ISSN: 2347-467X, Vol. 14, No. (1) 2026, Pg. 34-52

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Metabolomic Insights into Sheep Milk: Advancing the Development of Healthier Functional Foods

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Abstract

Sheep milk, a crucial health-promoting dairy product, holds nutraceutical significance in functional nutrition, as demonstrated by metabolomics studies. Compared to staples like cow and goat milk, sheep milk has a significantly higher concentration of various proteins, fats, vitamins, and bioactive components such as omega fatty acids, conjugated linoleic acid (CLA), and peptides with antioxidative and anti-inflammatory properties. Metabolomics, an advanced analytical tool that captures the multivariate profiles of biological samples, is essential for understanding the bioactive characteristics of sheep milk. The metabolite profile of fresh sheep milk varies significantly based on breed, stage of lactation, diet fed to the animals, and environmental factors, presenting promising opportunities for functional food development and genetic identification tailored to individual nutrition. Both specific and non-specific metabolomics approaches, including Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), Liquid Chromatography – MS (LC-MS), and Gas Chromatography-MS (GC-MS), facilitate hypothesis-driven and hypothesis-generating studies, aiding in the understanding of the nutritional values and presence of novel bioactive compounds in sheep and goat milk. Future directions for metabolomics research may focus on enhancing existing metabolomics techniques, developing a more extensive database of metabolomic

Article History
Received: 22 April 2025
Accepted: 15 October 2025

Keywords
Bioactive Compounds;
Functional Foods;
Metabolomics;
Nutritional Profile;
Sheep Milk.

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Doi: http://dx.doi.org/10.12944/CRNFSJ.14.1.3

data, and correlating metabolomics outcomes with other omics data. Improvements in high-resolution MS and bioinformatics could unveil more information about sheep milk for the dairy industry, particularly regarding the functional properties of milk to meet distinct health needs. This review advocates for greater standardization in sheep milk research to effectively harness its nutritional and therapeutic potential in consumer healthcare products.

Abbreviations

ACE	Angiotensin-Converting Enzyme
BCAAs	Branched-Chain Amino Acids
CLA	Conjugated Linoleic Acid
GC-MS	Gas Chromatography-Mass Spectrometry
HRMS	High-Resolution Mass Spectrometry
LC-MS	Liquid Chromatography - Mass Spectrometry
MCFAs	Medium-Chain Fatty Acids
MS	Mass Spectrometry
MT	Metric Tonne
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares-Discriminant Analysis
SCFAs	Short-Chain Fatty Acids
SVM	Support Vector Machines
XCMS	eXtensible Computational Mass Spectrometry

Introduction

Sheep milk has been a staple of Middle Eastern and Mediterranean cuisine for several centuries, and in the past decade, it has garnered increasing scientific attention due to its distinctive nutritional composition, characterized by higher protein, essential amino acids, medium-chain fatty acids, and bioactive peptides, as well as its potential health benefits. Sheep and goat milk amount to nearly 3.5% of global milk production, and by 2040, the sheep milk statistics are projected to reach 30 MT globally.[1] Over the past 50 years (1970–2022), dairy goats (DG; 214.01 million heads) inventory has grown 182%, and it will likely increase by 53.37% over the following 28 years (2023–2050). DGP increased 196% from 1970 to 2022 and is expected to reach 71.29% by 2050.[2] Sheep milk has more calcium, protein, vitamins A, D, and B12, and healthy fatty acids like conjugated linoleic acid (CLA), short- and medium-chain fatty acids (C4:0–C12:0) that improve digestibility, and long-chain polyunsaturated fatty acids (PUFAs) that promote cardiometabolic health than cow milk.[3] Also, sheep milk is a desired source of bioactive substances for communities with special dietary requirements, such as low lactose levels and high medium-chain fatty acids for improved digestibility. Its consumption also helps avoid cow milk sensitivity since sheep milk's casein profile is different (25–30% αs1, 10–12% αs,[2] 45–50% β, and around 10–12% κ). Additionally, in whey proteins, peptide release during digestion, and overall allergenic potential—although casein is not the sole factor responsible for milk protein sensitivity.[4] Sheep milk's antibacterial activity[5] (against pathogens such as *Escherichia coli* MTCC 1687, *Bacillus cereus* MTCC 12, *Salmonella Typhimurium* ATCC 14028, *Enterococcus fecalis* ATCC 29212) and anti-inflammatory activities[5] (inhibition/reduced production of TNF-α, IL-6, & IL-1β) are increasingly of interest to researchers and health-conscious customers alike. This is because fermented sheep milk is rich in bioactive peptides (viz. KADEKKFW, LDQWLCEK, HKEMPFPK, FAWPQYLK and ITMPLW), proven beneficial to human health.[6] These peptides improve nutritional value by making sheep milk an antioxidant, antibacterial, and antihypertensive functional food produced by digestion or fermentation of sheep milk. Sheep milk is less likely to cause allergies, as it has lower amount of αs1-casein (25-30%), and levels of α-lactalbumin and β-lactoglobulin known to cause allergy.[7] Due to its smaller fat globules, greater medium-chain fatty acids and lower contents of alpha s1-casein, the sheep milk can be tolerated by some people, who are intolerant to proteins of cow milk, better. Nevertheless, this is not the case with lactose intolerance, since sheep milk has just as much lactose as cow milk, and thus is typically inappropriate in lactose-intolerant people unless lactose is eliminated or hydrolysed.

Metabolomics helps to study the biochemical complexity of milk, including ovine milk.[8] It is the exhaustive study of molecules (metabolites) in a biological sample, helping to probe complex metabolic pathways and discover specific substances with nutritional and medicinal potential.[9] In addition, metabolomics techniques allow for identifying metabolites that affect the flavor, nutritional content, and possible health advantages of sheep milk.[10] They help determine bioactive substances (such as peptides) and assess the impact of environment, breed, and nutrition on milk composition. It can drive dairy innovation by discovering novel bioactive and enhancing the quality of high-value dairy foods such as Feta cheese and yogurt.[11]

Studying the metabolomics of sheep milk has been critical in highlighting the effects of different farming methods, different feed types, and different geographical locations on the nutritional contents.[12] Metabolomics analysis has revealed the presence of over 200 unique metabolites and nearly ten essential amino acids. Moreover, it provides insights into how the milk is metabolized, which indicates its possibility for consumption as a functional food and its advantages to human health.[13]

This review discusses current research in sheep milk metabolomics, particularly on nutritional composition research and its potential impact on health. Possible research opportunities include identifying existing and potential metabolomics in sheep milk products and enhancing their nutritional quality and health benefits, thus expanding their applications further in the food industry. This review aims to share the recent findings of sheep milk metabolomics and contribute to developing functional dairy products and customized nutrition solutions for scientists, business experts, and healthcare professionals.

Role of Metabolomics

Metabolomics, the comprehensive study of small molecules (typically <1,500 Da) within a biological system, has become a powerful technique in dairy research, particularly in studying the breakdown of milk components. It provides a snapshot of the metabolic activity within milk and the metabolites, such as intermediates and final products of cellular processes.[14] This method helps scientists identify small changes in profiles of metabolites and discover how environment, genetics, food, and lactation phases all affect the nutritional richness and quality of the milk. By applying metabolomics to dairy science, we have enhanced our understanding of milk's nutritional and functional qualities with implications for quality control, dairy production, and human health.[15]

Sheep milk is a complex biofluid high in proteins, lipids, carbohydrates, vitamins, and minerals, whose composition varies depending on multiple factors. Unique milk compositions from sheep, goats, and cows have been demonstrated by metabolomics research to be tailored to meet the physiologic requirements of their species' progeny. Cow milk is the most investigated and owned milk globally, so its metabolic profile is well-known.[16] It has been proven, though, that cow milk contains high amounts of lactose, and some fatty acids (such as conjugated linoleic acid, omega-3s) beneficial to human health.[17] The metabolic profile of goat milk is known for its digestibility and hypoallergenic qualities and includes higher amounts of easily absorbed medium-chain fatty acids (such as caproic and capric).[18] Although produced in lower tonnages, sheep milk has better nutritional profile than cow or goat milk, usually having a higher level of protein (5.4%), fat (7.1%), and mineral (0.9%).[19] Of those, the most important is its high concentration of bioactive compounds such as lactoferrin, lysozyme, casein-derived peptides, immunoglobulins, and oligosaccharides that have anti-inflammatory and antibacterial properties.[20]

Sheep milk is relatively richer in protein than cow and goat milk and is rich in particular fatty acids like Omega-3 and Omega-6.[21] Sheep milk is also exceptionally well suited for making cheese and yogurt, which, like most other foods, depend on their metabolism for flavor, texture, and nutritional value.[22] Metabolomics can help identify the metabolite(s) associated with these sensory and dietary attributes that may be used to improve the product, its further development, and quality control in the dairy processing industry.[23]

Nevertheless, there is scope to address the difficulties posed by using metabolomics on sheep milk. Firstly, it is produced in lesser quantities than cow milk, making it challenging to obtain enough for research purposes, which contributes to limited studies. Additionally, the complex metabolism of sheep milk can be analytically challenging because its full range of metabolites may not be successfully recorded, requiring complex equipment and techniques. A second difficulty lies in the variation of metabolite profiles induced by breed differences, feed content, and environmental factors, which may complicate data interpretation and standardization of findings across studies. However, advancements in analytical methods and data interpretation are overcoming these challenges and providing a framework for determining the health benefits and quality of sheep milk through metabolomics.

Metabolomics has provided comprehensive insight into the molecular makeup of milk from various species and may provide new impetus for dairy science. By comparing the metabolic profile of milk from cows, goats, and sheep, researchers can determine which distinct bioactive components contribute to each type of milk's nutrition and functional value.[24] While many challenges exist, particularly in studies of sheep milk, metabolomics has enormous potential to increase the manufacturing of dairy products and improve human health through tailored dietary recommendations. Sheep milk is high in total solids and a good source of nutrients, making it ideal for cheese manufacturing.[25]

Sheep Milk Composition

In comparison to cow, goat, and human milk, sheep milk is a nutrient-dense dairy product due to its high concentration of casein and whey proteins, which are 4.2–5.2 g/100 g and 1.02–1.3 g/100 g, respectively. The levels of caproic (C6:0), caprylic (C8:0), capric (C10:0), and lauric (C12:0) acids in sheep milk are significantly higher than those in bovine milk.[16] The distinctive flavours of cheeses are linked to these short- and medium-chain fatty acids. Furthermore, sheep milk is distinguished by a higher concentration of ω-3 fatty acids (FAs) and butyric acid (C4:0) than other ruminant milk.[19] It is abundant in lipids (with C10:0, C14:0, C16:0, C18:0, and C18:1 accounting for over 75% of all fatty acids), proteins (4.2% casein), polysaccharides, and distinctive bioactive compounds (including ACE inhibitory and antihypertensive peptides).[26] Sheep milk is utilised for commercial, medicinal, and nutritional purposes due to its composition. Additionally, its benefits render it an ideal addition to functional foods and tailored eating habits. In addition, ewe’s milk fat contains a moderate proportion of trans fatty acids (≈4.9% of total FAME), predominantly trans-C18:1 isomer mainly trans-11 with relatively low levels of potentially deleterious trans-9 and trans-10 isomers, further contributing to its distinctive lipid profile.[27]

Fats

The richness and creaminess that sheep milk imparts come from 6-8% fat levels, compared with just 3.2 % in cow milk.[28] These fats include medium-chain fatty acids, such as caproic, caprylic, and capric acids, which are easier to digest, are not stored easily, and provide energy without weight gain.[29] In addition, sheep milk contains polyunsaturated fats ranging from 2.6-7.3 %, including omega-3 and omega-6 fatty acids, which are cardiovascular protective and may have anti-inflammatory qualities.[30] CLA is a bioactive lipid present in abundant levels in sheep milk and has anti-obesity, anti-diabetic and anti-carcinogenic effects.[31]

Proteins

Sheep milk contains about 5–6% protein, which is significantly higher than cow milk (3–4%). Sheep milk has less αs1-casein than cow milk and thus may be less allergic.[19] The whey proteins of sheep milk contain bioactive peptides that aid digestion and have antibacterial, anti-inflammatory, and immune-modulating properties.[32] Second, the increased protein content also means that the resulting cheese will have better yields because caseins are required for curd development and constituents of cheese themselves. Sheep milk proteins contain more essential amino acids like leucine, lysine, isoleucine, and valine than cow milk has but only slightly higher content of non-essential amino acids like glutamic acid and proline which are extremely important for muscle growth and repair.[33] Sheep milk proteins also help the immune system grow, making it nutritious for adults and babies.

Carbohydrates

In sheep milk lactose is the primary sugar, and this is approximately 4-5 percent, a rather close but a bit lower proportion than in cow milk. Although such difference does not remove the intolerance among lactase-deficient individuals, it can make sheep milk slightly more tolerable among people with mild lactose intolerance.[34] The lactose in sheep milk is still high enough to be used as an energy source, but some people with lactose intolerance cannot cope. Moreover, it is of interest that sheep milk contains prebiotic-like oligosaccharides similar to those found in human milk. Prebiotic carbohydrates, which promote the development of helpful gut bacteria, may help improve gut and perhaps even immune health.[35] This characteristic makes sheep milk a good source of prebiotics, which improves gastrointestinal health and preserves microbiota balance.

Specialized Bioactive Substances and Metabolites

The list of crucial vitamins for health, skin, eyesight, and the immune system includes vitamins A, D, and E. Bone health and metabolic processes are associated with mineral content, especially calcium, magnesium, and phosphorus. Bioactive peptides from sheep milk proteins may reduce the risk of developing chronic diseases (such as cardiovascular and neurodegenerative) because of their antihypertensive, and antioxidant properties.[36] Besides the globulins and nucleotides found in sheep milk[37] that promote healing and provide immunity to tissue, sheep milk also contains essential immunoglobulins.[38] Its amount of antioxidants, including glutathione, is responsible for its capacity to eliminate free radicals and reduce oxidative stress.[39] The nutritional and bioactive components in sheep milk and their health benefits are depicted in Table 1 as below.

Table 1: Nutritional and Bioactive Components in Sheep Milk and Their Health Benefits

Component	Sheep milk	Cow milk	Description	Health Benefits
Proteins (%)	5.4-6.0%	3.0 – 4.0	High-quality protein, rich in leucine, lysine	Muscle growth, tissue repair[30]
Omega-3 and Omega -6 Fatty Acids	< 1%	< 0.5%	Polyunsaturated fats with anti-inflammatory properties	Cardiovascular health, inflammation reduction[40]
Conjugated Linoleic Acid (CLA)	0.5-1.0%	0.3-0.6%	Bioactive lipid with anti-obesity, anti-carcinogenic properties	Weight management, cancer prevention[30]
Lactose	4.5-5.0%	4.8-5.0	Primary carbohydrate; less prone to cause allergies than cow milk lactose	Energy source, gut health for lactose-sensitive individuals[19]
Bioactive Peptides	-		Compounds formed during protein hydrolysis; antimicrobial and antioxidant properties	Immune support, digestive health[30]
Oligosaccharides	<0.1%		Complex carbohydrates with prebiotic effects	Gut health, promotes beneficial gut bacteria[41]

Metabolomics and Important Metabolites Found in Sheep Milk

Preparing and Gathering Samples

Several variables, including nutrition, stage of lactation, and sheep breed, influence the milk's metabolomic profile. Each breed's milk contains a differing nutritional and metabolic makeup, which affects which metabolites are present.[36] For example, lipidomics analysis can be affected by the increased lipid content of the breeds. The nutritional content of milk is a function of the lactation stage. Early and late lactation milk is very different because of changes in dietary needs and milk production levels. It is also important to note the collection period during lactation for consistency.[42] The sheep's diet affects the metabolomic makeup of their milk because milk reflects the body's contents and bioactive substances they consume. For example, a diet high in grass produces a different metabolome compared to one based on grain. In metabolomics, keeping a consistent diet ensures uniformity and minimizes variability in study results. Often, samples are frozen immediately upon collection to preserve the metabolites. Next, they are processed by eliminating lipids and proteins, often by centrifugation or chemical treatments, to keep the focus on the relevant metabolites. The metabolomic techniques in analyzing sheep milk composition are mentioned in Table 2.

Table 2: Metabolomic Techniques in Analyzing Sheep Milk Composition

Technique	Description	Applications
Nuclear Magnetic Resonance (NMR)[43]	Non-destructive technique that provides structural and quantitative information on metabolites	Quantifies proteins, fatty acids, organic acids
Mass Spectrometry (MS)[44]	Sensitive, specific analysis of metabolites via molecular mass	Detects small molecules and volatile compounds
Liquid Chromatography-MS (LC-MS)[45]	Separates and identifies polar and semi-polar compounds	Analyzes larger biomolecules and lipids
Gas Chromatography-MS (GC-MS)[45]	Analyzes volatile, non-polar metabolites	Identifies fatty acids, flavor compounds
XCMS[45]	Bioinformatics tool for peak alignment and detection in MS data	Aligns peaks, corrects batch effects
MetaboAnalyst[46]	Provides biological interpretation and visualization	Pathway analysis, clustering, data normalization

Analytical techniques reveal the variety of lipids, small molecules, and other substances in the sheep milk metabolome. In terms of gathering information, mass spectrometry (MS) and NMR spectroscopy are the two ‘founding fathers’ of the discipline, but each method has its specific uses and insights.

Spectroscopy using NMR

Structural and quantitative details of metabolites in sheep's milk could be achieved by non-destructive nuclear magnetic resonance (NMR) spectroscopy. NMR is particularly good at quantifying lactose, proteins and fatty acids. Since it does not destroy the sample, it is possible to preserve the integrity of the milk for further studies. Small molecules added to the bottle have been detected by NMR including organic acids, amino acids, carbohydrates, all of which are essential to understanding the functional and nutritional properties of sheep milk.[9] NMR is required to understand milk components' bioactivity due to the complexity of the metabolite structure. It also tells us about molecular interactions and stability.[43] Despite these drawbacks, NMR is less effective than mass spectrometry in identifying low-abundance metabolites.[47,48]

Mass Spectrometry (MS)

Mass spectrometry is excellent in terms of sensitivity and specificity for the analysis of the metabolome of sheep milk. Various molecular types are also explained using methods like gas chromatography-mass spectrometry (GC-MS), or liquid chromatography-mass spectrometry (LC-MS). LC-MS technique is excellent for investigating polar and semi-polar substances. Sheep milk is rich in lipids, organic acids, and amino acids, which can be profiled.[42] LC-MS can analyze larger biomolecules and can be used for analyzing potential peptides and larger lipid compounds. However, the analysis of non-polar metabolites and volatile substances, fatty acids, and distinct lipids is generally done using GC-MS. By successfully separating and detecting tiny, volatile molecules, it gives insight into fragrance components that determine the sensory qualities of sheep milk.[44] These MS-based methods do not offer true sensitivity and precision in detection unless the measurement is subjected to chemical modification or ample derivatization.[8,16,21,32]

Metabolomics: Targeted vs Untargeted

Sheep milk metabolomics techniques fall into two categories: targeted and untargeted. There are many applications for each. Targeted Metabolomics commonly involves measuring of certain metabolites (i.e., concentration of known biological significance such as, for example, some lipids or amino acids). The specificity that can be targeted metabolomics into sheep's milk can illuminate specific metabolic pathways or nutritional characteristics.[49] Specific metabolites can be exactly quantified using their accuracy and low detection limits.

On the other hand, untargeted metabolomics profiles every metabolite found in the sample and, therefore, provides an overall picture (metabolome).[50] In particular, this method is beneficial for the identification of new metabolites or metabolic pathways specific to sheep milk, and it provides information about the biochemical details of this complex substrate.[12] Nevertheless, more sophisticated data processing and statistical analysis are needed to identify relevant metabolites.

Often, targeted procedures rather than untargeted ones are used based on the study's goals. For example, baseline, targeted methods might be used to validate certain nutritional qualities, where data might affirm or nullify a proposed way or platform.

Analyzing and Processing Data

Interpretation requires processing and analysis of complicated metabolomic data from sheep milk research. Statistical methods and bioinformatics tools are applied to handle large datasets, extract pertinent data, and draw significant biological insights.

Bioinformatics Methods

These are used to preprocess the raw data from NMR and MS to identify metabolite, peak alignment, and normalization. Various tools are used, such as XCMS, Metabo Analyst, NMR Suite, etc. XCMS is used frequently to align and detect peaks in the MS data. In addition to batch correction and quality control features, which are essential for dealing with big metabolomic datasets, it also provides consolidation of multiple analysis methods into a browser for ease of operation.[51] Statistical methods such as route analysis, clustering, and data normalization are available for the metabolomic data analysis of Metabo Analyst. Therefore, it provides biological interpretation and visualizations for both Targeted and Untargeted metabolomics.[46] NMR data can be explicitly processed for spectral alignment, metabolite identification, and quantification using the NMR-Suite. It is capable of handling large NMR datasets and thereby simplifies precise metabolite identification.[52]

Statistical Analysis

It is normally essential when analyzing metabolomic data. Techniques include multivariate analysis, univariate analysis, pathway analysis, machine learning, etc. Usually, researchers use methods like Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) to find patterns in metabolomic data.[53] Such techniques enable us to distinguish between groups and to reduce data dimensionality, for example, the milk samples from sheep in various stages of lactation and with different diet.

ANOVA and t-tests are applied to individual metabolites to identify significant differences in contrast to other groups. Where univariate methods are helpful for comparing levels of some metabolite in different circumstances, this is referred to as targeted metabolomics.[54]

Pathway analysis techniques connect detected compounds to known metabolic pathways and deliver the data with a biological context. And this method can explain how certain metabolic pathways are switched on at certain times, such as during lactation or after eating certain foods.[55]

More recently, newer machine learning methods are being used more, such as Random Forest or Support Vector Machines (SVM), for example, to predict metabolomics data with the aim of finding biomarkers related to that determined sheep breed or raw sheep milk quality.[56]

Metabolomics research in sheep milk provides important information about the nutritional and bioactive substances present in the milk, which are dependent on breed, diet, and lactation stage.[57] Targeted and untargeted methods serve different research goals, yet analytical techniques such as NMR and MS provide complementary insights into metabolomic patterns. Statistical techniques and bioinformatics technologies establish a strong framework for analyzing complex data within datasets, and statistical analysis remains essential for managing and interpreting the collected data. Together, these methods enhance our understanding of the metabolome of sheep milk and its potential applications in nutrition, food science, and health.

Metabolomic Applications in Sheep Milk

The various factors affecting metabolite composition in sheep milk is shown in Table 3.

Table 3: Factors Affecting Metabolite Composition in Sheep Milk

Factor	Description	Impact on Composition
Sheep Breed[48]	Genetic variations lead to different nutrient and bioactive compound levels	Alters protein, lipid, amino acid profiles
Diet[58]	Type of feed (grass vs. grain) impacts lipid and bioactive compound content	Higher CLA and omega-3 with grass-fed diets
Lactation Stage[48]	Variations in metabolite levels depending on lactation timing	Early lactation milk differs in protein and lipid composition
Environmental Factors[59]	Climate, grazing conditions, and seasonal changes affect mineral and vitamin content	Alters nutrient density, bioactive profiles
Storage and Processing[60]	Methods such as freezing, pasteurization impact metabolite stability	Bioactive compound retention varies

Sheep Milk Metabolites

Sheep milk is distinct among mammalian milks for the higher amounts of vital nutrients, bioactive substances, and metabolites.[32] Various profiles of lipids, amino acids, peptides, carbohydrates, and organic acids make it an essential source of nutrition and functional benefits. Metabolomic investigations have identified a wide range of metabolites, each with distinct functions in nutrition and health. Metabolite profiles significantly influence the bioactivity and nutritional content of sheep milk and exhibit high variability due to various factors, including diet, breed, and environment, as shown by untargeted UHPLC-QTOF/MS revealing pasture-induced enrichment of amino acids, fatty acids, and flavonoids in Sarda sheep milk,[61] GC–MS demonstrating differences in nucleosides, organic acids, and long-chain fatty acids under contrasting grazing systems,[62] and LC–MS metabolomics linking feed efficiency with alterations in glucose, lipid, and sphingolipid pathways in Assaf ewes.[63]

Metabolites - The Main Classes

Lipids

Sheep milk's flavor profile and high-calorie density are due to its lipid content. The major fraction of sheep milk's lipid composition consists of triglycerides, phospholipids, cholesterol, and minor lipid components.[64] Additionally, short- and medium-chain fatty acids abound to allow for energy metabolism and digestion. Bioactivity associated with sheep milk is due to the presence of critical fatty acids, linoleic and alpha-linolenic acid, known to have anti-inflammatory properties.[65] Sheep milk lipids also include conjugated linoleic acid (CLA), a bioactive lipid with potential anti-inflammatory, anticancer, and immunomodulatory properties.

The fatty content of sheep milk is related to their nutrition and surroundings. For instance, milk from sheep-fed grasses from natural pasture has tended to be higher in CLA and healthy omega-3 fatty acids compared to the milk of sheep on grain-based diets.[66] This diversity is particularly important when comparing health effects of sheep milk produced in extensive vs intensive farming methods.

Amino Acids

Being a complete protein source because its amino acids are plentiful and well balanced, sheep milk is a source of all the amino acids the human body needs. Branched-chain amino acids (BCAAs), such as valine, isoleucine, and leucine, have high potential to support metabolic health, as well.[67] Glutamine, an essential amino acid for gut health and immunological function, is also present in very high concentrations in sheep milk. However, it is especially important for promoting intestinal repair after infections or injuries.

An interesting thing to note is that lysine and other specific amino acids are more plentiful in sheep milk than in cow or goat mil.[68] Because sheep milk has a greater lysine content, it is especially good for bone health and tissue healing, but mostly because of this greater lysine content, it's good for collagen production, good for calcium absorption.[69]

Peptides

Biologically active peptides are produced when enzymes in sheep milk hydrolyze milk proteins. Sheep milk is associated with bioactivity due to critical fatty acids, linoleic and alpha-linolenic acid, known to have anti-inflammatory properties. Sheep milk lipids also include conjugated linoleic acid (CLA), a bioactive lipid with potential anti-inflammatory, anticancer, and immunomodulatory properties.[70] Thus, sheep milk peptides may be produced in preparing or digesting food, taken into the bloodstream, and have systemic effects. Bioactive peptides in sheep milk are also studied as a possible involvement in gut health since it has been shown that some peptides stimulate the growth of beneficial bacteria in the gut microbiome.[71] Two examples of variables that can influence the amount and profile of these bioactive peptides are the genetic composition of the sheep, which influences the milk protein composition, and the heat treatment during processing or storage.

Carbohydrates

The principal sources of carbohydrates in sheep milk are lactose, oligosaccharides, and traces of monosaccharides. Lactose is the main carbohydrate of sheep milk, which provides energy and helps absorb nutrients such as calcium and magnesium.[47] Sheep milk has less lactose than cow milk, but because people with lactose intolerance can tolerate a bit less lactose, sheep milk is better for them.

Prebiotic complex carbohydrates called oligosaccharides are present in lower amounts and promote beneficial gut microbiota development.[72] With their many structural variations, sheep milk oligosaccharides act as decoy receptors to which pathogens do not adhere to the intestinal lining due to their immunological functionality. Despite less scrutiny in human milk, sheep milk oligosaccharides have shown potential for gut health in both neonates and adults.[73] The carbohydrate composition of sheep milk can vary depending on the breed and feed, so, for example, the amount of lactose will affect the milk's availability and, consequently, its glycaemic impact and digestibility.

Organic Acids

Organic acids such as lactic acid, citric acid, and short-chain fatty acids are present in sheep milk and are essential for flavor, preservation, and metabolic health. The sour taste of sheep milk products is also partly because lactic acid, produced during fermentation, is an inexpensive natural preservative that inhibits bacterial growth. One other organic acid in sheep milk is citric acid, with known heart health benefits and natural antioxidant properties.[61]

Sheep milk contains short-chain fatty acids (SCFAS), which are essential for gut health and inflammation reduction. Acetate, propionate, and butyrate are included. SCFAs are well known to have immune modifying, gut barrier protecting, and colon cell energy-inducing properties. In sheep's milk, the amount of organic acids can vary depending on the sheep's nutrition, lactation stage, and even season, which affect the milk flavor, shelf life, and health benefits.[74]

Bioactive Compounds

The bioactive components in sheep milk are becoming popular as more of their functions become known. The main bioactive substances in sheep milk are CLA, peptides, SCFAs, and oligosaccharides, which offer many health benefits. CLA in sheep milk has been associated with reducing the risk of obesity, heart disease, and cancer because it changes lipid metabolism and decreases inflammation. The bioactive peptides of sheep milk are well known for their antibacterial, antioxidant, and antihypertensive activity. Potential uses of these peptides in therapeutic applications include oxidative stress, blood pressure, and immunological function. It is known that sheep milk oligosaccharides promote immunity and gut health by acting as decoy receptors for infections and stimulating the proliferation of probiotic bacteria. The SCFAs in sheep milk promote good bacteria against inflammation, which supports good gut health. Thus, sheep milk is good for people with inflammatory gut disorders.[75]

Metabolite Profile Variations

Sheep breed, ambient conditions, and nutrition determine the metabolite composition of sheep milk. Sheep milk contains high nutritional value, and producers have agreed that the type and quality of feed play a significant role. CLA combined with omega-3 PUFA is enhanced by diets created from pasture, with fresh grass and legumes[76]—however, diets containing grains raise other lipids while lowering these healthy substances. Also, irrespective of breed type, sheep milk originating from animals fed with diets high in fiber content tends to contain higher levels of the organic acids and SCFAs that enhance the good standing of the gut.

A study revealed that the nutrient profiles of the studied sheep milk and its mineral and vitamin content depend on the grazing situation. Mineral contents may also vary during certain seasons since changes in pasture content, in combination with meteorological conditions, influence the amount of carbohydrates and proteins; however, milk produced from sheep grazing in mineral-rich soils contains larger amounts of essential minerals.[77]

The fat, protein, and carbohydrate levels found in milk differ between sheep of different breeds. Certain breeds are programmed genetically to derive milk with higher fat, which gives it a rich flavor and creamy finish. Moreover, through breed-specific genetic factors, the percentage composition of bioactive peptides and amino acids may affect the medicinal types of sheep milk.[40]

Fats, proteins, free amino acids and peptides, carbohydrates, and organic acids are present in massive amounts in sheep milk, and all these macromolecules have specific health-promoting benefits and utility factors. CLA, peptides, oligosaccharides, and SCFAs in sheep milk act as immunological, gastrointestinal, and cardiovascular health-promoting bioactive molecules. Based on dietary, environmental, and breed differences, enhancing the beneficial aspects of the sheep milk metabolites in terms of health points of view may be possible.

Comparative Metabolomics Across Species

High degrees of biochemical variation between species, with particular reference to sheep, cows, and goats, are brought out using comparative metabolomics to obtain information concerning the potential nutritional uses and species-selective health benefits. As it possesses physiological, environmental, and genetic components, milk is a highly nutritional food for the offspring and a carrier of biochemical signals. Understanding these metabolomics changes can help in modifying dairy products to meet specific demands, for instance, because of an allergy or lack of needed nutrients, or to better capitalize on health benefits that are associated with the occurrence of certain metabolites.

Metabolic Profiles

Feeding, metabolism, and species-specific pathways influence the metabolic profiles of milk from cows, goats, and sheep. There is a clear difference between sheep, cow, and goat milk because sheep milk is rich in fat and protein, which causes variations in the lipid and amino acids profile. While goat milk that is consumed for everyday drinks by lactose intolerant people contains unique bioactive compounds, cow milk, which is mainly used for the production of dairy products, is mainly responsible for the extensive analysis in metabolomics.[14]

Amino Acids and Peptides

The amino acid and peptide content also differ according to the kind of milk. For instance, branched-chain amino acids, such as leucine and isoleucine, are indispensable for muscle synthesis and energy production, and bioactive peptides are identified at higher concentrations in sheep's milk. Besides, sheep milk is richer in taurine than cow or goat milk, which might benefit infants' neurodevelopment and other health benefits.[78]

Lipids

The percentage of MCFAs, which are easier to digest and are supposed to be useful in energy metabolism, was found to be higher in goat milk. Other lipid compounds, such as phospholipids and sphingolipids, are more complicated molecules that play a significant role in the signalling and structure of the cells of sheep milk. Such lipids have anti-inflammatory properties and are essential to the health of the gastrointestinal tract and brain. Polyunsaturated fatty acids present in sheep milk are also higher than those in other species, which play an essential role in anti-inflammatory and cognitive health. Cow and goat milk, however, contain more saturated and monounsaturated fatty acids; these are healthy but not as bioactive as the lipids found in sheep milk.[79]

Carbohydrates

Compared with cow milk, goat and sheep milk contain higher concentrations and numbers of different oligosaccharides, which are carbohydrates beneficial for the gut.[62] They are helpful in building good gut flora, which is necessary for efficient digestion and the immune system. This study has shown that goat and sheep milk contains higher levels of oligosaccharide fractions, particularly the fucosylated and sialylated oligosaccharides, than cow milk. Thus, goat and sheep milk would be preferred for use in newborn formula replacements to human milk.

Specific Components of Sheep Milk

In functional food and nutraceutical formulations, sheep milk is more desirable because it contains many special metabolites absent in cow or goat milk. Betaine is found in high concentrations in sheep milk, which functions in methylation and osmoregulation. It benefits individuals searching for nutrients that can improve the livers and cardiovascular health.[80]

Sheep milk contains several metabolites associated with the urea cycle ornithine and fatty acid transfer, such as carnitine. As ornithine assists in freeing extra nitrogen, it benefits athletes or people undergoing physical labour exercise.[81] Nonetheless, because carnitine facilitates the breakdown of fat, sheep milk is in order for individuals with energy metabolism disorders. Sheep milk contains a significantly higher number of various types of complex lipids than other types of milk. Sphingolipids have been shown to possess neuroprotective health benefits or effects, while phospholipids are responsible for the structural stability of the cell membrane. They enhance the functional role of sheep milk to fit into functional foods that promote brain and mental health.

Greater Conjugated Linoleic Acid (CLA) Concentrations

Sheep milk contains more CLA, which is considered effective in cutting down body fat and improving the health of the heart.[41] As CLA also possesses anti-carcinogenic properties, sheep milk is also good for consumption with diets to prevent chronic diseases. An understanding of these comparative metabolomics findings allows producers to develop new products containing these nutrients and adapt dairy products to consumers' needs. In addition, the characteristics of sheep milk might enhance potential utility in therapeutic diets and nutraceuticals. Possible questions like the active transport and bioavailability of the peptides, the interaction of the released peptides and its metabolites to enhance the action, and the long-term effect of the milk ingested to the body may be answered by identifying more metabolites in the sheep milk.

Research Limitations and Challenges

Technical Difficulties

Sheep milk contains several complex components that make sampling and analyzing metabolites challenging. Ideal extraction methods should allow for the complete separation of metabolites without loss, but achieving a balance between selectivity and efficiency remains difficult. The fat content in sheep milk complicates extraction since the lipids expected to be separated can obstruct the process.

Sensitivity and Detection Limits

Another disadvantage is the sensitivity of analytical tools because sheep milk contains relatively low levels of metabolites, which may be undetectable using approaches other than NMR spectroscopy or high-resolution mass spectrometry. Although these methods are precise, they are expensive and require professional handling, thus limiting their potential applications.

Reproducibility

In metabolomics studies, achieving a high result replication level is desirable and possible. Potential issues include sampling errors, biases, and sample handling, storage, and preparation inconsistencies. The high lipid content in sheep milk also diminishes reproducibility, as lipids may interfere with other metabolites or degrade over time. Thus, obtaining consistent data across different research investigations presents a challenge.

Variability in Biology

To this end, the compositional components of milk or the metabolomic profile depends on the genetic makeup of different breeds of sheep. The composition of total milk can vary due to breed metabolic rates, genomic potential for milk production, and tolerance to milch animal's environment. For example, the overall score of metabolites may be wholly off because some breeds produce larger volumes of certain bioactive chemicals in the milk. In lactation, there are differences in metabolite concentrations, especially in sheep's bodies. The protein, lipid, and carbohydrate composition described in the milk produced during the early stages of lactation is different from that produced in later stages. Milk yield may differ depending on the time chosen for lactation, so data collected using samples cannot be brought to a common base concerning the research rate; how samples are collected has to be planned with great care.

Environmental Factors

The metabolite content, also known as the profile of metabolites, varies depending on climate, nutrition, and farming practices. For instance, due to nutritional shifts, sheep's raised in cold areas may produce milk with a different lipid concentration. Because of this environmental impact, it is challenging to establish standard metabolomic fingerprints; variations in diet or season can dramatically affect the metabolomic profile.

Complexity of Data

Metabolomic investigations produce vast amounts of data, and analyzing such big data could be challenging. In sheep milk, many metabolites, including individuals possessing different functional properties and interconnected relations, have been detected. This involves understanding metabolic pathways and having trustworthy bioinformatics tools to handle such high-dimensional data.

Standardization and Absence of Reference Data

Unlike human or cow's milk, sheep milk does not benefit from established reference metabolomic databases. This is why comparing results across studies is so difficult and why making sense of data is so tedious, as there are no benchmarks. Developing a sound metabolomic database, particularly for sheep milk, could reduce variability and increase the reliability of the studies.

Integration with Other Omics

Another challenge is compatibility between metabolomic data and the proteins, sugars, and genes data. While glycaemic and proteomics can provide frameworks for understanding metabolomic data, merging these datasets requires certain expertise in bioinformatics. While this integration is still not well developed in sheep milk studies, it is crucial for understanding the biological effects of metabolites.

From these obstacles, it is seen that coordination of methodological and technological efforts is needed. Where genetic studies and environmental standardization minimize biological variation, technical problems can be solved by improved sample preparation, higher-quality instruments, and complex data analysis. To prepare a considerable basis for the analytical data on the metabolites of sheep milk, which in turn would help manufacturers and researchers harness the novelty biomarkers of sheep milk for their benefit. These limitations can, however, be transcended by metabolomic studies of sheep milk to help further explain aspects of health, nutrition as well as possible uses of dairy products towards the treatment of illnesses.

Future Research and Development Opportunities

The two features that are beginning to draw consumers' and investors' attention to sheep milk are the questions of emergent technologies and the available nutrition values, which also define major breakthroughs in sheep milk metabolomics. One such approach that could improve the analytical sensitivity and specificity of detection is high-resolution mass spectrometry (HRMS). Electrophilic compounds such as lipids and carbohydrates in sheep milk as simple or multiple-order structures are characterized using HRMS linked with GC and LC techniques. This enhanced resolution is important in detecting differences of even one part per trillion in fats, proteins, or lactose that may be caused by changes in the environment, diet or breed.

Another crucial aspect of metabolomics is the advancement in bioinformatics. The large volume of current high-throughput metabolomics analysis data requires improved bioinformatics tools for handling, analysis, and interpretation. Our understanding of the significant differences in the metabolic profile of sheep milk can be enhanced through data preprocessing, machine learning, and artificial intelligence, which can assist in identifying and quantifying biomarkers in sheep milk. They also contribute to improving and expanding data integration between metabolomics and other omics data, including proteomics and genetics, providing a clearer picture of sheep milk composition.

The option of personalized nutrition is another intriguing direction. Once metabolomics identifies the bioactive compounds in sheep milk, the nutrients and their functionality can be better tailored to meet the needs of each individual. For instance, milk from sheep contains various fatty acids, peptides, and antioxidants that support immunity, metabolism, and digestion, derived from the naturally occurring glands in the animal's udder. Scientists may design viable probiotics through enhanced metabolomic profiling, which could be beneficial for individuals with restricted diets, diseases, or sensitivities who prefer specialized dairy products with specific nutritional values. This strategy may also expand the market for other sheep milk-related products, such as cheese and butter.

These gaps or deficiencies must be addressed for the advancement of the field to progress to the next level. A broader limitation is that the availability of comparable procedures for sheep milk metabolomics concerning sample pre-treatment and analysis, as well as data acquisition, is essential. Non-convergence methods may hinder the reproduction of results and complicate comparison across studies. There is a necessity to examine the breed, season, and lactation period related to the metabolome of sheep milk. A longitudinal study might help clarify these factors, aiding farmers in optimizing milk production and quality. Moreover, as metabolomics data becomes increasingly complex, integrating results with biological interpretation will be vital, requiring cross-disciplinary collaboration among fields such as nutrition, dairy science, and biochemistry.

Discussion

The comprehensive analysis in this review highlights the considerable potential of sheep milk as an essential ingredient for the advancement of functional foods, a potential predominantly identified through metabolomic research. Sheep milk has a better nutritional profile than traditional dairy sources like cow and goat milk. This is because it has more proteins, lipids, essential minerals, and vitamins. Metabolomics has emerged as an essential instrument for clarifying this difficult biochemical matrix, offering a comprehensive overview of the small molecules that enhance the health-promoting attributes of sheep milk.

A review of the literature illustrates that sheep milk is not only a denser source of macronutrients, but it also contains a lot of bioactive compounds that could be used for medicine. These consist of elevated concentrations of omega-3 and omega-6 fatty acids, conjugated linoleic acid (CLA), and particular casein-derived peptides.[3] The fatty acid profile, especially the high levels of short- and medium-chain fatty acids (MCFAs), not only gives derived foods like cheese their unique taste, but it also makes them easier to digest.[19] The unique casein profile, which has less αs1-casein than cow's milk, may also make it less likely to cause allergies in some people.[6] The presence of bioactive peptides with proven antioxidant, anti-inflammatory, and antihypertensive properties enhances its classification from a mere nutritional source to a functional ingredient.[7]

Metabolomics has been key in helping us understand sheep milk in more detail than just its basic composition. Utilising robust analytical techniques such as NMR, LC-MS, and GC-MS, both targeted and untargeted methodologies have facilitated the identification of more than 200 distinct metabolites.[13] These studies have effectively connected differences in milk composition to variables including breed, diet, lactation stage, and environment.[12] For example, studies have shown that diets based on pasture can add beneficial compounds like CLA and omega-3 fatty acids to sheep milk, while diets based on grain change the lipid profile in a different way.[66] This ability to link outside factors to specific metabolic outputs is important for making production more consistent and improving milk for health benefits like increasing cheese yield or making personalised nutrition products.[25]

Subsequent research ought to concentrate on surmounting these constraints. Improvements in high-resolution mass spectrometry (HRMS) and bioinformatics are likely to make it easier to analyse data and draw conclusions. The next important steps are to make complete databases and standardise protocols. In addition, combining metabolomic data with other "omics" fields, like genomics and proteomics, will give us a complete picture of how biological systems work. These kinds of integrated approaches will be very helpful in getting the most out of sheep milk for making personalised functional foods and nutraceuticals that meet specific health needs. This will make sheep milk an even more important part of consumer healthcare.

Conclusion

Studies on the metabolomics of sheep milk have provided us with the possibility of acquiring increased awareness of the unique nutritional and bioactive profile of sheep milk compared to cow and goat milk. Because of the existence of higher levels of essential amino acids, bioactive peptides, fatty acids, and antioxidants, sheep milk provides an appropriate platform for the production of functional dairy products. A powerful tool made available by metabolomics is the identification of health-promoting substances that may be utilized in the production of special foods in accordance with the requirements of individual nutrition and the requirements of consumers who are concerned about their health. In the future, investigations will be conducted using high-resolution mass spectrometry and advanced bioinformatics to further elucidate the metabolic dynamics of sheep milk. This will allow milk to be subjected to a greater variety of uses in human nutrition and improve its commercial application as a functional food.

Acknowledgement

The authors are thankful to Parul University, Vadodara, Gujarat, India for providing the Article Processing Charges and necessary facilities to carry out this work, and are also grateful to ICAR-National Dairy Research Institute, Karnal, Haryana, India for their support and guidance.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from Other Sources

Not Applicable.

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