles	Indicating meat flavor through volatilomics; studying complex food matrices
LC-MS	Food metabolites identification	High resolution of molecular mass, direct analysis without extensive pretreatment	Requires significant sample preparation, is subject to ionization and matrix effects, and demands expert data analysis and regular maintenance	Analyzing a large number of metabolites; identifying lipids and bioactive compounds
NMR spectroscopy	Analysis of metabolome and organic compounds	Broad profiling information, simple sample pretreatment, rapid detection, high repeatability, nondestructive	Low resolution and sensitivity	Studying potential biomarkers of diet, geographical origin, detecting adulteration in meat
HPLC-UV	Separating, identifying and quantifying metabolites based on UV light absorption	Useful for analyzing compounds with known UV absorbance, contributes to comprehensive profiling	Limited to UV-absorbing compounds	Targeted metabolite analysis in dairy and meat products
GC × GC-ToF-MS	Studying various complex food matrices	Enhanced resolution and sensitivity, ability to analyze complex mixtures, structured chromatograms and faster analysis times	More complex and expensive than single-dimensional GC-MS, requires expert operation and interpretation	Detailed profiling of volatile and semi-volatile compounds, differentiating chemically similar species, environmental analysis, food flavor and fragrance chemistry
IMS	Already noted as profiling VOCs of food	Rapid analysis times, operates at atmospheric pressure, can be coupled with other mass spectrometry techniques for enhanced selectivity	Limited resolution compared to other mass spectrometry techniques, sensitivity to moisture and temperature	Fast method to profile VOCs
HS-SPME/GC–MS	Meat species discrimination	Allows for the concentration and analysis of volatile compounds from complex matrices without solvent use, making it environmentally friendly and sensitive	Tedious sample processing and derivatization required	Analyzing volatile flavor compounds in foods and beverages, environmental monitoring, forensic applications
Combined GC-MS and LC-MS	Enhancing metabolite detection and accurate identification	Complementary strengths of both techniques	Requires access to multiple analytical platforms	Increasing the number of identified metabolites, enhancing phenotype-related metabolite identification

Chicken, Chevon, Beef and Donkey meat (Akhtar et al. 2021). Recently, three quantitative NMR metabolomics analysis methods (ultrafiltration, solvent precipitation with either acetonitrile/acetone/methanol or chloroform/methanol) with excellent protein removal, high concentrations of metabolites and high reproducibility are recommended to be used for lamb meat metabolome analysis (Samuelsson et al. 2021).

The choice of technique depends on the study's goals: GC-MS is preferred for analyzing volatile compounds, LC-MS for complex mixtures with high precision, NMR for broader profiling with simpler sample preparation, and HPLC-UV for targeted analysis of UV-absorbing compounds (Table 2). Combining these techniques can improve metabolome coverage and enhance the identification of metabolites, providing a more complete understanding of the biochemical processes under study. The integration of GC-MS, LC-MS, NMR, and HPLC-UV allows researchers to capitalize on the strengths of each method, thereby increasing the breadth and depth of metabolite detection and characterization, ultimately yielding a more comprehensive metabolic profile.

Nowadays, relying solely on a single platform poses significant challenges in identifying compounds, and the accuracy of data prediction is also limited. Combining GC-MS and LC-MS techniques is common in metabolomics studies to broaden the scope of detected metabolites. This integration aims to improve the accuracy, precision, and comprehensiveness of identifying phenotype-related metabolites (Zeki et al. 2020). These three methods collectively identified 353 metabolites, with only 65 detected by the GC-MS component. This underscores how employing diverse instruments in metabolomic profiling can augment the number of identified metabolites (Simon-Manso et al. 2013). A comprehensive milk metabolome profiling was conducted, employing chemical isotope labeling and LC-MS techniques. Specifically, dansylation labeling was utilized to target the amine/phenol submetabolome, allowing for a focused analysis of these compounds, illustrating the complexity of the milk metabolome (Mung and Li 2017).

Targeted and untargeted approaches

In metabolomic approaches utilizing GC-MS and LC-MS platforms, it is crucial to delineate between targeted and untargeted methods. Targeted approaches focus on identifying and quantifying a specific set of known metabolites, typically ranging from tens to hundreds. These may include common marker compounds

Table 2. The choice of analytical platform for ruminant foods

Ruminant food type	Preferred platform	Reasons
Milk	NMR	Nondestructive, minimal prep, broad profiling. Suitable for overall quality assessment.
	LC-MS	High precision for nonvolatile metabolites like amino acids and lipids.
	HPLC-UV	Cost-effective for specific UV-absorbing compounds (e.g., vitamins).
Meat	GC-MS	High sensitivity for volatile flavor compounds, important for aroma evaluation.
	LC-MS	Comprehensive analysis of nonvolatile compounds, ideal for nutritional profiling.
	NMR	General metabolic profiling, useful for comparisons. Limited by low sensitivity.
Fermented milk (cheese)	GC-MS	Ideal for detecting volatile fermentation by-products contributing to flavor.
	LC-MS	Suitable for a wide range of fermentation metabolites (organic acids, vitamins).
	NMR	Nondestructive, good for overall profiling and comparison. Limited in detecting low-abundance compounds.

pivotal in clinical or technological analyses (Chen et al. 2020; Lelli et al. 2021). Conversely, untargeted approaches aim to gather extensive information by annotating metabolites and examining both known and unknown metabolic alterations (Guo and Huan 2020; Lelli et al. 2021).

In untargeted metabolomics studies, two commonly utilized data acquisition methods exist. The first relies on full scan MS-only acquisitions to provide accurate mass measurements for individual molecules (raw mass features), facilitating multivariate statistical calculations. Subsequently, data-dependent acquisition is employed for identification purposes, generating fragmentation patterns for the metabolites with the highest signal intensity (Guo and Huan 2020). Another untargeted metabolomics strategy involves data independent acquisition, which integrates full scan MS-only acquisition with MS/MS fragmentation for all precursor ions, either concurrently or within specific mass ranges (Guo and Huan 2020; Wang et al. 2019a). A previous study systematically evaluated the advantages and disadvantages of targeted and nontargeted metabolomics approach (Lelli et al. 2021). It was due to the lack of standard pure compounds these days; most metabolites cannot be detected using targeted metabolomics. However, unlike targeted metabolomics, nontargeted approaches present the opportunity to discover new biomarkers, albeit with potentially lower robust accuracy due to the risk of false identification of metabolites or bias/signal drift induced by matrix effects.

Targeted metabolomics focuses only on a preselected set of known metabolites, typically ranging from tens to hundreds. This limited scope can be a drawback in food safety monitoring, where unexpected contaminants or novel metabolites may need to be detected. For example, in cases of food adulteration or contamination with unknown toxins, targeted metabolomics may miss important compounds simply because they are not part of the target list (Sarmad et al. 2023). Targeted approaches require standard pure compounds for calibration and identification, which may not always be available, especially for newly emerging contaminants. In food safety, this poses a significant challenge, as many potential contaminants or toxins may lack commercially available standards (Lelli et al. 2021). The inability of targeted metabolomics to identify unknown or emerging contaminants limits its effectiveness in detecting unexpected food safety issues, such as novel pesticide residues or chemical contaminants that may appear due to changes in farming or food processing practices.

Untargeted metabolomics has lower accuracy compared to targeted approaches because of the risk of false identification (Cajka and Fiehn 2016). In food safety monitoring, this lack of robustness can lead to misidentification or the inclusion of false positives, which can complicate or hinder regulatory decision-making (McGrath et al. 2018). Untargeted metabolomics generates large datasets, and interpreting these data can be challenging and time-consuming. Food safety monitoring often requires rapid response, but the complexity of data analysis in untargeted studies can delay actionable outcomes. In untargeted approaches, matrix effects and signal drift can significantly affect the results, making it difficult to achieve consistent quantification across different sample matrices (Watrous et al. 2017). This inconsistency is problematic in food safety monitoring, where reliable, quantitative information is essential for determining the level of risk posed by a contaminant. Unlike targeted metabolomics, untargeted approaches are less effective at accurate quantification of detected metabolites, particularly when the concentrations of specific contaminants are low. For food safety monitoring purposes, precise quantification is crucial for determining whether contaminant levels exceed regulatory thresholds.

Targeted metabolomics is always used for the detection of preselected set of known metabolites, typically ranging from tens to hundreds. However, in food safety monitoring, the unexpected contaminants or novel metabolites may need to be detected (Sarmad et al. 2023) as many potential contaminants or toxins may lack commercially available standards (Lelli et al. 2021). In the meantime, untargeted metabolomics has much more metabolites than can be detected but some of them with lower accuracy compared to targeted approaches because of the risk of false identification (Cajka and Fiehn 2016). In untargeted approaches, matrix effects and signal drift can significantly affect the results, making it difficult to achieve consistent quantification across different sample matrices (Watrous et al. 2017). Thus, these two methods can be combined to mitigate the drawbacks.

Metabolome database

Nowadays, Human Metabolome Database (HMDB) (http://www.hmdb.ca/) (Wishart et al. 2022) and Bovine Metabolome Database (BMDB) (Foroutan et al. 2020) represent two of the most comprehensive databases to work on metabolomics in ruminants biology. PubChem compounds of NCBI and KEGG COMPOUND also provide a reference metabolite in further details. An online database (http://www.lmdb.ca) includes data on the analytical platform(s), experimental conditions, field of research, and animal breed used in acquiring the metabolomic data (Table 3). To enhance consistency, concentrations of all metabolites with quantitative data were converted into a standardized unit, such as μM. Currently, five levels of confidence in identification have been established with the highest confidence of validated identification (Level 1), a putative identification

Table 3. Metabolome database used in metabolomics studies

Database	Focus Area	Features
Human Metabolome Database (HMDB)	Human metabolomics	Comprehensive data on human metabolites, including structure, function and concentrations.
Bovine Metabolome Database (BMDB)	Ruminant biology, specifically cattle	Detailed information on bovine metabolites for research in ruminant biology.
PubChem Compounds of NCBI	General reference for chemical compounds	Extensive database of chemical molecules and their biological activities.
KEGG COMPOUND	Biochemical compounds involved in metabolic pathways	Offers detailed biochemical pathways and molecular interaction networks.
Livestock Metabolome Database (LMDB)	Livestock metabolomics	Includes data on analytical platforms, experimental conditions and animal breeds in metabolomic studies.

(Level 2), preliminary identifications (Level 3), molecular formula candidates (Level 4), and de-convoluted experimental *m*/*z* features (Level 5) (Rocchetti and O'Callaghan 2021). Moreover, a review of livestock metabolomic studies encompassing cattle, sheep, goats, horses, and pigs detected and/or quantified a total of 1070 metabolites (Goldansaz et al. 2017).

Ruminant milk and dairy product

Classification and function of milk

Milk, an important biofluid of animals, is often called the "perfect food," rich in key nutrients such as proteins (Albenzio et al. 2016). Milk stands as one of the most extensively consumed beverages globally, with 927 million tons produced in 2023 (OECD/FAO 2024). Comprising primarily water (85–87%), bovine milk also contains fats (3.8–5.5%), proteins (2.9–3.5%), and carbohydrates (5%) at the macronutrient level. Additionally, it harbors various bioactive compounds such as vitamins, minerals, biogenic amines, organic acids, nucleotides, oligosaccharides, and immunoglobulins at the micronutrient level (Foroutan et al. 2019).

Global milk production and commercial milk products is dominated by five ruminant species with 81% of total milk production coming from cows, followed by buffaloes with 15%, 4% for goat, sheep and camel milk combined (OECD/FAO 2024). Dairy cow milk is the most prevalent choice among consumers, whereas sheep, goat, and camel milk are significantly rarer in the market (Ahamad et al. 2017; Akhtar et al. 2021; Caboni et al. 2019; Foroutan et al. 2019). Thus, we can refer to these as ruminant milk products. Bovine milk, a biofluid rich in nutrients and chemically intricate, encompasses a multitude of diverse components. The hydrolytic AA content was found to be greater in bovine colostrum compared to human colostrum, suggesting a need for further investigation into AA metabolomics and its implications for infant formula development (Liang et al. 2018). A Web-accessible database called the Milk Composition Database (MCDB, http://www.mcdb.ca/) was constructed based on 2355 identified metabolites in bovine milk (Foroutan et al. 2019).

Factors affect milk metabolome

Milk and milk products are globally consumed and renowned for their nutritional richness. Consequently, safeguarding their nutritional quality and ensuring product safety have emerged as paramount concerns in ruminant food research. Metabolites serve as indicators of milk and milk products' quality, encompassing aspects such as nutritional value, authenticity, and safety (Suh 2022). Till now, several metabolites such as choline, citrate, valine, hippuric acid, 2-butanone, lactate and some FAs have been used as robust biomarkers for milk quality, traceability and safety studies (Zhu et al. 2021b). Milk metabolome studied is not only a sign of milk quality but also a metabolic indicator of animal performance (Sun et al. 2017, 2015). It was commonly known that milk composition varies with the cattle breed (i.e., Holstein, Jersey, Brown Swiss, etc.), stage of lactation, level of parity, number of viable pregnancies, and processing after milk collection (Boudonck et al. 2009; Yang et al. 2016). The fluctuations in milk metabolites are influenced by various factors such as dietary nutrition, genetics, dairy animal species, lactation stage, as well as external factors like season, geographic origin, disease, and processing and storage conditions (Fig. 1) (Goetsch et al. 2011; Klein et al. 2010). Following metabolomic analyses, potential ruminant milk biomarkers indicative of these factors was summarized (Table 4).

Feed factor

To enhance the health attributes of dairy products, dietary feed regulation has been made to increase their contents of unsaturated FA (UFA), especially that of omega-3 polyunsaturated fatty acids (*n*3-PUFA). Additionally, rumen protection techniques are applied to ensure that PUFAs are not degraded by rumen microbes, thereby validating the effects of dietary supplementation (Lanier and Corl 2015). Untargeted metabolomics was used to investigate that both feed-derived (such as phenolic metabolites) and animal-derived compounds (such as FAs) are potential biomarkers associated with dairy cows fed different feeding regimens (Rocchetti et al. 2020). Validation of these biomarkers involves using standardized analytical methods such as ultra-high-performance liquid chromatography–high-resolution mass spectrometry (UHPLC-HRMS) to ensure reproducibility and accuracy (Rocchetti et al. 2020). Alternatively, grazing has been found to increase the content of *n*3-PUFA in goat milk. Controlled feeding comparisons and the application of consistent analytical platforms such as LC-MS have been used to validate these observations, ensuring the robustness of biomarkers like *n*3-PUFA (Argov-Argaman et al. 2021). Milk sourced from animals grazing on pasture is commonly (Hadaya et al. 2020), albeit not always, considered healthier. Biomarker validation here involves repeated studies to confirm the effect of grazing on milk FA content, with standardization achieved by using consistent sampling and analysis methods, such as GC-MS and LC-MS. In addition, many plants or plant extracts were shown to potentially change the milk flavor or taste by upregulate or downregulate milk-specific metabolites, such as essential oils (Sundekilde et al. 2015), purple perilla leaf (Wang et al. 2021a), berry extracts (Prestel et al. 2020), and bamboo leaf extract (Zhan et al. 2021). For instance, using LC-MS/MS, supplementation of *Perilla frutescens* leaf could potentially modify the milk metabolome with elevated levels of oleanolic acid, nucleotides, PE-NMe (18:1(9Z)/18:1(9Z)), and DG (18:0/20:4(5Z,8Z,11Z,14Z)/0:0), influencing pathways like pyrimidine metabolism and the biosynthesis of UFA in dairy cows (Wang et al. 2021a). The validation of these biomarkers is

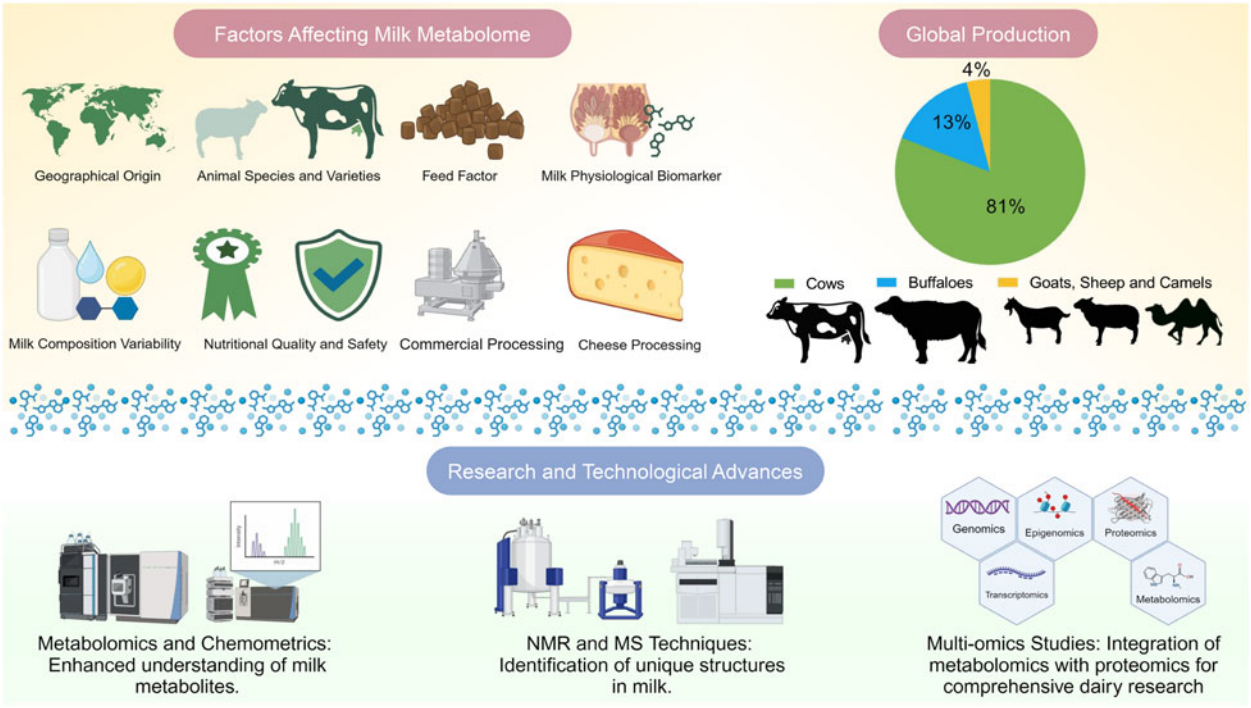

Figure 1. Overview of ruminant milk and dairy product classification and factors influencing the milk metabolome. (Created in BioRender. Zhang, B. (2024) BioRender.com/s41o643).

performed through controlled supplementation trials, followed by standardized metabolomic analysis using LC-MS/MS to ensure reproducibility. Organic bovine milk exhibits a significantly higher concentration of beneficial FAs, including conjugated (9-cis,11-trans)18:2 linoleic acid (CLA), α-linolenic acid, linoleic acid, and total UFA, alongside a reduction in caproleic acid levels. The validation process for these biomarkers includes multivariate analysis, such as principal component analysis, partial least squares discriminant analysis, and receiver operating characteristic analysis, applied to NMR metabolomics data, which allows for consistent differentiation between organic and conventional milk profiles (Tsiafoulis et al. 2019). These distinctions were revealed through the application of 1H-NMR and 1D TOCSY NMR techniques, highlighting the impact of agricultural practices on the nutritional composition of milk.

Animal species and varieties

The dairy animals (cows, buffaloes, sheep, goats, camel) have their respective share (81% from cow, 15% from buffaloes and the rest of 4% from goat, sheep and camel) in overall milk yield of the world and an increase has been seen over years (Bittante et al. 2022). Bovine milk products are the main predominantly probiotic carrier in dairy foods (Ranadheera et al. 2017). The consumption of milk and dairy products obtained from sheep and goat is expected to increase by 26% and 53% respectively until 2030 (Bittante et al. 2022; Pulina et al. 2018). By integrating NMR with chemometrics, researchers identified 10 metabolites – carnitine, *N*-acetylcarbohydrates, acetate, choline, ethanolamine, citrate, creatine, lecithin, D-lactose, and D-sucrose – as reliable markers for detecting milk adulteration. This approach enhances the ability to safeguard the integrity and authenticity of milk product (Li et al. 2017a). Yak milk has a richer composition than Holstein milk, with higher levels of fat, protein, solids-not-fat, and calcium. Metabolomics has been applied to explore these differences, demonstrating yak milk's potential for specialized dairy products like cheese (Zhang et al. 2020b). Traditional fermented yak milk, produced by Tibetan herders in Gannan, contains a diverse microbial community, including *Streptococcus salivarius, Lactobacillus helveticus*, and *Kluyveromyces marxianus*. Metabolomics helps link these microbes to flavor compounds, with O2PLS analysis identifying key bacterial and fungal genera contributing to flavor. This insight supports the development of traditional fermented yak milk products with enhanced flavor profiles (Li et al. 2024a). Yak milk from the Tibetan Plateau is known for its high nutritional value, containing high protein, fat, lactose, and bioactive components than cow milk. Metabolomics has identified essential AAs, UFAs, and bioactive peptides, which contribute to antioxidant and immune-boosting effects. These qualities make yak milk ideal for functional dairy products that promote health benefits such as anti-fatigue and hypoxia resistance, particularly useful in high-altitude regions (Li et al. 2023). Recent metabolomic research has highlighted the unique nutritional profiles of different animal species and varieties of milk. Yak colostrum contains high levels of inositol, glycine, and carnitine, along with a favorable essential AAs to total AA ratio, contributing to its superior nutritional value (Zhang et al. 2024b). Yak milk also has elevated levels of creatine, lipoprotein lipase, and specific bioactive proteins that reflect its adaptation to high-altitude environments, making it particularly rich in health-promoting compounds (Li et al. 2024b). Buffalo colostrum is rich in primary bile acids and bioactive peptides, enhancing its medicinal properties, such as antihypertensive, antioxidant, and anti-inflammatory effects, while supporting the survival of probiotic bacteria in fermented dairy products (Li et al. 2024b; Zhang et al. 2024b). In comparison, cow milk is characterized by higher concentrations of iminostilbene and osteopontin, which support bone health and immune function, though it has lower concentrations

Table 4. Summary of screened potential biomarkers for ruminant milk are shown when available

Item	Target	Metabolites	Pathways	Platform	Source
Breed					
Goat, cow, soy	Raw milk	Rich in short- and medium-chain fatty acids (MCFA), USFA, ω-6 FA, ω-3 FA, EPA and DHA of goat milkrich in Cer, TG and DG of cow milk rich in phospholipids of soymilk		UPLC-Q-Exactive Orbitrap Mass Spectrometry based lipidomics	(Li et al. 2017b)
Yak	Raw milk	Rich in protein, fat, lactose and bioactive components such as essential amino acids, CLA, EPA, DHA		UPLC-Q-Exactive Orbitrap Mass Spectrometry based lipidomics	(Li et al. 2023)
Yak	Raw milk	High levels of fat, protein, solids-not-fat, calcium, larger casein micelles		Dynamic Light Scattering (DLS), Optical Microrheology Analysis	(Zhang et al. 2020b)
Yak	Fermented milk	17 amino acids, 52 volatile compounds (including ketones, esters, aldehydes, alcohols, alkenes, fatty acids and others), higher levels of lactic acid, minerals and vitamins B and C	Glycolysis, proteolysis, lipolysis, KEGG pathways	Gas chromatography with ion mobility spectrometry (GC-IMS), liquid chromatography mass spectrometry (LC-MS)	(Li et al. 2024a)
Sheep, goat	Raw milk	Sheep's milk exhibited higher abundance of arabitol, citric acid, α-ketoglutaric acid, glyceric acid, myo-inositol and glycine. Conversely, goat's milk displayed elevated levels of mannose-6-phosphate, isomaltulose, valine, pyroglutamic acid, leucine and fucose		GC-MS	(Caboni et al. 2019)
Goat, cow	Raw milk	Valine and glycine were found exclusively in goat milk, while talose and malic acid were unique to cow milk		Gas chromatograph–mass spectrometry (GC-MS)	(Scano et al. 2014)
Milk adulteration	Soymilk, goat milk, bovine milk	D-lactose, D-sucrose, choline, citrate, lecithin, ethanolamine, *N*-acetylcarbohydrates, acetate, creatine and carnitine		Nuclear magnetic resonance (NMR)	(Li et al. 2017a)
Camel, human, bovine	Raw milk	Human milk is rich in TGs containing LA, SM containing ultra-long-chain FAs and PLs containing ARA/DHA/DGLACaprine milk is rich in PLs, including HexCer, Hex2Cer, SM, Cer and PC. Bovine milk is rich in PC and CL.		UHPLC-Q-TOF-MS-based lipidomics	(Wang et al. 2020)
Camel vs. bovine	Fermented milk	The bioaccessible fraction of fermented camel milk displayed enhanced biological functionality in comparison to fermented bovine milk		Ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight (UPLC-QTOF)	(Ayyash et al. 2021)
Italian buffalo vs. cow mozzarella	Cheese	Italian buffalo mozzarella cheese were higher in threonine, serine, valine, and lower in orotic acid and urea		GC-MS	(Pisano et al. 2016)
Feed					
Different feeding regimens	Cow milk	Feed-derived (such as phenolic metabolites) but also animal-derived compounds (such as fatty acids) are the potential biomarkers		Ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS)	(Rocchetti et al. 2020)
Grazing vs. confined	Goat milk	Grazing increased omega 3 fatty acid and phospholipid		Gas chromatograph + HPLC combined with an evaporative light-scattering detector	(Argov-Argaman et al. 2021)

(*Continued*)

Table 4. (*Continued.*)

Item	Target	Metabolites	Pathways	Platform	Source
Essential oils from caraway	Cow milk	Increased creatinine, choline, omega citrate, decreased *N*-acetyl hexosamine, glutamate, carnitine and hippurate		NMR spectrometry	(Sundekilde et al. 2015)
Purple perilla leaf	Cow milk	More PE-NMe (18:1(9Z)/18:1(9Z)) and DG (18:0/20:4(5Z,8Z,11Z,14Z)/0:0), oleanolic acid and nucleotides	Pyrimidine metabolism and biosynthesis of unsaturated fatty acids	UHPLC-QTOF-MS	(Wang et al. 2021a)
Berry extracts	UHT cow milk	Reduced Maillard reaction product *N* ε-(carboxymethyl)-L-lysine during UHT processing		GC-MS	(Prestel et al. 2020)
Bamboo leaf extract	Cow milk	Upregulated glycerophospholipids and fatty acyls, and downregulated moracetin, sphinganine and lactulose	Sphingolipid signaling, glycerophospholipid metabolism, sphingolipid metabolism and necroptosis	LC-MS	(Zhan et al. 2021)
Organic vs. conventional	Bovine milk	Increased content of caproleic acid, α-linolenic acid, linoleic acid, conjugated (9-cis,11-trans)18:2 lLnoleic acid (CLA), total unsaturated fatty acids (UFA), allylic protons and decreased content for unsaturated fatty acids		1H-NMR and 1D TOCSY NMR methods	(Tsiafoulis et al. 2019)
Geographical origin	Goat milk	38 and 19 lipid molecules		UPLC-Q-Exactive Orbitrap MS	(Liu et al. 2020)
Processing					
Pasteurized vs. UHT	Goat and cow milk	Hydroxyglutaric acid		GC-MS	(Scano et al. 2014)
UHT vs. reconstituted milk	Commercial milk purchased from supermarkets	60 marker metabolites from three categories of peptides, lipids and nucleic acids		UHPLC-QTOF-MS	(Tan et al. 2021)
UHT milk vs. raw milk vs. pasteurized milk		2-Hydroxymyristic acid, 3-hydroxytetradecanoic acid, 3-hydroxyhexadecanoic acid, 5-hydroxyeicosatetraenoic acid, 7 oxylipids (9-hydroxydecanoic acid, 12-hydroxydodecanoic acid and 10-hydroxyoctadecanoic)		UHPLC-QTOF-MS	(Zhang et al. 2018)
UHT and reconstituted milk	Bovine milk	L-carnitine, succinate and acetate		NMR	(Cui et al. 2019)
Yoghurt	Brown goat milk	Organic acid, peptide and medium- and long-chain fatty acid contents increased		UPLC-Quadrupole-Orbitrap HRMS based lipidomics	(Jia et al. 2021b)
rRnd inclusion	Cheese	2-Hydroxyadenine and argininic acid and 5-hydroxyindole acetaldehyde		Untargeted metabolomics analysis based on UHPLC-Orbitrap-HRMS and Peptidomics profiling by UHPLC-QTOF-HRMS	(Rocchetti et al. 2021)
Both ripening time and anomalous rind inclusion	Cheese	Medium-chain aldehyde 4-hydroperoxy-2-nonenal		Untargeted metabolomics analysis based on UHPLC-Orbitrap-HRMS and Peptidomics profiling by UHPLC-QTOF-HRMS	(Rocchetti et al. 2021)

of functional components compared to yak and buffalo milk (Li et al. 2024b). These insights emphasize the distinct health benefits of each milk type, offering potential for developing specialized dairy products that cater to various health needs, including infant nutrition and functional foods.

The production of various milk and dairy products from goat (*Capra hircus*) and sheep (*Ovis aries*) milk is on the rise, though their global market share remains significantly smaller in comparison to cow and buffalo milk. In certain regions, the issue of a "goaty" or "mutton" taste in milk products may arise, affecting consumer preference and acceptance (Pandya and Ghodke 2007; Watkins et al. 2021). However, goat and sheep milk and related dairy products have gained increasing attention from both consumers and the industry due to their superior digestibility and higher concentrations of bioactive substances compared to cow or human milk. Studies on goat milk, particularly involving infant formula, were based on populations that included 62 infants in a randomized controlled trial, 200 infants in a 12-month study, and 79 infants in another trial, demonstrating the nutritional adequacy of goat milk formula compared to cow milk formula (Prosser 2021). Sheep milk studies included various experimental models, including in vivo trials using Wistar rats to evaluate the health benefits of fermented sheep milk products, highlighting its anticancer, gastrointestinal health, and other health-promoting properties (Flis and Molik 2021; Prosser 2021). Research has identified that valine and glycine are unique to goat milk, whereas talose and malic acid are distinctive markers of cow milk (Scano et al. 2014). For instance, the goat milk not only has more digestible proteins and fats but contains higher contents of short- and medium-chain FAs (MCFA), UFA, *n*6 FA, *n*3 FA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than cow milk, which results in increasing particular interest by consumers, especially to infants and elder people (Li et al. 2017b). In the comparison between sheep and goat milk, through a GC-MS-based metabolomics approach, it was discovered that arabitol, citric acid, α-ketoglutaric acid, glyceric acid, myo-inositol, and glycine are predominantly found in sheep's milk. Conversely, goat's milk exhibited elevated levels of mannose-6-phosphate, isomaltulose, valine, pyroglutamic acid, leucine, and fucose, highlighting the distinct metabolic profiles between these two types of milk (Caboni et al. 2019), but there is still a lack of whole picture regarding the goat and sheep milk metabolite profiles as the animal varieties (Caboni et al. 2019). Camel milk is celebrated for its nutritional richness, containing all essential nutrients alongside compounds that may possess anticarcinogenic, antihypertensive, antioxidant, hypoallergenic, and cholesterol-lowering properties, making it a unique and beneficial addition to the diet (Buchilina and Aryana 2021). Camel milk is a specific local food used in some countries and regions of Southeast Asian, Middle East, and African continent, showing with immunomodulatory effects and good for human health, and is easily digested and well tolerated by lactose intolerance people (Ahamad et al. 2017; Al-Awadi and Srikumar 2001). The bioaccessible fraction of fermented camel milk has been shown to possess enhanced biological functionality compared to that of fermented bovine milk, highlighting its superior nutritional and health-promoting qualities (Ayyash et al. 2021).

The lipidome and FA composition in human, bovine and caprine milk were analyzed and compared using UHPLC-QTOF-MS and GC–MS. Human milk is rich in triglycerides (TG) containing linoleic acid (C18:2), sphingomyelin (SM) containing ultra-long-chain FAs and phospholipids (PLs) containing arachidonic acid (ARA)/DHA/ dihomo-γ-linolenic acid (DGLA). Caprine milk is rich in PLs, including hexosylceramide (Hexcer), Hex2Cer, SM, ceramide (Cer), and phosphatidylcholine (PC). Bovine milk is rich in PC and CL. The detailed examination of the lipid profiles of Chinese human, bovine, and caprine milk contributed valuable insights that could assist in formulating infant nutrition that is more precisely tailored to meet the dietary needs of Chinese babies (Wang et al. 2020). These findings have significant real-world applications beyond infant formula production. Understanding the unique lipid and FA profiles in these different milk types allows for better nutritional interventions for specific populations, such as elderly individuals or those with particular dietary requirements (Mollica et al. 2021). By leveraging the specific bioactive components found in caprine and bovine milk, it is possible to develop specialized nutritional products that address deficiencies in essential FAs or PLs, enhance cognitive function or support immune health (Eggersdorfer et al. 2022). The identification of FA like DHA and ARA, which are crucial for neural and visual development, is particularly useful for designing targeted nutritional supplements and functional foods (Fan et al. 2024). Furthermore, these insights can be applied to improve the nutritional quality of dairy products aimed at supporting growth and development in young children, as well as enhancing the health benefits of dairy-based functional foods for adults.

Geographical origin

The NMR metabolomic technique has been validated as an effective method for determining the origin of authentic products, demonstrating its applicability across both narrowly defined geographic regions, such as the Mugello valley, and broader areas encompassing large-scale distribution networks (Rocchetti and O'Callaghan 2021; Tenori et al. 2018). Using an untargeted approach that combines UPLC-Q-Exactive Orbitrap MS with multivariate statistical analysis, researchers identified 38 lipid molecules as potential indicators for determining the geographical origins of goat milk, and 19 lipid molecules for discerning its lactation stages, showcasing the method's precision in tracing the provenance and physiological status of goat milk (Liu et al. 2020).

Commercial processing

Metabolomics is useful to evaluate changes caused by food processing and can be seen as a crucial tool to support academia and industry on revealing the transformation of raw animal materials into ready-to-eat products (Utpott et al. 2022). The manufacturing and processing of milk commercially is for pasteurized beverage, Ultra-high-temperature (UHT), evaporated milk, ice cream, butter, milk powder and cheese, whey protein concentrate, paneer, ghee, traditional milk products, even soaps, lotions, and sweets, besides the popular cheeses and yoghurt (Pandya and Ghodke 2007). Fermentation and thermal processing enhance the flavor and texture of dairy foods, making them more appealing (Dos Santos Rocha et al. 2022). Hydroxyglutaric acid is a biomarker and unique metabolite of pasteurized goat or cow milk (Scano et al. 2014). In recent years, there are 60 marker metabolites from three categories of peptides, lipids, and nucleic acids that were detected for distinguishing between UHT and reconstituted milk by UPLC-Q-TOF-MS (Tan et al. 2021). Applying the same technique, researchers identified seven oxylipids – 9-hydroxydecanoic acid, 12-hydroxydodecanoic acid, 2-hydroxymyristic acid, 3-hydroxytetradecanoic acid, 5-hydroxyeicosatetraenoic acid, 3-hydroxyhexadecanoic acid, and 10-hydroxyoctadecanoic acid – as effective markers for differentiating UHT milk from raw and pasteurized varieties (Zhang et al. 2018). Furthermore, L-carnitine,

succinate, and acetate were pinpointed as biomarkers to differentiate UHT and reconstituted milk, based on comparisons with standard NMR-spectra databases (Cui et al. 2019). Post-pasteurization, the percentage of SM in milk saw an increase, whereas fermentation into yogurt did not affect its levels (Argov-Argaman et al. 2021). Following the fermentation of brown goat milk, there was a notable increase in the contents of organic acids, peptides, medium- and long-chain FAs, and heterocyclic compounds through a comprehensive approach that integrated lipidomics and metabolomics (Jia et al. 2021b).

Cheese, a fermented dairy delight, hosts a variety of microbial communities that evolve over time and differ based on the cheese variety and the specific starter and adjunct cultures used in its production. The milk of all ruminant species can be used to make cheese, but to improve efficiency, cheese-making procedures need to be optimized to take into account the large differences in their coagulation, curd-firming, and syneresis properties (Bittante et al. 2022). The deployment of metatranscriptomics, metaproteomics, and metabolomics – collectively referred to as "cheesomics" – utilizes a multi-omics approach to enhance our comprehension of cheese's microbial makeup and predict cheese characteristics such as flavor, quality, texture, and safety, as well as uncovering bioactive metabolites that may impact human health (Afshari et al. 2020). However, untargeted metabolomic approach is the most commonly used method to detect the cheese quality. For instance, the chemical fingerprints distinguishing Protected Designation of Origin (PDO) Grana Padano cheeses from non-PDO "Grana-type" cheeses have been established using UHPLC/QTOF-MS. This analysis revealed that lipids (FAs and their derivatives, PLs and monoacylglycerols), AAs, and oligopeptides, along with plant-derived compounds, emerged as the markers with the highest potential for discrimination (Rocchetti et al. 2018). Utilizing a synergistic metabolomics and peptidomics strategy, researchers identified potential markers to detect counterfeit cheeses, particularly those with an excessive rind content (>18%). The compounds 2-hydroxyadenine and argininic acid, along with 5-hydroxyindole acetaldehyde, were pinpointed as the most effective indicators of rind inclusion. Furthermore, the medium-chain aldehyde 4-hydroperoxy-2-nonenal emerged as a common marker indicative of both the cheese's ripening duration and abnormal rind inclusion (Rocchetti et al. 2021). During the Mongolian cheese storage, it was found that the bitter AAs, bitter peptide (Phe-Ile), and organic acids (sinapic acid, butyric acid) increased accompanied with the increased contents of short-chain FAs, 2-undecanone and ethyl esters, which increased the cheese unpleasant smell and decreased the overall acceptability (Zhang et al. 2022). Metabolomic analysis of the commercial processing of milk highlights several significant findings regarding fermentation and co-fermentation, as well as cheese production. co-fermentation with *Bifidobacterium adolescentis* and other probiotics improved the stability of probiotic fermented beverages compared to single-strain fermentation, enhancing levels of health-promoting metabolites such as gamma-aminobutyric acid and L-malic acid even after 30 days of storage (Guo et al. 2024a). In cheese production, various fermentation methods, including the use of probiotics like *Lactobacillus plantarum* and *Lactobacillus helveticus*, have been shown to enhance the production of AAs, Fas, and other bioactive compounds, improving both the nutritional quality and sensory properties of cheese. These probiotics also influence key metabolic pathways related to flavor and bioactivity, leading to health-promoting effects such as anti-inflammatory benefits, thereby providing opportunities for the development of functional dairy products (Ma et al. 2024).Thus, metabolomics is useful for assessing the cheese quality through the changing of small molecule compounds.

Dairy cow physiological condition

Diseases such as mastitis, lameness, and rumen acidosis are very common in dairy cows, beef cattle, sheep, and goat, exerting huge economic loss. Heat stress and mastitis represent significant financial challenges within the dairy industry. The metabolomics approach is helpful for better understanding the pathobiology of these diseases (Xi et al. 2017; Zhu et al. 2021a; Zwierzchowski et al. 2020). The analysis of whole raw milk from Holstein dairy cows affected by lameness, utilizing direct inject/LC-MS and NMR techniques, has proven beneficial for identifying and potentially mitigating lameness-associated pathological processes (Zwierzchowski et al. 2020). Significant variations of metabolome were found between healthy and mastitis cows by a novel metabolomics technique based on LC-MS. Milk arginine and Leu-Leu were increased in both the clinical and subclinical mastitis groups compared to healthy cows, indicating these metabolites were the potential biomarkers (Xi et al. 2017). Furthermore, the enrichment of the tricarboxylic acid cycle and the biosynthesis pathways of phenylalanine, tyrosine, and tryptophan were identified to elucidate the mechanisms behind the variation in the metabolome of mastitic milk, employing an untargeted 1H-NMR approach (Zhu et al. 2021a). NMR-based metabolomics analysis showed an increase in lactate, butyrate, isoleucine, acetate, and β-hydroxybutyrate levels, while levels of inosine and fumarate decreased in milk exhibiting high somatic cell counts (Sundekilde et al. 2013b).

Milk choline, phosphocholine, *N*-acetylcarbohydrates, lactate, and β-hydroxybutyrate have been identified as potential markers of inflammation, exhibiting varying patterns dependent on the ambient temperature (Salama et al. 2020). An integrative metabolomics investigation employing LC-MS and 1H NMR spectroscopy identified several biomarkers in milk – lactate, pyruvate, creatine, acetone, β-hydroxybutyrate, trimethylamine, oleic acid, linoleic acid, lysophosphatidylcholine 16:0, and PC – that effectively indicate heat stress (Tian et al. 2016). Changes in concentrations of individual milk metabolites (volatile metabolites, and nonvolatile metabolites) can be related to the ruminal CH_4 production pathway (van Gastelen et al., 2018). Furthermore, multi-omics studies represent an important gap revealing in livestock research (Sun et al. 2020; Xue et al. 2020, 2022). The combination of metabolomics and proteomics was always used in dairy milk characteristics (Lu et al. 2013, 2015).

Ruminant meat metabolomes

Meat quality is generally focused on the phenotypes, including nutrient contents, meat color, tender, juiciness, etc., which may be dependent on the subjective preference by consumers and have limited information for the food characteristics. However, the meat metabolome could show the basic fingerprint of food and help consumers and health professionals make informed decisions (Fig. 2). Metabolomics, supported by bioinformatics, identifies biomarkers in muscle and meat that enhance animal production and meat quality, benefiting both producers and consumers (Muroya 2023). The breed of the animal, its diet, and even the specific part of the meat influence meat quality and its metabolites. Consequently, potential biomarkers in ruminant meat indicative of these variables have been systematically compiled (Table 5).

The compounds such as *N′*-formylkynurenine, kynurenine, and kynurenic acid (all part of tryptophan metabolism) and the

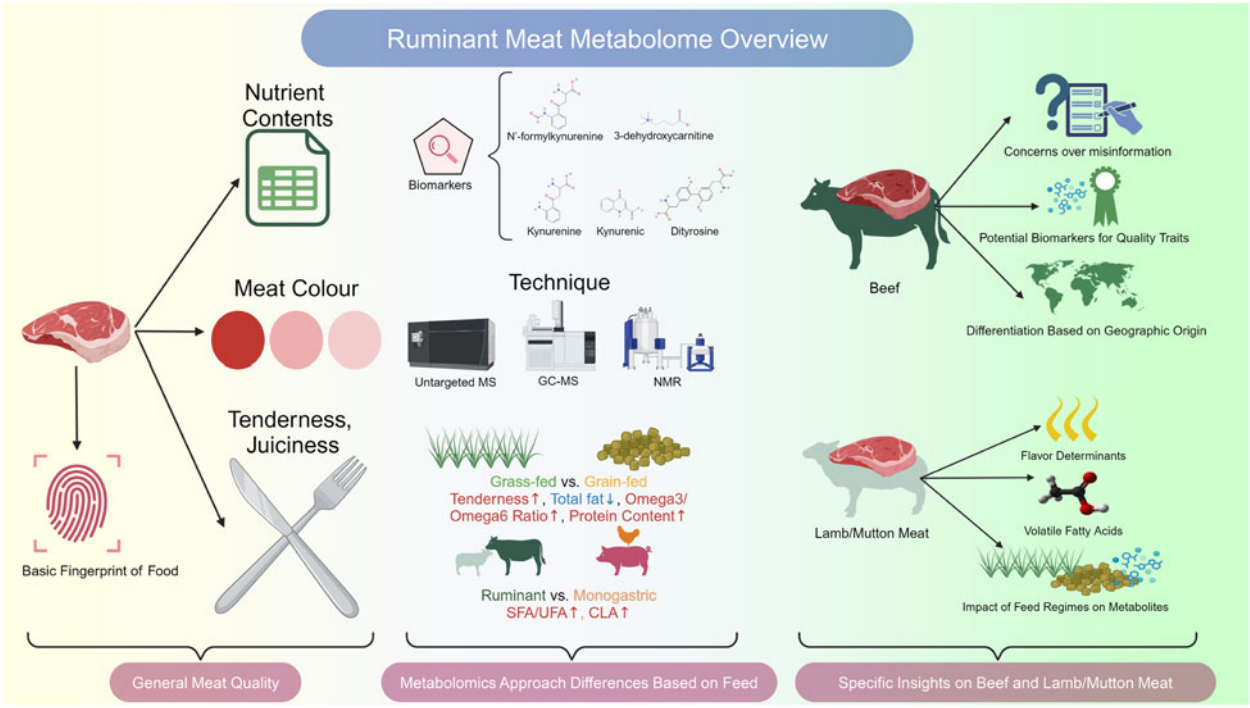

Figure 2. Metabolomic insights into ruminant meat quality, nutritional traits and authenticity assessment. (Created in BioRender. Zhang, B. (2024) BioRender.com/m65h597).

oxidative stress indicator dityrosine and 3-dehydroxycarnitine are mechanistically connected to pathways associated with red meat, distinguishing them from those linked to white meat (Rombouts et al. 2017). Ruminant meat is typically red meat, high in saturated FA (SFA), and red meats from ruminants exhibit a higher SFA to UFA ratio compared to meat products derived from monogastric animals and fish (Vahmani et al. 2015). However, ruminant meat, especially for beef and mutton, is high in CLA. The cis-9, trans-11 isomer stands out as the primary dietary form of CLA present in products from ruminants. This particular isomer is generated through partial biohydrogenation of linoleic acid in the rumen or via endogenous synthesis within the tissues themselves (Webb 2021). Boosting the CLA content in meat could enhance its nutritional and therapeutic benefits. To elevate CLA intake in the human diet, options include either consuming more ruminant-derived foods or increasing the CLA levels in milk and meat products (Dhiman et al. 2005). On the negative outcome side, due to the rumen biohydrogenation, the beef and mutton are always showing lower UFA, especially of PUFA compared to the pork, chicken, and fishes. Nowadays, the enhancement of PUFA, especially for *n*3-PUFA in beef and mutton are mainly through supplementing of *n*3-PUFA enriched feed sources but with relative lower transformation rate, or using rumen protected methods to release the *n*3-PUFA in gut (Ebrahimi et al. 2014). Till now, differences in meat between ruminant animals and monogastric animals have been conducted by GC-MS based metabolomics, such as the comparison between beef and pork (Ueda et al. 2019). However, this study did not use fresh muscle samples. Another limitation is that the animal species and sample number are not enough to show the meat metabolome differences using same analyzed metabolomics method. Future research could address these limitations by using fresh muscle samples to ensure that the metabolomic profiles accurately reflect the native biochemical state of the meat. Moreover, increasing the number of animal species and sample sizes would provide a more comprehensive understanding of metabolome variations. Standardizing the metabolomics protocols across different species would also enhance comparability and reproducibility, leading to more robust conclusions about ruminant and monogastric meat differences.

Furthermore, the mixing of high-price meat species with low-quality/-price meat can be seen in food market or catering industry (Akhtar et al. 2021). The ruminant meat is commonly in higher price than other livestock meat; thus, metabolomics can be used in adulteration of beef or mutton. NMR-based metabolomics has emerged as an effective technique for identifying unique signatures (potential biomarkers) to distinguish meats from different sources. This method holds promise for quality control applications, offering a way to differentiate between meat types – an important factor for ensuring food safety and addressing public health concerns (Akhtar et al. 2021).

Beef

Beef, a staple food in numerous countries, faces increasing scrutiny due to misinformation regarding beef products. Concerns include false claims about origin, species, and production methods, highlighting the need for accurate information and transparency in the beef industry (Man et al. 2021; Visciano and Schirone 2021). Metabolomics has been utilized to explore potential biomarkers associated with meat quality traits, concentrating on factors like the genetic background of the animal, sensory characteristics, feeding systems, and formulations. This research also encompasses processes such as postmortem storage and hygiene management, aiming to comprehensively understand how these elements influence meat quality (Akhtar et al. 2021). The volatile compounds like 2-methylfuran-3-thiol, 3-sulfanylpentan-2-one, furan-2-ylmethanethiol, 2-propylpyrazine, 1-furan-2-ylpropan-2-one, 1H-pyrrole, 2-methylthiophene, and

Table 5. Summary of screened potential biomarkers for ruminant meat are shown when available

Item	Target/Treat	Metabolites	Pathways	Platform	Source
Ruminant meat	Ruminant compared to monogastric animals and fishes	Higher in the ratio of saturated fatty acids (SFA) than unsaturated fatty acids (UFAs); high in conjugated linoleic acid (CLA)			(Ebrahimi et al. 2014; Ueda et al. 2019; Visciano and Schirone 2021)
Beef					
Beef stock	Glutathione-Maillard reaction products	1H-pyrrole, 2-methylthiophene, 2-propylpyrazine, 2-(furan-2-ylmethyldisulfanylmethyl) furan, 2-methylfuran-3-thiol, 3-sulfanylpentan-2-one, furan-2-ylmethanethiol and 1-furan-2-ylpropan-2-one		Gas chromatography–time-of-flight mass spectrometry (GC-TOF/MS)	(Castejón et al. 2015)
Beef	Geographical origins and feeding regimes	Amino acids, several sugar metabolites and several PCs and PEs		UPLC-Orbitrap-MS and GC-MS	(Lee et al. 2011)
Beef	Geographical origin of beef from four countries: Australia, Korea, New Zealand and the United States	Various amino acids and succinate		NMR	(Jung et al. 2010)
Beef, strip loin	Aging type (dry and wet aging)	Lactic acid, alanine, methionine, fumaric acid, inosine, inosine monophosphate, creatine, betaine, carnosine and hypoxanthine		1H-NMR spectroscopy	(Khan et al. 2015)
Marbled beef of Japanese Black cattle	Tissues types (muscle, intramuscular fat and intermuscular fat)	Medium-chain fatty acids were uniquely detected in fat tissue, but decanoic acid, uric acid, elaidic acid and 3-phosphoglyceric acid are potential biomarkers for intramuscular fat to assess marbling levels		Chromatography–mass spectrometry (GC-MS)	(Visciano and Schirone 2021)
Chinese Jinjiang yellow cattle	Longissimuss lumborum vs. psoas major	Pyruvate, and numbers of metabolites involved in tricarboxylic acid cycle	Tricarboxylic acid cycle	UPLC-MS/MS	(Qiu et al. 2012)
Farmed Jiulong yaks	The longissimus thoracis vs. biceps femoris	Higher concentrations of carnosine and formate and lower concentrations of mannose, inosine, threonine, IMP, alanine, valine, isoleucine, tyrosine, phenylalanine and leucine		NMR	(Zhu et al. 2020)
Lamb/mutton					
Hu sheep	Castration	Hypoxanthine metabolites and volatile compounds of 1-octen-3-ol and hexanal		LC-MS and GC-MS	(Cônsolo et al. 2020)
Sunit sheep	Paster	Higher 1-octen-3-ol and 2,3-octanedionone		UPLC-Q-TOF/MS	(Wang et al. 2021b)
Tan sheep	Artificial pasture grazing	Increased *N*-acetyl-L-aspartic acid, *N*-acetylaspartylglutamate, acetylcarnitine and L-carnitine, but decreased carnosine and creatinine	Linoleic acid metabolism	LC-MS	(Hu et al. 2017)
Dorper sheep	Calcium soap of palm fatty acids and prilled fat plus lecithin diets	decreased total cholesterol, esterified cholesterol, choline, glycerophosphocholine and glycerophospholipids		NMR	(Wang et al. 2021d)
Dorper sheep	Calcium soap of palm fatty acids and prilled fat plus lecithin diets	Higher glycerol and sphingomyelin		NMR	(Wang et al. 2021d)
Tan sheep	Boiled approach	More losses of sphingomyelin, less losses of phosphatidylcholine and lysophosphatidylcholine		UPLC-Q-Orbitrap HRMS	(Sivadier et al. 2009)

2-(furan-2-ylmethyldisulfanylmethyl)furan are potential key contributors to beef-related attributes and flavor in glutathione-Maillard reaction products (Lee et al. 2011). Both UPLC-Orbitrap-MS and GC–MS analytical platforms were used and the geographical origins and feeding regimes could be differentiated by the potential biomarkers including AAs, several sugar metabolites, and a number of PCs and phosphatidylethanolamines (PEs), which offers a method to identify the geographical origin of beef

at any point along the supply chain and could be used to develop a verifiable traceability system (Man et al. 2021). Various AAs and succinate emerge as potential biomarkers for discriminating the geographical origin of beef sourced from four countries: Australia, Korea, New Zealand, and the United States (Jung et al. 2010). Utilizing NMR-based metabolomics with High Resolution Magic Angle Spinning (HR-MAS) enables the classification of meat samples based on their storage time (Castejón et al. 2015). This study represents the first metabolomic investigation of Japanese Black cattle using GC/MS analysis, comparing metabolites across different muscle and intramuscular fat (intermuscular fat) in marbled beef. MCFA implicated in triacylglycerol synthesis were exclusively detected in fat tissue. Additionally, decanoic acid, uric acid, elaidic acid, and 3-phosphoglyceric acid emerged as potential biomarkers for IMF, indicative of marbling levels. Notably, decanoic acid and glutamine were identified as potential biomarkers associated with oily flavor, wagyu beef aroma, and overall sensory evaluations (Ueda et al. 2019). Differences in enzymatic activities (lactate dehydrogenase, malate dehydrogenase, and succinate dehydrogenase), pyruvate contents, and the number of metabolites associated with the tricarboxylic acid cycle were observed between *longissimus lumborum* and psoas major muscles from Chinese Jinjiang yellow cattle (Yu et al. 2019). Yak (*Bos grunniens*), a special beef cattle, is mainly located in the Himalayan highlands region (Qiu et al. 2012), the longissimus thoracis from locally farmed Jiulong yaks had higher concentrations of carnosine and formate and lower concentrations of mannose, inosine, threonine, alanine, valine, isoleucine, tyrosine, phenylalanine, and leucine compared to biceps femoris by an untargeted NMR metabolomic approach (Zhu et al. 2020). Beef from grass-fed animals is characterized by tenderness, lower total fat content, a higher omega-3/omega-6 ratio, and superior protein content compared to grain-fed counterparts (Carrillo et al. 2021, 2016). Metabolomics has been extensively used to understand the biochemical changes in beef during processing and storage, with particular emphasis on color stability and tenderness. The metabolomic investigation of fresh beef, lamb, and venison using NMR spectroscopy highlighted the metabolite changes affecting color stability in meat under retail display conditions. It was found that the stability of fresh beef color was greater than that of lamb and venison, with metabolites such as leucine, isoleucine, valine, succinate, inosine monophosphate, and choline playing key roles in these differences (Kanokruangrong et al. 2024). In another study, LC-MS-based metabolomics revealed that superchilling treatment of beef accelerated the degradation of μ-calpain and caspase 3, leading to improved tenderness. The metabolomic profiles of superchilled beef were distinguished from other treatments, indicating changes in pathways such as arginine and proline metabolism, which are associated with tenderness (Chen et al. 2024). Additionally, metabolomics has been employed to investigate the effects of chilling regimes on the metabolome of beef, showing that superchilling improved the tenderness of beef by affecting key metabolic pathways, including ATP and its degradation products, which were influenced during the early post-mortem period (Guo et al. 2024b).

On the other hand, the occurrence of dark cutting meat has a notable impact on meat quality attributes and concentrations of *post-mortem* glycolytic metabolites in Angus Í Nellore crossbreed cattle, which was associated with mitochondrial activity and energetic metabolic pathways (Cônsolo et al. 2021). Significant differences in lactic acid, alanine, methionine, fumaric acid, inosine, inosine monophosphate, creatine, betaine, carnosine, and hypoxanthine were observed based on aging type (dry and wet aging) in the beef metabolome (Bischof et al. 2021).

Lamb/mutton meat

Meat flavor, one of the most important sensory characteristics and main attributes that determines consumers' decisions to purchase a meat, is attributed to some volatile compounds (Khan et al. 2015). In mutton, either from sheep or goat, a specific mutton dodur exists due to the volatile medium- and short-chain FAs such as 4-methyloctanoic acid, 4-ethyloctanoic acid and 4-methylnonanoic acid (Wang et al. 2021c). It was revealed that significant variations in FAs, aldehydes, ketones, lactones, alkaloids, flavonoids, phenolics, and drug residues among three types of goat meat: Lubei white goats, Boer goats, and Jining grey goats. This underscores how untargeted LC-MS can elucidate the subtle differences in flavors and sensory attributes among these varieties (Wang et al. 2019b). Among the lamb breeds studied, (E)-2-hexenal was exclusively detected in Tan lambs, whereas (E)-2-nonenal and (E, E)-2,4-nonadienal were only present in Dorper lambs. Hu lambs exhibited the fewest volatile compounds. Analysis demonstrated that Dorper lambs had a higher proportion of PUFA, AAs, and volatile compounds compared to Tan and Hu lambs. However, specific PUFA derivatives in Dorper lambs were found to have a negative influence on the odor profile (Zhang et al. 2020a). Using lipidomics and targeted metabolomics, hypoxanthine metabolites and volatile compounds of 1-octen-3-ol and hexanal were significantly increased by castration, which might be beneficial in lamb quality (Li et al. 2020). NMR spectroscopy was employed to delve into the confinement odor phenomenon in lamb meat, establishing correlations between this odor and specific meat and drip metabolites. These included tyramine, formate, alanine, carnosine, urea, proline, aspartate, glutathione, and nicotinate, which are substrates or products of glucose fermentation and AA catabolism (Cônsolo et al. 2020). The study on Mongolian sheep highlighted the dynamic changes in metabolites during early postmortem chilled aging, with significant alterations in AAs, fatty acyls, and glycerophospholipids, which are crucial for the flavor and quality of meat. Metabolites like AAs and small peptides accumulated significantly, enhancing flavor through pathways like AA metabolism and protein digestion (Zhang et al. 2024a).

From the view of lipids composition, low IMF leads to the decrease of flavor precursors in lamb (Bravo-Lamas et al. 2018). Lipids such as PC, PE, and TG and their structures with SFA and UFA are critical to the thermal oxidative capacity of glycerol chain-based lipids (Wu and Wang 2019). A validated UPLC-Q-Orbitrap HRMS combined lipid screening strategy method-based lipidomics was used for identification of Tan sheep meat products with different thermal processing methods. For atherosclerosis patients, the boiled cooking method proved to be preferable due to greater losses of SM compared to Cer in meat. Conversely, the steamed approach resulted in fewer losses of PC and lysophosphatidylcholine, indicating that Tan sheep meat prepared in this manner might be more suitable for elderly and infant populations (Jia et al. 2021a).

Volatile biomarkers, including alkanes, ketones, terpenes, and 2,3-octanedione, were found in ruminant tissues could distinguish exclusive pasture diets from exclusive concentrate diets (Sivadier et al. 2009). The feed regimes affect the metabolites found in *longissimus dorsi* muscle of sheep, showing that total cholesterol, esterified cholesterol, choline, glycerophosphocholine, and glycerophospholipids were significantly lower in calcium soap of palm

FAs and prilled fat plus lecithin diets, while glycerol and SM were significantly higher in calcium soap of palm FAs and prilled fat plus lecithin diets (Behan et al. 2021). An untargeted metabolomic and lipidomic method based on UPLC-Q-TOF/MS provided a basis for differentiation of meat from pasture-fed and concentrate-fed sheep/goats (Wang et al. 2021d). It was also found that the contents of 1- octen-3-ol and 2,3-octanedionone in mutton from pasture-fed animals were significantly higher (Wang et al. 2021d). Using both untargeted and targeted metabolomics, the main increased *N*-acetyl-L-aspartic acid, *N*-acetylaspartylglutamate, acetylcarnitine, and L-carnitine, but decreased carnosine and creatinine were the main newly found grazing feeding regime associated metabolites, which might contribute to the improved lamb meat quality under artificial pasture grazing (Wang et al. 2021b). Incorporating *P. frutescens* seeds into Tan lamb diets enhances *n*3-PUFA levels and flavor, marked by increased volatile compounds like acetaldehyde and 1,2,4-trimethyl-benzene. This inclusion boosts beneficial lipids and metabolites such as PG(18:1/18:1), PG(18:2/18:2), and 5′-inosinic acid, while reducing lysophosphatidylcholine, guanidinosuccinic acid, 3-hydroxydecanoic acid, and 2-methylbutyroylcarnitine (Yu et al. 2024). It has been summarized previously that volatile alcohols, like 1-octen-3-ol, and ketones, such as 2-heptanone and 3-hydroxy-2-butanone, contribute to the distinctive aroma of lamb. Studies indicate that compounds like 1-octen-3-ol, which imparts a "mushroom-like" note, are often appreciated by consumers for enhancing the umami aspects of cooked meat, leading to increased consumer preference. The aldehyde content, including hexanal, nonanal and heptanal, contributes significantly to the characteristic mutton odor. Compounds like (E)-2-nonenal and (E)-2-octenal impart fresh, fatty and slightly green aroma notes, which are desirable for a well-rounded lamb flavor, and the 4-methyloctanoic acid and 4-ethyloctanoic acid, which are responsible for the specific "mutton" flavor (Khan et al. 2015). Volatile compounds like phenylacetaldehyde and methional have been noted to provide antioxidant properties, which help in reducing oxidative stress. These antioxidants are significant for maintaining the overall nutritional quality of lamb meat and may contribute to the health benefits of reducing the risks associated with high-fat diets. Castration in lambs has been found to alter the concentration of volatile compounds, like hexanal and 1-octen-3-ol, reducing off-odors and enhancing the flavor profile, which might positively impact consumer preferences while also increasing the levels of beneficial lipids (Li et al. 2020). Such dietary adjustments suggest significant metabolomic advantages and potential for healthier lamb meat production, as analyzed by HD-mix LC-MS/MS for lipid and metabolite profiling.

Metabolomics for ruminant food alternatives

Synthetic/artificial milk

With the gradual increase in world milk consumption and concerns about the safe production of ruminant products under modern intensive farming conditions, some problems caused by efficient intensive farming, such as animal health, environmental pollution, animal welfare, etc. are closely related to "One Health" concept contradicts (Hu et al. 2017). In this context, the new technology of "lab-grown food" "replace an extremely inefficient, input-intensive and waste-producing traditional agricultural sector with precise, targeted and easy-to-process systems" production system by using small land and drastically reduces water and nutrient requirements (Monbiot 2020). To produce ruminant food, the most direct way to think of is to use controllable artificial conditions and the technical basis of laboratory cultivation to synthesize meat, eggs and milk. Synthetic milk has emerged as a new potential alternative to cow's milk, unlike plant-based oat, nut, and soy milks, which are designed to replicate its taste, appearance, and mouthfeel. Synthetic milk may have a smaller carbon footprint than dairy production, cause less pollution, and apparently eliminate animal welfare concerns (George 2023). Here we need enough information to understand the composition of naturally produced or real milk to make synthetic milk closer to natural milk. Metabolomics as a powerful tool can play a crucial role in understanding the real composition of milk for lab-grown milk production. The application of metabolomics has strong advantages in understanding the chemical characteristics and dynamics of dairy products, as well as potential biomarkers for differentiating the consumption of different dairy products and identifying milk quality, traceability and safety (Goldansaz et al. 2017; Zhu et al. 2021b). Applications of NMR-based metabolomics in milk research include linking milk metabolite analysis to nutritional aspects and technical quality of milk. Identification of novel metabolites through metabolomics as biomarkers or bioactive compounds (Sundekilde et al. 2013a). The application of metabolomics can provide a detailed understanding of milk composition, reveal changes in metabolite levels related to lactation, breed, diet, and other factors, and provide more valuable information to produce synthetic milk.

Ruminant meat alternatives

A new wave of plant-based or cell-based meat alternatives, designed to replicate the taste and nutritional profile of red meat, has garnered significant consumer interest, research focus, and media attention. Plant-based diets exclude or substantially limit the consumption of meat and animal products and are of growing interest to many due to their sustainability and health benefits (Rubio et al. 2020; Wang et al. 2021b). Nowadays, the plant-based meat is an artificial processed meat that are in evolution with the developed understanding and upgrading knowledge of meat metabolites and structure (Allen and Locasale 2021). The clearer revealing of the meat metabolome contributes to that the artificial meat is more nearly taste and flavor close to the real animal feeding meat (Fig. 3) (Allen and Locasale 2021; Munekata et al. 2022; van Vliet et al. 2021).

The metabolomics analysis found that metabolite abundances between the plant-based meat alternative and grass-fed ground beef differed by 90%. Nutrients such as DHA, niacinamide (vitamin B3), glucosamine, hydroxyproline, and antioxidants including allantoin, anserine, cysteamine, spermine and squalene was exclusively present in beef. Conversely, ascorbate (vitamin C), phytosterols, and several phenolic antioxidants such as loganin, sulfurol, syringic acid, tyrosol, and vanillic acid were only found in the plant-based meat alternatives (van Vliet et al. 2021). For the future study of ruminant meat, metabolomics in targeting animal blood and meat can be used as a noninvasive technology to prediction of meat quality (Muroya 2023).

Spatial metabolomics and future artificial meat

The development of plant-based and cell-cultured meat has accelerated in recent years due to the advancements in metabolomics, 3D printing, and cell culture technologies. Specifically, 3D printing techniques play a crucial role in the production of ruminant meat alternatives (K. Handral et al. 2022; Rubio et al. 2020). To

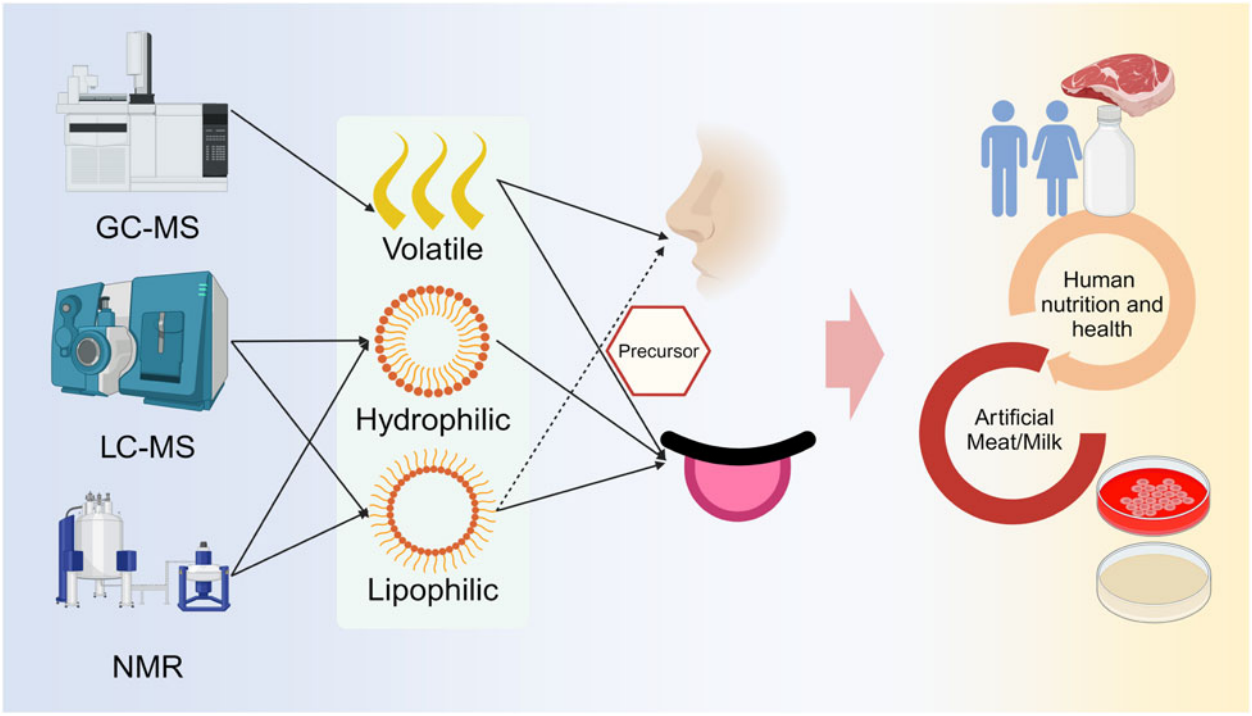

Figure 3. Metabolomic strategies for the development and Quality Control of Ruminant Food Alternatives. (Created in BioRender. Zhang, B. (2024) BioRender.com/l16r397).

bridge the gap between traditional meat and artificial meat, spatial metabolomics provides detailed information about the localization and chemistry of small molecules in individual cells (Taylor et al. 2021), tissues (Bourceau et al. 2022), and the host–microbe interface (Geier et al. 2020). For instance, spatial metabolomics has been used to map PL distributions in the tissues of marine bivalves (Bourceau et al. 2022), revealing insights that can also be applied to understand the deposition of intramuscular fat and specific lipids in beef and mutton. Furthermore, imaging MS allows spatial metabolomics to identify the localization of limited content compounds in plants (Nakabayashi et al. 2021). Thus, the use of spatial metabolomics offers a potential bridge between traditional and synthetic food production by providing a detailed map of the food metabolites.

Ruminant food metabolites on human health

As is well known, ruminant has a unique rumen that contributes to a large number greenhouse gas production, which brings increasing attention due to its threat to the world climate change and human being living safety (McMichael et al. 2007). Ruminant animal-based foods not only have high social and climate costs but have strong effects on human health. For instance, the CLA that is a special ruminant FA has beneficial effects on human health (Badawy et al. 2023; Mcguire and Mcguire 2000). In our previous study, we found that indoxyl sulfate in lamb meat was increased by feeding high energy diet but flavory AAs such as L-glutamine, L-serine, L-glutamate, and oleic acid were decreased (Wang et al. 2022). The L-glutamine, L-serine, L-glutamate and oleic acid were good taste or health beneficial (Sales-Campos et al. 2013) but sulfate compounds were potentially harmful for human health and easily result in human liver and kidney diseases (Vanholder et al. 2014). One Health and Global Health are based on the idea that human health and animal health are interdependent as well as being linked to the health of the ecosystems of which they are part. Thus, ruminant production is a key procedure in One Health that is the collaborative effort of multiple health science professions to attain optimal health for people (Hernando-Amado et al. 2019).

Furthermore, antibiotics are a class of secondary metabolites mainly produced by microorganisms. The abuse of antibiotics in livestock would easily result in antimicrobial resistance, and the antibiotic residues in animal derived food would accelerate the spread of resistant bacteria within and between these sectors and around the globe (Hernando-Amado et al. 2019). The application of different analytical techniques for metabolomics have been successfully used in environmental, food or health sciences with various advantages and drawbacks (Fraga-Corral et al. 2022). Living systems encompass microorganisms, plants, animals, and humans, with food systems directly impacting nutrition and human health, which is from farm to human (Kim et al. 2016). The ruminant food metabolomics are helping to interpret and connect the front-end animal production to back-end human life and open a window toward a better understanding of the complex interactions between food and human health (Scalbert et al. 2014). However, very limited research did the traceability and metabolites flow from planting to animal food, and then to human health.

The emergence of metabolomics enables the discovery of biomarkers that enhance the deposition of functional substances during nutritional interventions, thereby improving meat quality (Zhong et al. 2022). Simultaneously, it facilitates the detection and regulation of harmful compounds, ensuring the safety of food products (Lu et al. 2013; Ye et al. 2023). By constructing a comprehensive metabolite spectrum, metabolomics surpasses traditional methods in capturing the complexity of food quality and safety. Traditional approaches to evaluating food quality and safety often focus on individual components, such as protein,

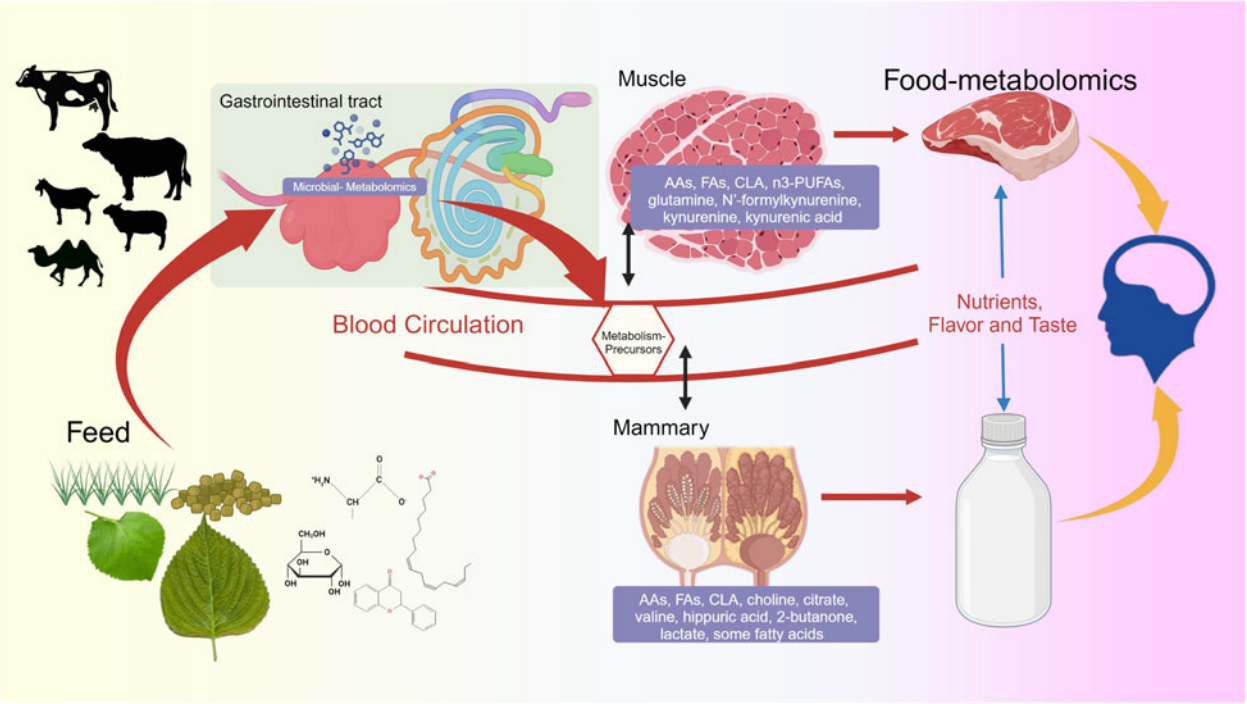

Figure 4. The whole workflow and key points in determining ruminant meat and milk production. (Created in BioRender. Zhang, B. (2024) BioRender.com/k75h157).

fat or specific contaminants. While effective, these methods fail to address the intricate interplay of metabolites that collectively define food quality and safety (Meijer et al. 2021). In contrast, metabolomics integrates a broad array of metabolites, including AAs, FAs, organic acids, vitamins, and bioactive compounds. This holistic approach provides a more nuanced and accurate representation of food quality and safety (Liu et al. 2023). By aligning with the goals of modern food science, metabolomics ensures the production of high-quality, safe, and trustworthy food products for consumers. However, as metabolomics databases for ruminant-derived foods continue to expand, this field holds the potential for even greater precision and comprehensiveness in identifying and regulating food quality and safety.

Conclusions

Metabolomics has been optimally and effectively utilized in the field of ruminant food science and nutrition research, facilitating the identification of crucial metabolites including AAs, *n*3-PUFA, and various organic acids. These metabolites serve either as precursors or as critical determinants in assessing food quality, flavor, and taste, which in turn influence consumer preferences (Fig. 4). The development of instrumentation would make metabolite detection and quantification more sensitive, accurate, robust, automated, and comprehensive, while promising advances need to improve and replenish the relevant metabolome. The most difficulties for metabolomics analysis are potentially the new metabolites or compounds identification, and the internal relationship among these metabolites in the food generation. For many of the small molecules, such as AAs and FAs, are part of the macronutrients such as protein and fat. Many more reference spectral or chromatographic databases on food components need to be developed and the name of metabolites should be uniform with chemical name and trivial name. A specific database related to the nutritional and ruminant food relevant compounds can be routinely identified or quantified.

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References

Afshari R, Pillidge CJ, Dias DA *et al.* (2020) Cheesomics: The future pathway to understanding cheese flavour and quality. *Critical Reviews in Food Science and Nutrition* **60**, 33–47.

Ahamad SR, Raish M, Ahmad A *et al.* (2017) Potential health benefits and metabolomics of camel milk by GC-MS and ICP-MS. *Biological Trace Element Research* **175**, 322–330.

Akhtar MT, Samar M, Shami AA *et al.* (2021) (1)H-NMR-based metabolomics: An integrated approach for the detection of the adulteration in chicken, chevon, beef and donkey meat. *Molecules* **26**, 4643.

Al-Awadi FM and Srikumar TS (2001) Trace elements and their distribution in protein fractions of camel milk in comparison to other commonly consumed milks. *Journal of Dairy Research* **68**, 463–469.

Albenzio M, Santillo A, Ciliberti MG *et al.* (2016) Milk from different species: Relationship between protein fractions and inflammatory response in infants affected by generalized epilepsy. *Journal of Dairy Science* **99**, 5032–5038.

Allen AE and Locasale JW (2021) Metabolomics: Insights into plant-based diets. *EMBO Molecular Medicine* **13**, e13568.

Amarnath K, Amarnath V, Amarnath K *et al.* (2003) A specific HPLC-UV method for the determination of cysteine and related aminothiols in biological samples. *Talanta* **60**, 1229–1238.

Argov-Argaman N, Glasser T, Muklada H *et al.* (2021) Lipidome changes, with a focus on phospholipids, due to feeding systems and processing in goat milk. *Food Chemistry* **340**, 127938.
Atapattu SN and Temerdashev A (2023) Recent advances in gas chromatography injection port derivatization in analytical method development. *TrAC Trends in Analytical Chemistry* **168**, 117334.
Ayyash M, Abdalla A, Alhammadi A *et al.* (2021) Probiotic survival, biological functionality and untargeted metabolomics of the bioaccessible compounds in fermented camel and bovine milk after in vitro digestion. *Food Chemistry* **363**, 130243.
Badawy S, Liu Y, Guo M *et al.* (2023) Conjugated linoleic acid (CLA) as a functional food: Is it beneficial or not? *Food Research International* **172**, 113158.
Behan AA, Akhtar MT, Loh TC *et al.* (2021) Meat quality, fatty acid content and NMR metabolic profile of dorper sheep supplemented with bypass fats. *Foods* **10**, 1133.
Bischof G, Witte F, Terjung N *et al.* (2021) Analysis of aging type- and aging time-related changes in the polar fraction of metabolome of beef by (1)H NMR spectroscopy. *Food Chemistry* **342**, 128353.
Bittante G, Amalfitano N, Bergamaschi M *et al.* (2022) Composition and aptitude for cheese-making of milk from cows, buffaloes, goats, sheep, dromedary camels, and donkeys. *Journal of Dairy Science* **105**, 2132–2152.
Bizzaro G, Vatland AK and Pampanin DM (2022) The One-Health approach in seaweed food production. *Environment International* **158**, 106948.
Boudonck KJ, Mitchell MW, Wulff J *et al.* (2009) Characterization of the biochemical variability of bovine milk using metabolomics. *Metabolomics* **5**, 375–386.
Bourceau P, Michellod D, Geier B *et al.* (2022) Spatial metabolomics shows contrasting phosphonolipid distributions in tissues of marine bivalves. *PeerJ Analytical Chemistry* **4**, e21.
Bravo-Lamas L, Barron LJR, Farmer L *et al.* (2018) Fatty acid composition of intramuscular fat and odour-active compounds of lamb commercialized in northern Spain. *Meat Science* **139**, 231–238.
Buchilina A and Aryana K (2021) Physicochemical and microbiological characteristics of camel milk yogurt as influenced by monk fruit sweetener. *Journal of Dairy Science* **104**, 1484–1493.
Caboni P, Murgia A, Porcu A *et al.* (2019) A metabolomics comparison between sheep's and goat's milk. *Food Research International* **119**, 869–875.
Cajka T and Fiehn O (2016) Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics. *Analytical Chemistry* **88**, 524–545.
Carrillo JA, He Y, Li Y *et al.* (2016) Integrated metabolomic and transcriptome analyses reveal finishing forage affects metabolic pathways related to beef quality and animal welfare. *Scientific Reports* **6**, 25948.
Carrillo J, Bai Y, He Y *et al.* (2021) Growth curve, blood parameters and carcass traits of grass-fed Angus steers. *Animal* **15**, 100381.
Castejón D, García-Segura JM, Escudero R *et al.* (2015) Metabolomics of meat exudate: Its potential to evaluate beef meat conservation and aging. *Analytica Chimica Acta* **901**, 1–11.
Chen L, Zhong F and Zhu J (2020) Bridging targeted and untargeted mass spectrometry-based metabolomics via hybrid approaches. *Metabolites* **10**, 348.
Chen X, Mao Y, Liang R *et al.* (2024) LC-MS-based metabolomics reveals metabolite dynamic changes of beef after superchilling early post-mortem. *Food Research International* **183**, 114208.
Cônsolo NRB, Olivecrona N, Samuelsson LM *et al.* (2020) Investigation of metabolites associated with confinement odour in chilled vacuum-packed lamb by proton nuclear magnetic resonance ((1)H NMR) spectroscopy. *Meat Science* **169**, 108207.
Cônsolo NRB, Rosa AF, Barbosa L *et al.* (2021) Preliminary study on the characterization of Longissimus lumborum dark cutting meat in Angus × Nellore crossbreed cattle using NMR-based metabolomics. *Meat Science* **172**, 108350.
Cui J, Zhu D, Su M *et al.* (2019) The combined use of (1) H and 2D NMR-based metabolomics and chemometrics for non-targeted screening of biomarkers and identification of reconstituted milk. *Journal of the Science of Food & Agriculture* **99**, 6455–6461.
Dhiman TR, Nam SH and Ure AL (2005) Factors affecting conjugated linoleic acid content in milk and meat. *Critical Reviews in Food Science and Nutrition* **45**, 463–482.
Dos Santos Rocha C, Magnani M, Ramos G *et al.* (2022) Emerging technologies in food processing: Impacts on sensory characteristics and consumer perception. *Current Opinion in Food Science* **47**, 100892.
Ebrahimi M, Rajion MA and Goh YM (2014) Effects of oils rich in linoleic and alpha-linolenic acids on fatty acid profile and gene expression in goat meat. *Nutrients* **6**, 3913–3928.
Eggersdorfer M, Berger MM, Calder PC *et al.* (2022) Perspective: Role of micronutrients and omega-3 long-chain polyunsaturated fatty acids for immune outcomes of relevance to infections in older adults—a narrative review and call for action. *Advances in Nutrition* **13**, 1415–1430.
Fan L, Wang X, Szeto IM-Y *et al.* (2024) Dietary intake of different ratios of ARA/DHA in early stages and its impact on infant development. *Food & Function* **15**(7), 3259–3273.
Fiehn O (2016) Metabolomics by gas chromatography-mass spectrometry: Combined targeted and untargeted profiling. *Current Protocols in Molecular Biology* **114**, 30–34.
Flis Z and Molik E (2021) Importance of bioactive substances in sheep's milk in human health. *International Journal of Molecular Sciences* **22**, 4364.
Foroutan A, Fitzsimmons C, Mandal R *et al.* (2020) The bovine metabolome. *Metabolites* **10**, 233.
Foroutan A, Guo AC, Vazquez-Fresno R *et al.* (2019) Chemical composition of commercial cow's milk. *Journal of Agricultural and Food Chemistry* **67**, 4897–4914.
Fraga-Corral M, Carpena M, Garcia-Oliveira P *et al.* (2022) Analytical metabolomics and applications in health, environmental and food science. *Critical Reviews in Analytical Chemistry* **52**, 712–734.
Geier B, Sogin EM, Michellod D *et al.* (2020) Spatial metabolomics of in situ host–microbe interactions at the micrometre scale. *Nature Microbiology* **5**, 498–510.
George AS (2023) The promises and challenges of cell-based dairy: Assessing the viability of lab-grown milk as a sustainable alternative. *Partners Universal International Research Journal* **2**, 218–233.
German JB, Hammock BD and Watkins SM (2005) Metabolomics: Building on a century of biochemistry to guide human health. *Metabolomics* **1**, 3–9.
Goetsch A, Zeng S and Gipson T (2011) Factors affecting goat milk production and quality. *Small Ruminant Research* **101**, 55–63.
Goldansaz SA, Guo AC, Sajed T *et al.* (2017) Livestock metabolomics and the livestock metabolome: A systematic review. *PloS One* **12**, e0177675.
Guo J and Huan T (2020) Comparison of full-scan, data-dependent, and data-independent acquisition modes in liquid chromatography-mass spectrometry based untargeted metabolomics. *Analytical Chemistry* **92**, 8072–8080.
Guo S, Sun Y, Wu T *et al.* (2024a) Co-fermented milk beverage has better stability and contains more health-promoting amino acid metabolites than single-strain-fermented milk beverage over one-month storage. *Food Chemistry* **430**, 136840.
Guo T, Zhang Q, Wang X *et al.* (2024b) Targeted and untargeted metabolomics reveals meat quality in grazing yak during different phenology periods on the Qinghai-Tibetan Plateau. *Food Chemistry* **447**, 138855.
Hadaya O, Landau SY, Glasser T *et al.* (2020) Producing pasture-like milk from goats in confinement. *Livestock Science* **236**, 104056.
Hernando-Amado S, Coque TM, Baquero F *et al.* (2019) Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nature Microbiology* **4**, 1432–1442.
Herzallah SM (2009) Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chemistry* **114**, 1141–1146.
Hu Y, Cheng H and Tao S (2017) Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. *Environment International* **107**, 111–130.
Jia W, Li R, Wu X *et al.* (2021a) UHPLC-Q-Orbitrap HRMS-based quantitative lipidomics reveals the chemical changes of phospholipids during thermal processing methods of Tan sheep meat. *Food Chemistry* **360**, 130153.

Jia W, Liu Y and Shi L (2021b) Integrated metabolomics and lipidomics profiling reveals beneficial changes in sensory quality of brown fermented goat milk. *Food Chemistry* **364**, 130378.

Jung Y, Lee J, Kwon J *et al.* (2010) Discrimination of the geographical origin of beef by (1)H NMR-based metabolomics. *Journal of Agricultural and Food Chemistry* **58**, 10458–10466.

Kanokruangrong S, Kebede B, Carne A *et al.* (2024) Metabolomic investigation of fresh beef, lamb and venison using nuclear magnetic resonance spectroscopy in relation to colour stability. *Food Chemistry* **463**, 141447.

Khan MI, Jo C and Tariq MR (2015) Meat flavor precursors and factors influencing flavor precursors–A systematic review. *Meat Science* **110**, 278–284.

K. Handral H, Hua Tay S, Wan Chan W *et al.* (2022) 3D printing of cultured meat products. *Critical Reviews in Food Science and Nutrition* **62**, 272–281.

Kim S, Kim J, Yun EJ *et al.* (2016) Food metabolomics: From farm to human. *Current Opinion in Biotechnology* **37**, 16–23.

Klein MS, Almstetter MF, Schlamberger G *et al.* (2010) Nuclear magnetic resonance and mass spectrometry-based milk metabolomics in dairy cows during early and late lactation. *Journal of Dairy Science* **93**, 1539–1550.

Klein MS, Buttchereit N, Miemczyk SP *et al.* (2012) NMR metabolomic analysis of dairy cows reveals milk glycerophosphocholine to phosphocholine ratio as prognostic biomarker for risk of ketosis. *Journal of Proteome Research* **11**, 1373–1381.

Korchazhkina O, Jones E, Czauderna M *et al.* (2006) HPLC with UV detection for measurement of vitamin E in human milk. *Acta Chromatographica* **16**, 48.

Lanier JS and Corl BA (2015) Challenges in enriching milk fat with polyunsaturated fatty acids. *Journal of Animal Science and Biotechnology* **6**, 26.

Lee SM, Kwon GY, Kim KO *et al.* (2011) Metabolomic approach for determination of key volatile compounds related to beef flavor in glutathione-Maillard reaction products. *Analytica Chimica Acta* **703**, 204–211.

Lelli V, Belardo A and Timperio AM (2021) From targeted quantification to untargeted metabolomics. In Xianquan Z ed, *Metabolomics—Methodology and Applications in Medical Sciences and Life Sciences* London, United Kingdom: BoD – Books on Demand 2021, 15–30.

Li A, Liu C, Han X *et al.* (2023) Tibetan Plateau yak milk: A comprehensive review of nutritional values, health benefits, and processing technology. *Food Chemistry: X* **20**, 100919.

Li H, Yang X, Tang D *et al.* (2024a) Exploring the link between microbial community structure and flavour compounds of traditional fermented yak milk in Gannan region. *Food Chemistry* **435**, 137553.

Li J, Tang C, Zhao Q *et al.* (2020) Integrated lipidomics and targeted metabolomics analyses reveal changes in flavor precursors in psoas major muscle of castrated lambs. *Food Chemistry* **333**, 127451.

Li Q, Wang X, Zhang Q *et al.* (2024b) Multi-omics analysis of five species of milk and specific composition links within each species. *Food Chemistry* **457**, 140028.

Li Q, Yu Z, Zhu D *et al.* (2017a) The application of NMR-based milk metabolite analysis in milk authenticity identification. *Journal of the Science of Food & Agriculture* **97**, 2875–2882.

Li Q, Zhao Y, Zhu D *et al.* (2017b) Lipidomics profiling of goat milk, soymilk and bovine milk by UPLC-Q-Exactive Orbitrap Mass Spectrometry. *Food Chemistry* **224**, 302–309.

Li W, Chen YP, Blank I *et al.* (2021) GC x GC-ToF-MS and GC-IMS based volatile profile characterization of the Chinese dry-cured hams from different regions. *Food Research International* **142**, 110222.

Liang X, Han H, Zhao X *et al.* (2018) Quantitative analysis of amino acids in human and bovine colostrum milk samples through iTRAQ labeling. *Journal of the Science of Food & Agriculture* **98**, 5157–5163.

Liu H, Guo X, Zhao Q *et al.* (2020) Lipidomics analysis for identifying the geographical origin and lactation stage of goat milk. *Food Chemistry* **309**, 125765.

Liu J, Zhao H, Yin Z *et al.* (2023) Application and prospect of metabolomics-related technologies in food inspection. *Food Research International* **171**, 113071.

Lu J, Antunes Fernandes E, Páez Cano AE *et al.* (2013) Changes in milk proteome and metabolome associated with dry period length, energy balance, and lactation stage in postparturient dairy cows. *Journal of Proteome Research* **12**, 3288–3296.

Lu J, Boeren S, van Hooijdonk T *et al.* (2015) Effect of the DGAT1 K232A genotype of dairy cows on the milk metabolome and proteome. *Journal of Dairy Science* **98**, 3460–3469.

Lu X, Zhao X, Bai C *et al.* (2008) LC–MS-based metabonomics analysis. *Journal of Chromatography B* **866**, 64–76.

Ma Q, Liu L, Jiao Y *et al.* (2024) Insights into flavor quality and metabolites profiles of fresh cheese with different probiotics by SPME-GC-MS and untargeted metabolomics. *Food Research International* **197**, 115154.

Madrid-Gambin F, Brunius C, Garcia-Aloy M *et al.* (2018) Untargeted (1)H NMR-based metabolomics analysis of urine and serum profiles after consumption of lentils, chickpeas, and beans: An extended meal study to discover dietary biomarkers of pulses. *Journal of Agricultural and Food Chemistry* **66**, 6997–7005.

Man KY, Chan CO, Tang HH *et al.* (2021) Mass spectrometry-based untargeted metabolomics approach for differentiation of beef of different geographic origins. *Food Chemistry* **338**, 127847.

McGrath TF, Haughey SA, Patterson J *et al.* (2018) What are the scientific challenges in moving from targeted to non-targeted methods for food fraud testing and how can they be addressed?–Spectroscopy case study. *Trends in Food Science and Technology* **76**, 38–55.

Mcguire MA and Mcguire MK (2000) Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. *Journal of Animal Science and Biotechnology* **77**, 3.

McMichael AJ, Powles JW, Butler CD *et al.* (2007) Food, livestock production, energy, climate change, and health. *Lancet* **370**, 1253–1263.

Meijer GW, Lähteenmäki L, Stadler RH *et al.* (2021) Issues surrounding consumer trust and acceptance of existing and emerging food processing technologies. *Critical Reviews in Food Science and Nutrition* **61**, 97–115.

Mollica MP, Trinchese G, Cimmino F *et al.* (2021) Milk fatty acid profiles in different animal species: Focus on the potential effect of selected PUFAs on metabolism and brain functions. *Nutrients* **13**, 1111.

Monbiot G (2020) Lab-grown food will soon destroy farming–and save the planet. *The Guardian* **8**.

Munekata PE, Pateiro M, Rocchetti G *et al.* (2022) Application of metabolomics to decipher the role of bioactive compounds in plant and animal foods. *Current Opinion in Food Science* **46**, 100851.

Munekata PES, Pateiro M, López-Pedrouso M *et al.* (2021) Foodomics in meat quality. *Current Opinion in Food Science* **38**, 79–85.

Mung D and Li L (2017) Development of chemical isotope labeling LC-MS for milk metabolomics: Comprehensive and quantitative profiling of the amine/phenol submetabolome. *Analytical Chemistry* **89**, 4435–4443.

Muroya S (2023) An insight into farm animal skeletal muscle metabolism based on a metabolomics approach. *Meat Science* **195**, 108995.

Nakabayashi R, Hashimoto K, Mori T *et al.* (2021) Spatial metabolomics using imaging mass spectrometry to identify the localization of asparaptine A in *Asparagus officinalis*. *Plant Biotechnology* **38**, 311–315.

OECD/FAO (2024) *OECD-FAO Agricultural Outlook 2024-2033*. Paris/FAO, Rome: OECD Publishing.

Pandya AJ and Ghodke KM (2007) Goat and sheep milk products other than cheeses and yoghurt. *Small Ruminant Research* **68**, 193–206.

Pavlidis DE, Mallouchos A, Ercolini D *et al.* (2019) A volatilomics approach for off-line discrimination of minced beef and pork meat and their admixture using HS-SPME GC/MS in tandem with multivariate data analysis. *Meat Science* **151**, 43–53.

Pisano MB, Scano P, Murgia A *et al.* (2016) Metabolomics and microbiological profile of Italian mozzarella cheese produced with Buffalo and cow milk. *Food Chemistry* **192**, 618–624.

Plumb RS, Gethings LA, Rainville PD *et al.* (2023) Advances in high throughput LC/MS based metabolomics: A review. *TrAC Trends in Analytical Chemistry* **160**, 116954.

Prestel S, de Falco B, Blidi S *et al.* (2020) Evaluation of the effect of berry extracts on carboxymethyllysine and lysine in ultra-high temperature treated milk. *Food Research International* **130**, 108923.

Prosser CG (2021) Compositional and functional characteristics of goat milk and relevance as a base for infant formula. *Journal of Food Science* **86**, 257–265.

Pulina G, Milan MJ, Lavin MP *et al.* (2018) Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. *Journal of Dairy Science* **101**, 6715–6729.

Qiu Q, Zhang G, Ma T *et al.* (2012) The yak genome and adaptation to life at high altitude. *Nature Genetics* **44**, 946–949.

Ranadheera CS, Vidanarachchi JK, Rocha RS *et al.* (2017) Probiotic delivery through fermentation: Dairy vs. non-dairy beverages. *Fermentation* **3**, 67.

Ren JL, Zhang AH, Kong L *et al.* (2018) Advances in mass spectrometry-based metabolomics for investigation of metabolites. *RSC Advances* **8**, 22335–22350.

Rocchetti G, Gallo A, Nocetti M *et al.* (2020) Milk metabolomics based on ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry to discriminate different cows feeding regimens. *Food Research International* **134**, 109279.

Rocchetti G, Lucini L, Gallo A *et al.* (2018) Untargeted metabolomics reveals differences in chemical fingerprints between PDO and non-PDO Grana Padano cheeses. *Food Research International* **113**, 407–413.

Rocchetti G, Michelini S, Pizzamiglio V *et al.* (2021) A combined metabolomics and peptidomics approach to discriminate anomalous rind inclusion levels in Parmigiano Reggiano PDO grated hard cheese from different ripening stages. *Food Research International* **149**, 110654.

Rocchetti G and O'Callaghan TF (2021) Application of metabolomics to assess milk quality and traceability. *Current Opinion in Food Science* **40**, 168–178.

Rombouts C, Hemeryck LY, Van Hecke T *et al.* (2017) Untargeted metabolomics of colonic digests reveals kynurenine pathway metabolites, dityrosine and 3-dehydroxycarnitine as red versus white meat discriminating metabolites. *Scientific Reports* **7**, 42514.

Rubio NR, Xiang N and Kaplan DL (2020) Plant-based and cell-based approaches to meat production. *Nature Communications* **11**, 6276.

Salama AAK, Contreras-Jodar A, Love S *et al.* (2020) Milk yield, milk composition, and milk metabolomics of dairy goats intramammary-challenged with lipopolysaccharide under heat stress conditions. *Scientific Reports* **10**, 5055.

Saleem F, Bouatra S, Guo AC *et al.* (2013) The bovine ruminal fluid metabolome. *Metabolomics* **9**, 360–378.

Sales-Campos H, Souza PR, Peghini BC *et al.* (2013) An overview of the modulatory effects of oleic acid in health and disease. *Mini-Reviews in Medicinal Chemistry* **13**, 201–210.

Samuelsson LM, Olivecrona N, Cônsolo NNB *et al.* (2021) Preparation of drip samples from leg of lamb with extended shelf life for nuclear magnetic resonance metabolomics studies. *Meat Science* **172**, 108304.

Sarmad S, Viant MR, Dunn WB *et al.* (2023) A proposed framework to evaluate the quality and reliability of targeted metabolomics assays from the UK Consortium on Metabolic Phenotyping (MAP/UK). *Nature Protocols* **18**, 1017–1027.

Scalbert A, Brennan L, Manach C *et al.* (2014) The food metabolome: A window over dietary exposure. *The American Journal of Clinical Nutrition* **99**, 1286–1308.

Scano P, Murgia A, Pirisi FM *et al.* (2014) A gas chromatography-mass spectrometry-based metabolomic approach for the characterization of goat milk compared with cow milk. *Journal of Dairy Science* **97**, 6057–6066.

Simon-Manso Y, Lowenthal MS, Kilpatrick LE *et al.* (2013) Metabolite profiling of a NIST Standard Reference Material for human plasma (SRM 1950): GC-MS, LC-MS, NMR, and clinical laboratory analyses, libraries, and web-based resources. *Analytical Chemistry* **85**, 11725–11731.

Sivadier G, Ratel J and Engel E (2009) Latency and persistence of diet volatile biomarkers in lamb fats. *Journal of Agricultural and Food Chemistry* **57**, 645–652.

Suh JH (2022) Critical review: Metabolomics in dairy science - evaluation of milk and milk product quality. *Food Research International* **154**, 110984.

Sun HZ, Shi K, Wu XH *et al.* (2017) Lactation-related metabolic mechanism investigated based on mammary gland metabolomics and 4 biofluids' metabolomics relationships in dairy cows. *BMC Genomics* **18**, 936.

Sun H-Z, Wang D-M, Wang B *et al.* (2015) Metabolomics of four biofluids from dairy cows: Potential biomarkers for milk production and quality. *Journal of Proteome Research* **14**, 1287–1298.

Sun HZ, Zhou M, Wang O *et al.* (2020) Multi-omics reveals functional genomic and metabolic mechanisms of milk production and quality in dairy cows. *Bioinformatics* **36**, 2530–2537.

Sundekilde U, Clausen M, Lejonklev J *et al.* (2015) Addition of essential oils to cows' feed alters the milk metabolome-NMR spectroscopic studies of "Nature's perfect food". In Francesco C, Luca L and Peter SB (eds), *Magnetic Resonance in Food Science: Defining Food by Magnetic Resonance*. Thomas Graham House, Science Park, Milton Road.Cambridge CB4 0WF,UK: Royal Society of Chemistry, 161–170.

Sundekilde UK, Larsen LB and Bertram HC (2013a) NMR-based milk metabolomics. *Metabolites* **3**, 204–222.

Sundekilde UK, Poulsen NA, Larsen LB *et al.* (2013b) Nuclear magnetic resonance metabonomics reveals strong association between milk metabolites and somatic cell count in bovine milk. *Journal of Dairy Science* **96**, 290–299.

Tan D, Zhang X, Su M *et al.* (2021) Establishing an untargeted-to-MRM liquid chromatography-mass spectrometry method for discriminating reconstituted milk from ultra-high temperature milk. *Food Chemistry* **337**, 127946.

Taylor MJ, Lukowski JK and Anderton CR (2021) Spatially resolved mass spectrometry at the single cell: Recent innovations in proteomics and metabolomics. *Journal of the American Society for Mass Spectrometry* **32**, 872–894.

Tenori L, Santucci C, Meoni G *et al.* (2018) NMR metabolomic fingerprinting distinguishes milk from different farms. *Food Research International* **113**, 131–139.

Tian H, Zheng N, Wang W *et al.* (2016) Integrated metabolomics study of the milk of heat-stressed lactating dairy cows. *Scientific Reports* **6**, 24208.

Tsiafoulis CG, Papaemmanouil C, Alivertis D *et al.* (2019) NMR-based metabolomics of the lipid fraction of organic and conventional bovine milk. *Molecules* **24**, 1067.

Tyndall SM, Maloney GR, Cole MB *et al.* (2022) Critical food and nutrition science challenges for plant-based meat alternative products. *Critical Reviews in Food Science and Nutrition* **64**, 1–16.

Ueda S, Iwamoto E, Kato Y *et al.* (2019) Comparative metabolomics of Japanese Black cattle beef and other meats using gas chromatography-mass spectrometry. *Bioscience Biotechnology and Biochemistry* **83**, 137–147.

Utpott M, Rodrigues E, Rios AO *et al.* (2022) Metabolomics: An analytical technique for food processing evaluation. *Food Chemistry* **366**, 130685.

Vahmani P, Mapiye C, Prieto N *et al.* (2015) The scope for manipulating the polyunsaturated fatty acid content of beef: A review. *Journal of Animal Science and Biotechnology* **6**, 1–13.

Vanholder R, Schepers E, Pletinck A *et al.* (2014) The uremic toxicity of indoxyl sulfate and *p*-cresyl sulfate: A systematic review. *Journal of the American Society of Nephrology* **25**, 1897–1907.

van Vliet S, Bain JR, Muehlbauer MJ *et al.* (2021) A metabolomics comparison of plant-based meat and grass-fed meat indicates large nutritional differences despite comparable Nutrition Facts panels. *Scientific Reports* **11**, 13828.

Visciano P and Schirone M (2021) Food frauds: Global incidents and misleading situations. *Trends in Food Science and Technology* **114**, 424–442.

Wang B, Sun Z, Tu Y *et al.* (2021a) Untargeted metabolomic investigate milk and ruminal fluid of Holstein cows supplemented with Perilla frutescens leaf. *Food Research International* **140**, 110017.

Wang B, Wang Y, Zuo S *et al.* (2021b) Untargeted and targeted metabolomics profiling of muscle reveals enhanced meat quality in artificial pasture grazing Tan lambs via rescheduling the rumen bacterial community. *Journal of Agricultural and Food Chemistry* **69**, 846–858.

Wang B, Zhao X, Zhang B *et al.* (2022) Assessment of components related to flavor and taste in Tan-lamb meat under different silage-feeding regimens using integrative metabolomics. *Food Chemistry-X* **14**, 100269.

Wang F, Gao Y, Wang H *et al.* (2021c) Analysis of volatile compounds and flavor fingerprint in Jingyuan lamb of different ages using gas chromatography-ion mobility spectrometry (GC-IMS). *Meat Science* **175**, 108449.

Wang J, Xu Z, Zhang H *et al.* (2021d) Meat differentiation between pasture-fed and concentrate-fed sheep/goats by liquid chromatography quadrupole time-of-flight mass spectrometry combined with metabolomic and lipidomic profiling. *Meat Science* **173**, 108374.

Wang L, Li X, Liu L *et al.* (2020) Comparative lipidomics analysis of human, bovine and caprine milk by UHPLC-Q-TOF-MS. *Food Chemistry* **310**, 125865.

Wang R, Yin Y and Zhu ZJ (2019a) Advancing untargeted metabolomics using data-independent acquisition mass spectrometry technology. *Analytical and Bioanalytical Chemistry* **411**, 4349–4357.

Wang W, Sun B, Hu P *et al.* (2019b) Comparison of differential flavor metabolites in Meat of Lubei White Goat, Jining Gray Goat and Boer Goat. *Metabolites* **9**, 176.

Watkins PJ, Jaborek JR, Teng F *et al.* (2021) Branched chain fatty acids in the flavour of sheep and goat milk and meat: A review. *Small Ruminant Research* **200**, 106398.

Watrous JD, Henglin M, Claggett B *et al.* (2017) Visualization, quantification, and alignment of spectral drift in population scale untargeted metabolomics data. *Analytical Chemistry* **89**, 1399–1404.

Webb EC (2021) Cis/trans-fatty acid content of red meats and the related effects on meat quality and human health. In Chhabi LR (ed), *Meat and Nutrition* London, United Kingdom: BoD – Books on Demand, 2021, 121–139.

Wishart DS (2008) Metabolomics: Applications to food science and nutrition research. *Trends in Food Science and Technology* **19**, 482–493.

Wishart DS, Guo A, Oler E *et al.* (2022) HMDB 5.0: The Human Metabolome Database for 2022. *Nucleic Acids Research* **50**, D622–D631.

Wittwer AE, Lee SG and Ranadheera CS (2023) Potential associations between organic dairy products, gut microbiome, and gut health: A review. *Food Research International* **172**, 113195.

Wu N and Wang XC (2019) Identification of important odorants derived from phosphatidylethanolamine species in steamed male Eriocheir sinensis hepatopancreas in model systems. *Food Chemistry* **286**, 491–499.

Xi X, Kwok LY, Wang Y *et al.* (2017) Ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry MS(E)-based untargeted milk metabolomics in dairy cows with subclinical or clinical mastitis. *Journal of Dairy Science* **100**, 4884–4896.

Xue MY, Sun HZ, Wu XH *et al.* (2020) Multi-omics reveals that the rumen microbiome and its metabolome together with the host metabolome contribute to individualized dairy cow performance. *Microbiome* **8**, 64.

Xue MY, Xie YY, Zhong Y *et al.* (2022) Integrated meta-omics reveals new ruminal microbial features associated with feed efficiency in dairy cattle. *Microbiome* **10**, 32.

Yang Y, Zheng N, Zhao X *et al.* (2016) Metabolomic biomarkers identify differences in milk produced by Holstein cows and other minor dairy animals. *Journal of Proteomics* **136**, 174–182.

Ye H, Yang J, Xiao G *et al.* (2023) A comprehensive overview of emerging techniques and chemometrics for authenticity and traceability of animal-derived food. *Food Chemistry* **402**, 134216.

Yu Q, Tian X, Shao L *et al.* (2019) Targeted metabolomics to reveal muscle-specific energy metabolism between bovine longissimus lumborum and psoas major during early postmortem periods. *Meat Science* **156**, 166–173.

Yu Y, Zhang B, Jiang X *et al.* (2024) Exploring the metabolomic landscape: Perilla frutescens as a promising enhancer of production, flavor, and nutrition in Tan lamb meat. *Meat Science* **209**, 109419.

Zeki OC, Eylem CC, Recber T *et al.* (2020) Integration of GC-MS and LC-MS for untargeted metabolomics profiling. *Journal of Pharmaceutical & Biomedical Analysis* **190**, 113509.

Zergiebel S, Ueberschaar N and Seeling A (2023) Development and optimization of an ultra-fast microextraction followed by HPLC-UV of tetracycline residues in milk products. *Food Chemistry* **402**, 134270.

Zhan J-W, Shen Y, Lin X *et al.* (2021) Microbiome and metabolic changes of milk in response to dietary supplementation with bamboo leaf extract in dairy cows. *Frontiers in Nutrition* **8**, 723446.

Zhang C, Zhang H, Liu M *et al.* (2020a) Effect of breed on the volatile compound precursors and odor profile attributes of lamb meat. *Foods* **9**, 1178.

Zhang J, Sun M, Elmaidomy AH *et al.* (2023) Emerging trends and applications of metabolomics in food science and nutrition. *Food & Function* **14**(20), 9050–9082.

Zhang J, Yang M, Cai D *et al.* (2020b) Composition, coagulation characteristics, and cheese making capacity of yak milk. *Journal of Dairy Science* **103**, 1276–1288.

Zhang M, Sun L, Su R *et al.* (2024a) Widely targeted metabolomic analysis reveals the dynamic changes of metabolites during postmortem chilled aging in Mongolian sheep. *Food Chemistry* **431**, 137035.

Zhang X, Li C, Huang J *et al.* (2024b) Characterization and comparison of metabolites in colostrum from yaks, buffaloes, and cows based on UPLC-QTRAP-MS metabolomics. *Food Chemistry* **463**, 141345.

Zhang X, Zheng Y, Zhou R *et al.* (2022) Comprehensive identification of molecular profiles related to sensory and nutritional changes in Mongolian cheese during storage by untargeted metabolomics coupled with quantification of free amino acids. *Food Chemistry* **386**, 132740.

Zhang YD, Li P, Zheng N *et al.* (2018) A metabolomics approach to characterize raw, pasteurized, and ultra-high temperature milk using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry and multivariate data analysis. *Journal of Dairy Science* **101**, 9630–9636.

Zhong P, Wei X, Li X *et al.* (2022) Untargeted metabolomics by liquid chromatography-mass spectrometry for food authentication: A review. *Comprehensive Reviews in Food Science and Food Safety* **21**, 2455–2488.

Zhu C, Petracci M, Li C *et al.* (2020) An untargeted metabolomics investigation of Jiulong Yak (Bos grunniens) meat by (1)H-NMR. *Foods* **9**, 481.

Zhu C, Tang K, Lu X *et al.* (2021a) An untargeted metabolomics investigation of milk from dairy cows with clinical mastitis by (1)H-NMR. *Foods* **10**, 1707.

Zhu D, Kebede B, McComb K *et al.* (2021b) Milk biomarkers in relation to inherent and external factors based on metabolomics. *Trends in Food Science and Technology* **109**, 51–64.

Zwierzchowski G, Zhang G, Mandal R *et al.* (2020) Milk metabotyping identifies metabolite alterations in the whole raw milk of dairy cows with lameness. *Journal of Agricultural and Food Chemistry* **68**, 4507–4514.