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COMPARATIVE COMPOSITIONAL, AMINO ACID AND ANTIOXIDANT PROFILING OF GOAT AND SHEEP MILK: IMPLICATIONS FOR THE DEVELOPMENT OF FUNCTIONAL MILK LOLLIES

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ABSTRACT
Goat and sheep milk are relatively underutilized dairy resources with distinct compositional characteristics that offer potential for the development of value-added frozen dairy products. This study compared the proximate composition, *in vitro* antioxidant activity and amino acid profiles of goat and sheep milk to establish their suitability for milk-lolly formulation. Sheep milk contained significantly ($p < 0.05$) higher fat (6.63 %), total solids (17.37 %), protein (4.57 %) and lactose (5.43 %) than goat milk (3.71 %, 12.35 %, 3.67 % and 4.36 %, respectively). ABTS radical-scavenging activity did not differ significantly between sheep and goat milk (12.14 % and 12.22 %, respectively), whereas sheep milk exhibited significantly higher DPPH radical-scavenging activity (21.12 %) than goat milk (14.60 %). Amino acid profiling revealed species-dependent differences in its distribution, with goat milk characterized by higher relative proportions of glutamic acid, valine and aspartic acid, whereas sheep milk contained higher relative abundances of glutamic acid, leucine, lysine and aspartic acid. The calculated relative proportions of essential amino acids and branched-chain amino acids were significantly higher in sheep milk (50.75% and 25.64%, respectively) than in goat milk (37.08% and 21.35%, respectively). These findings demonstrate that sheep milk provides a more concentrated nutrient with enhanced essential amino acid distribution and antioxidant activity, whereas goat milk offers a lower-fat alternative with comparable ABTS activity and a distinctive amino acid profile. The results provide a scientific basis for species-specific formulation of non-bovine milk lollies and other frozen dairy products.

KEYWORDS: ABTS; Amino acid profile; Antioxidant activity; DPPH; Functional dairy products; Goat milk; Milk lollies; UHPLC; Sheep milk

1. INTRODUCTION

Small-ruminant milk represents an underutilized source of dairy resource with compositional and physicochemical characteristics that differ from those of bovine milk (Clark & Mora García, 2017; Balthazar *et al.*, 2017; Siddiqui *et al.*, 2024). Sheep milk contains higher concentration of total solids, fat and protein, while both goat and sheep milk contain relatively high proportions of short- and medium-chain fatty acids that influence nutritional quality, digestibility, flavour development and processing behaviour. In both goat and sheep milk, variations in casein genotype, casein micelle structure, mineral equilibrium, fat globule size distribution and whey protein composition affect coagulation properties, water-binding capacity, rheological behaviour and the microstructure of dairy products (Park *et al.*, 2007; Roy *et al.*, 2020; Moatsou *et al.*, 2021; Li *et al.*, 2023). These intrinsic characteristics have stimulated growing interest in the utilization of small-ruminant milk for the development of value-added functional dairy products. In addition to their compositional advantages, goats and sheep are well adapted to semi-arid and resource-limited environments, efficiently converting low-quality forage into nutrient-dense milk while contributing to household nutrition, livelihood security and sustainable dairy production. Nevertheless, commercial utilization of small-ruminant milk remains largely restricted to traditional fermented products and specialty cheeses, whereas its application in frozen dairy products has received comparatively limited scientific attention.

Milk composition is a primary determinant of processing performance and product quality because the concentrations of fat, protein, lactose and total solids govern viscosity, freezing behaviour, ice-crystal formation, water immobilization, melting resistance and textural stability. A recent global meta-analysis reported average goat milk composition of approximately 12.7% total solids, 4.1% fat, 3.5% protein and 4.4% lactose, while emphasizing considerable variation attributable to breed, feeding system, geographical location, season and stage of lactation (Akshit *et al.*, 2024). Comparative

investigations across dairy species have consistently shown that sheep milk possesses the highest concentrations of total solids, fat, protein and casein among domesticated ruminants, resulting in higher nutrient density and enhanced processing efficiency (Rafiq *et al.*, 2015). The higher casein and total solids content of sheep milk promotes stronger protein network formation, increased serum-phase immobilization and improved structural characteristics, whereas the relatively lower solids content of goat milk produces distinct physicochemical behaviour during dairy processing. Such compositional differences are expected to influence the formulation, freezing characteristics and quality attributes of frozen dairy confections, indicating that goat and sheep milk should not be regarded as interchangeable raw materials.

Beyond proximate composition, amino acid composition provides a more detailed assessment of protein quality and nutritional value (FAO, 2013; Mathai *et al.*, 2017). Milk proteins supply all indispensable amino acids required for human nutrition, while branched-chain amino acids (BCAAs), particularly leucine, isoleucine and valine, play pivotal roles in skeletal muscle protein synthesis through activation of mammalian target of rapamycin (mTOR)-mediated signaling pathways (Drummond & Rasmussen, 2008; Kaspy *et al.*, 2024). Lysine contributes to tissue protein accretion and collagen synthesis, whereas threonine is involved in mucin biosynthesis and maintenance of intestinal integrity. Previous studies have demonstrated that goat and sheep milk possess comparable qualitative amino acid profiles but differ quantitatively according to species, breed and production system (Landi *et al.*, 2021; Rafiq *et al.*, 2015). Glutamic acid generally predominates among non-essential amino acids, whereas leucine, lysine, valine and threonine are constitute major components of the indispensable amino acid pool. Moreover, the amino acid composition of milk proteins influences the generation of bioactive peptides during gastrointestinal digestion and dairy processing, thereby affecting their nutritional and biological potential (Tagliazucchi *et al.*, 2018).

Milk also contains an antioxidant components including enzymatic antioxidants, sulfur-containing amino acids, antioxidant peptides, uric acid, phospholipids and other reducing compounds distributed within the casein micelles, whey fraction and milk-fat globule membrane (Khan *et al.*, 2019; Grażyna *et al.*, 2017; Nie *et al.*, 2024). The antioxidant activity measured in milk is influenced by assay chemistry, radical type, reaction kinetics and the accessibility of hydrophilic and lipophilic antioxidant constituents (Prior *et al.*, 2005; Cömert & Gökmen, 2018). Consequently, ABTS and DPPH radical-scavenging assays frequently produce different antioxidant activity because the ABTS radical is reactive toward both hydrophilic and lipophilic antioxidants, whereas DPPH preferentially measures compounds with higher affinity for organic media (Re *et al.*, 1999; Ozgen *et al.*, 2006; Floegel *et al.*, 2011). Comparative studies have reported species-dependent antioxidant activities among bovine, goat and sheep milk and demonstrated that antioxidant capacity differs among whole milk, casein and whey fractions (Bučević-Popović *et al.*, 2014; Yilmaz-Ersan *et al.*, 2018; Khaledian *et al.*, 2025). Therefore, simultaneous application of complementary radical-scavenging assays provides a more comprehensive characterization of milk antioxidant potential.

Frozen dairy confections prepared from goat and sheep milk offer an attractive opportunity to diversify small-ruminant dairy products while addressing consumer demand for convenient, nutrient-dense foods (Balthazar *et al.*, 2017; Siddiqui *et al.*, 2024). Unlike aerated ice cream, milk lollies are non-aerated frozen systems in which structural stability is governed primarily by the interactions among milk proteins, fat globules, lactose, stabilizers and the ice-crystal network (Goff, 1997; Goff, 2002; Muse & Hartel, 2004). Consequently, differences in milk composition directly influence viscosity, freezing point depression, ice recrystallization, melting behaviour and sensory quality (Bahramparvar & Tehrani, 2011; Muse & Hartel, 2004; Soukoulis & Fisk, 2016). Detailed characterization of the compositional and biochemical attributes of goat and sheep milk is therefore essential for designing species-specific formulations capable of maximizing nutritional quality and technological performance (Clark & Mora García, 2017; Park *et al.*, 2007; Balthazar *et al.*, 2017; Siddiqui *et al.*, 2024). Although several studies have independently described the composition, antioxidant properties or amino acid profiles of goat and sheep milk (Rafiq *et al.*, 2015; Landi *et al.*, 2021; Khaledian *et al.*, 2025), comparatively few investigations have integrated proximate composition, radical-scavenging activity and chromatographic amino acid profiling while interpreting these characteristics in relation to frozen dairy product development. Accordingly, the present study compared the proximate composition, *in vitro* antioxidant capacity determined by ABTS and DPPH radical-scavenging assays, and amino acid profiles of goat and sheep milk.

2. MATERIALS AND METHODS

MILK COLLECTION AND SAMPLE PREPARATION

Raw goat and sheep milk samples were obtained from healthy animals maintained by local dairy farmers in and around Amreli, Gujarat, India. Milk was collected under hygienic conditions into sterile food-grade containers and transported to the Department of Dairy Technology, College of Dairy Science, Kamdhenu University, Amreli, in insulated containers under refrigerated conditions (≤5 °C). Upon arrival, samples were immediately stored at 4 ± 1 °C and all physicochemical, antioxidant and amino acid analyses were initiated on the day of collection to minimize compositional changes during storage.

PROXIMATE COMPOSITION

Fat, total solids, protein and lactose contents were determined using a calibrated MilkoScan™ milk analyser (IndiFOSS Analytical Pvt. Ltd., India). The instrument was standardised before analysis, and each sample was mixed gently to ensure uniform distribution of milk constituents. Results were reported as percentage by mass.

ABTS RADICAL-SCAVENGING ACTIVITY

The antioxidant capacity of goat and sheep milk was determined using the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolourization assay according to Re *et al.* (1999), with minor modifications for milk samples as described by Chen *et al.* (2003) and Niero *et al.* (2017). ABTS (≥98%; Sigma-Aldrich, USA) was dissolved in deionized water to obtain a 7.0 mM solution and reacted with 2.45 mM potassium persulfate (analytical grade; Merck, Germany). The mixture was incubated in the dark at room temperature (22–25 °C) for 12–16 h to generate the ABTS radical cation ($ABTS^{\bullet+}$). Before analysis, the radical solution was diluted with 10 mM phosphate-buffered saline (PBS, pH 7.4) to obtain an absorbance of 0.700 ± 0.020 at 734 nm, measured using a Shimadzu UV-1900i UV–Visible spectrophotometer (Shimadzu, Japan) equipped with 1 cm quartz cuvettes.

Milk samples were homogenized and centrifuged at 5,000 × g for 15 min at 4 °C using a refrigerated centrifuge (Eppendorf Centrifuge). The cream layer was removed, and the clarified milk serum was collected for analysis. Frozen dairy samples were thawed at 4 °C and processed using the same clarification procedure. When necessary, samples were diluted with 10 mM PBS (pH 7.4) to ensure that the measured inhibition remained within the linear range of the assay.

For the reaction, 0.20 mL of clarified sample was mixed with 3.80 mL of freshly diluted $ABTS^{\bullet+}$ working solution, vortexed, and incubated in the dark at room temperature for 6 min. Absorbance was measured at 734 nm against the corresponding reagent blank. A reagent control containing $ABTS^{\bullet+}$ solution without sample and a sample blank containing sample extract without $ABTS^{\bullet+}$ were included to correct for background absorbance due to sample colour and residual turbidity.

ABTS radical-scavenging activity was calculated as:

$$\text{ABTS inhibition (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where, $A_{Control}$ is the absorbance of the $ABTS^{\bullet+}$ solution without sample, and A_{Sample} is the blank-corrected absorbance of the sample after reaction with the ABTS radical cation.

DPPH RADICAL-SCAVENGING ACTIVITY

The free radical-scavenging activity of goat and sheep milk was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Epsin *et al.* (2000) with minor modifications for milk samples. DPPH (≥95% purity; Sigma-Aldrich, USA) was dissolved in HPLC-grade ethyl acetate (Merck, Germany) to prepare a 0.10 mM stock solution. The reagent was freshly prepared before each analysis, protected from light, and diluted with methanol to obtain an absorbance at 515 nm, measured using a Shimadzu UV-1900i UV–Visible spectrophotometer (Shimadzu, Japan) equipped with 1 cm quartz cuvettes.

Milk samples were homogenized and centrifuged at 5,000 × g for 15 min at 4 °C using a refrigerated centrifuge (Eppendorf Centrifuge). After removal of the cream layer, 1.0 mL of the milk serum was extracted with 9.0 mL of 80% (v/v) HPLC-grade ethyl acetate, vortex-mixed for 1 min, incubated at room temperature in the dark for 30 min, and centrifuged again at 5,000 × g for 10 min. The clear supernatant was used for antioxidant analysis. Frozen dairy samples were thawed at 4 °C and processed using the same extraction procedure.

For the assay, 0.20 mL of the sample was mixed with 3.80 mL of the DPPH working solution, vortexed, and incubated for 30 min at room temperature in the dark. Absorbance was measured at 515 nm against ethyl acetate as the blank. A reagent control (DPPH solution without sample) and a sample blank (sample extract without DPPH) were included to correct for background absorbance arising from sample colour and turbidity. DPPH radical-scavenging activity was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{Control}} \times 100$$

Where, $A_{Control}$ is the absorbance of the control reaction mixture containing DPPH solution without sample extract, and A_{Sample} is the blank-absorbance of the sample reaction mixture after incubation with DPPH solution.

Amino acid profiling by UHPLC

The amino acid profiles of goat and sheep milk were determined using an UHPLC system following acid hydrolysis and pre-column derivatization, based on Henderson *et al.* (2000) and Rutherfurd and Gilani (2009), with modifications. Amino acids were identified by comparison with standard retention times. As external calibration standards were unavailable, the results were expressed as relative peak area percentage:

$$\text{Relative amino acid peak area (\%)} = \frac{\text{Peak area of individual amino acid}}{\text{Total peak area of all resolved amino acids}} \times 100$$

Identified amino acids were further grouped as essential, branched-chain, acidic, basic and aromatic amino acids.

Derived amino acid indices

Derived amino acid indices were calculated from the semi-quantitative UHPLC profiles to facilitate comparative evaluation of amino acid distribution between goat and sheep milk. Calculations were based exclusively on the identified chromatographic peaks and expressed as the relative percentage of the total integrated chromatographic peak area.

Essential amino acids (EAAs) were calculated as the sum of histidine, threonine, valine, methionine, phenylalanine, isoleucine, leucine and lysine according to the indispensable amino acid classification of WHO/FAO/UNU (2007). Tryptophan was excluded because it was not detected following conventional acid hydrolysis. Branched-chain amino acids (BCAAs) were calculated as the combined relative peak areas of valine, isoleucine and leucine. Acidic amino acids comprised aspartic acid and glutamic acid, whereas basic amino acids included histidine, arginine and lysine. Aromatic amino acids were calculated as the sum of phenylalanine and tyrosine.

The derived amino acid indices were calculated as follows:

$$\text{Relative EAA (\%)} = \text{His} + \text{Thr} + \text{Val} + \text{Met} + \text{Phe} + \text{Ile} + \text{Leu} + \text{Lys}$$

$$\text{Relative BCAA (\%)} = \text{Val} + \text{Ile} + \text{Leu}$$

$$\text{Relative acidic amino acids (\%)} = \text{Asp} + \text{Glu}$$

$$\text{Relative basic amino acids (\%)} = \text{His} + \text{Arg} + \text{Lys}$$

$$\text{Relative aromatic amino acids (\%)} = \text{Phe} + \text{Tyr}$$

Because these indices were derived from relative chromatographic peak areas rather than calibration-based amino acid concentrations, they were used solely to compare amino acid distribution between milk species. Accordingly, the derived indices should not be interpreted as absolute amino acid concentrations, amino acid scores, protein digestibility-corrected amino acid scores (PDCAAS), digestible indispensable amino acid scores (DIAAS), or direct measures of protein quality.

STATISTICAL ANALYSIS

Statistical analysis of proximate composition and antioxidant activity data was carried out using SPSS software, version 26.0. The experimental data were analysed following a completely randomized design. Results were expressed as mean ± standard deviation. Significant differences between milk species were assessed by analysis of variance (ANOVA), and mean separation was performed using Duncan's Multiple Range Test at $p < 0.05$, as described by Snedecor and Cochran (1989).

Amino acid chromatographic profiles were interpreted descriptively because replicate-based concentration data and associated variance estimates were not available in the present dataset. Therefore, amino acid results were presented as semi-quantitative relative peak-area profiles and derived amino acid-group indices, without inferential statistical comparison. The unidentified chromatographic peak was excluded from derived nutritional amino acid-group calculations.

3. RESULTS AND DISCUSSION

Proximate composition of goat and sheep milk

Considerable species-dependent differences were observed in the proximate composition of goat and sheep milk (**Table 1**). Sheep milk contained significantly ($p < 0.05$) higher concentrations of fat, total solids, protein and lactose than goat milk. The fat content of sheep milk (6.63 ± 0.48%) was approximately 1.79-fold higher than that of goat milk (3.71 ± 0.42%), while total solids increased from 12.35 ± 0.25% in goat milk to 17.37 ± 0.30% in sheep milk. Similarly, protein concentration was significantly higher in sheep milk (4.57 ± 0.08%) than in goat milk (3.67 ± 0.12%), whereas lactose exhibited a comparatively smaller but significant increase from 4.36 ± 0.17% to 5.43 ± 0.09%.

Table 1. Proximate composition of raw goat and sheep milk

Species	Fat (%)	Total solids (%)	Protein (%)	Lactose (%)
Goat milk	3.71 ± 0.42^{b}	12.35 ± 0.25^{b}	3.67 ± 0.12^{b}	4.36 ± 0.17^{b}
Sheep milk	6.63 ± 0.48^{a}	17.37 ± 0.30^{a}	5.43 ± 0.09^{a}	4.57 ± 0.08^{a}

Values are mean ± SE (n = 10). Superscript (a, b) indicates significant ($p<0.05$) differences among the species

The higher concentration of milk constituents in sheep milk reflects species-specific differences in mammary gland metabolism, nutrient partitioning and milk secretion efficiency (Balthazar *et al.*, 2017; Roy *et al.*, 2021). Sheep milk is characterized by a higher proportion of casein and milk solids, resulting in enhanced nutrient density and a higher concentration of structural and functional milk components (Rafiq *et al.*, 2015; Balthazar *et al.*, 2017; Roy *et al.*, 2021). In contrast, goat milk contains a higher proportion of water relative to total solids, yielding a comparatively lower total solids in milk (Akshit *et al.*, 2024; Roy *et al.*, 2021). These compositional differences arise from genetic background, breed characteristics, physiological status, feeding regime, stage of lactation and environmental conditions, all of which influence mammary synthesis of milk fat, proteins and lactose (Clark & Mora García, 2017; Akshit *et al.*, 2024). The compositional values obtained for goat milk were within the ranges reported in the global meta-analysis of Akshit *et al.* (2024), while the higher concentrations of fat, protein and total solids in sheep milk agree with previous comparative studies across domestic dairy species (Rafiq *et al.*, 2015; Balthazar *et al.*, 2017; Roy *et al.*, 2021).

From a technological perspective, the elevated total solids and protein content of sheep milk is expected to influence the physicochemical behaviour of frozen dairy products (Goff, 2002; Hartel *et al.*, 2017; Alvarez *et al.*, 2005). Higher protein concentrations increase water-binding capacity and promote the formation of a more continuous protein network, whereas increased total solids reduce the proportion of free water available for ice crystal formation (Goff, 2002; Alvarez *et al.*, 2005; Soukoulis & Fisk, 2016). These characteristics generally improve mix viscosity, reduce ice crystal growth during

frozen storage and enhance melting resistance, thereby contributing to improved body and texture of frozen dairy products (Muse & Hartel, 2004; BahramParvar & Tehrani, 2011; Soukoulis & Fisk, 2016). The higher lactose concentration may also contribute to freezing-point depression, although excessive lactose levels require careful formulation to minimize lactose crystallization during storage (Hartel *et al.*, 2017; Huppertz & Gazi, 2016). In addition, the higher fat content of sheep milk can improve creaminess and flavour release but necessitates appropriate fat standardization to achieve the desired nutritional profile and comply with product specifications (Roland *et al.*, 1999; Goff, 2002; Hyvönen *et al.*, 2003). Conversely, the comparatively lower total solids and protein content of goat milk produce a less concentrated colloidal system with reduced viscosity and lower solids recovery (Akshit *et al.*, 2024b; Rafiq *et al.*, 2015; Akshit *et al.*, 2024a). Consequently, formulation of goat milk-based frozen products may require optimization of milk solids, stabilizer concentration or protein supplementation to obtain physicochemical characteristics comparable with those of sheep milk formulations (Alvarez *et al.*, 2005; BahramParvar & Tehrani, 2011; Kurultay *et al.*, 2010). Nevertheless, the naturally lower fat content of goat milk provides higher flexibility for developing reduced-fat frozen dairy products while maintaining an entirely dairy-based formulation (Akshit *et al.*, 2024; Rafiq *et al.*, 2015; Roland *et al.*, 1999).

ANTIOXIDANT ACTIVITY AND ASSAY-SPECIFIC RESPONSE

The antioxidant activities of goat and sheep milk exhibited assay-dependent differences (**Table 2**). No significant difference ($p > 0.05$) was observed in ABTS radical-scavenging activity, with sheep and goat milk exhibiting inhibition values of 12.14 ± 0.09% and 12.22 ± 0.10%, respectively. In contrast, DPPH radical-scavenging activity differed significantly ($p < 0.05$) between the two species, with sheep milk (21.12 ± 0.19%) exhibiting approximately 44.7% higher inhibition than goat milk (14.60 ± 0.16%). These results indicate that the antioxidant behaviour of milk is dependent on the chemical characteristics of the radical system employed rather than representing a single intrinsic antioxidant property (Prior *et al.*, 2005; Apak *et al.*, 2016a, 2016b; Xiao *et al.*, 2020).

Table 2. Antioxidant profile of goat and sheep milk

Sample	ABTS inhibition (%)	DPPH inhibition (%)
Sheep milk	12.14 ± 0.09^{a}	21.12 ± 0.19^{a}
Goat milk	12.22 ± 0.10^{a}	14.60 ± 0.16^{b}

Values are mean ± SE (n =10); Superscript (a, b) indicates significant ($p<0.05$) differences among the species

The contrasting responses obtained with the ABTS and DPPH assays reflect their different reaction mechanisms and solvent environments (Brand-Williams *et al.*, 1995; Re *et al.*, 1999; Prior *et al.*, 2005; Apak *et al.*, 2016a). The ABTS radical cation ($ABTS^{\bullet+}$) is soluble in both aqueous and organic media and reacts readily with hydrophilic and lipophilic antioxidants through electron-transfer and hydrogen atom transfer mechanisms (Re *et al.*, 1999; Apak *et al.*, 2016a, 2016b). Consequently, the ABTS assay provides a broader estimate of the overall antioxidant capacity of milk. In contrast, DPPH is a relatively hydrophobic free radical dissolved in organic solvents, making its response more sensitive to compounds with higher lipophilic character and to antioxidants capable of rapid hydrogen atom donation (Brand-Williams *et al.*, 1995; Prior *et al.*, 2005; Apak *et al.*, 2016a). The comparable ABTS activities observed for goat and sheep milk therefore suggest that both species may possess a similar water- and lipid-soluble antioxidant constituents, whereas the significantly higher DPPH scavenging activity of sheep milk indicates differences in specific antioxidant components or their relative abundance.

The higher DPPH inhibition observed in sheep milk is consistent with its significantly higher protein, fat and total solids contents (**Table 1**). Milk proteins, particularly caseins and whey proteins, contain antioxidant amino acid residues including tyrosine, tryptophan, methionine, histidine and cysteine that can donate electrons or hydrogen atoms to neutralize free radicals (Chen *et al.*, 2003; Zulueta *et al.*, 2009; Khan *et al.*, 2019). In addition, sulfur-containing amino acids, phosphopeptides, vitamins, and endogenous antioxidant enzymes collectively contribute to the antioxidant activity of milk (Chen *et al.*, 2003; Khan *et al.*, 2019; Stobiecka *et al.*, 2022). Khaledian *et al.* (2025) reported higher antioxidant potential in sheep milk than in goat and bovine milk and attributed this difference to the higher concentrations of antioxidant proteins and bioactive components present in sheep milk.

For product development, the antioxidant activities reported here represent the properties of raw milk. Thermal processing, homogenization, ingredient incorporation, freezing and frozen storage can modify protein conformation, promote peptide release, induce lipid oxidation and alter the availability of antioxidant compounds, thereby changing the measured radical-scavenging activity (Chen *et al.*, 2003; Oh *et al.*, 2013; Khan *et al.*, 2019; Stobiecka *et al.*, 2022). Kalyan *et al.* (2021) demonstrated that thermal processing influenced the antioxidant capacity of goat milk, although the magnitude and direction of the response depended on processing conditions and the analytical assay employed. Consequently, the antioxidant characteristics of milk lollies should be determined after manufacture and throughout frozen storage to evaluate antioxidant retention under actual processing conditions.

Comparative amino acid profile of goat and sheep milk

The HPLC chromatographic profiles revealed differences between species in the relative distribution of amino acids between goat and sheep milk (**Figure 1**). In both species, glutamic acid was the predominant amino acid, accounting for 28.30% and 21.25% in goat and sheep milk, respectively. Aspartic acid represented the second major acidic amino acid,

contributing 13.09% in goat milk and 9.77% in sheep milk. The predominance of glutamic and aspartic acids is consistent with the amino acid composition of caseins and whey proteins, which are naturally enriched in acidic amino acid residues and play important roles in maintaining protein structure, calcium binding and functional properties of milk proteins (Rafiq *et al.*, 2015; Holt *et al.*, 2013; Landi *et al.*, 2021).

Despite this common pattern, marked differences were evident in the distribution of essential amino acids. Goat milk exhibited relatively higher proportions of valine (13.91%), whereas sheep milk was characterized by substantially higher proportions of leucine (13.61%) and lysine (13.15%). Compared with goat milk, the relative abundance of leucine and lysine in sheep milk increased by approximately 2.75-fold and 4.00-fold, respectively, whereas the relative valine content of goat milk was approximately 2.27-fold higher than that of sheep milk. Methionine was detected only in sheep milk (0.39%) under the chromatographic conditions employed, while the corresponding peak was not resolved in the goat milk chromatogram. These observations indicate species-specific differences in protein composition and amino acid distribution despite the broadly similar qualitative amino acid profiles (Rafiq *et al.*, 2015; Landi *et al.*, 2021).

Derived amino acid indices further emphasized these compositional differences (**Table 5)**. Sheep milk exhibited a substantially higher proportion of essential amino acids (50.75%) than goat milk (37.08%). Similarly, the relative abundance of branched-chain amino acids (BCAAs), comprising leucine, isoleucine and valine, was higher in sheep milk (25.64%) than in goat milk (21.35%). Basic amino acids also contributed a considerably larger proportion of the chromatographic profile in sheep milk (16.04%) than in goat milk (6.60%). Conversely, goat milk exhibited a higher proportion of acidic amino acids (41.39%), reflecting the higher relative abundance of glutamic and aspartic acids.

Table 5. Derived amino acid-group indices based on chromatographic peak area

Amino acid group	Goat milk (%)	Sheep milk (%)
Essential amino acids[1]	37.077	50.745
Branched-chain amino acids[2]	21.352	25.641
Acidic amino acids[3]	41.387	31.018
Basic amino acids[4]	6.601	16.035
Aromatic amino acids[5]	5.990	5.009

[1]His + Thr + Val + Met + Phe + Ile + Leu + Lys; [2]Val + Ile + Leu; [3]Asp + Glu; [4]His + Arg + Lys; [5]Phe + Tyr. Values are relative peak-area proportions and not absolute concentrations.

The observed amino acid distribution agrees with previous reports describing glutamic acid as the predominant non-essential amino acid in small-ruminant milk proteins. Rafiq *et al.* (2015) reported that glutamic acid constitutes the major amino acid residue within casein and whey protein fractions, while Landi *et al.* (2021) similarly identified glutamic acid/glutamine as the most abundant amino acid component in goat and sheep milk. However, previous studies have also

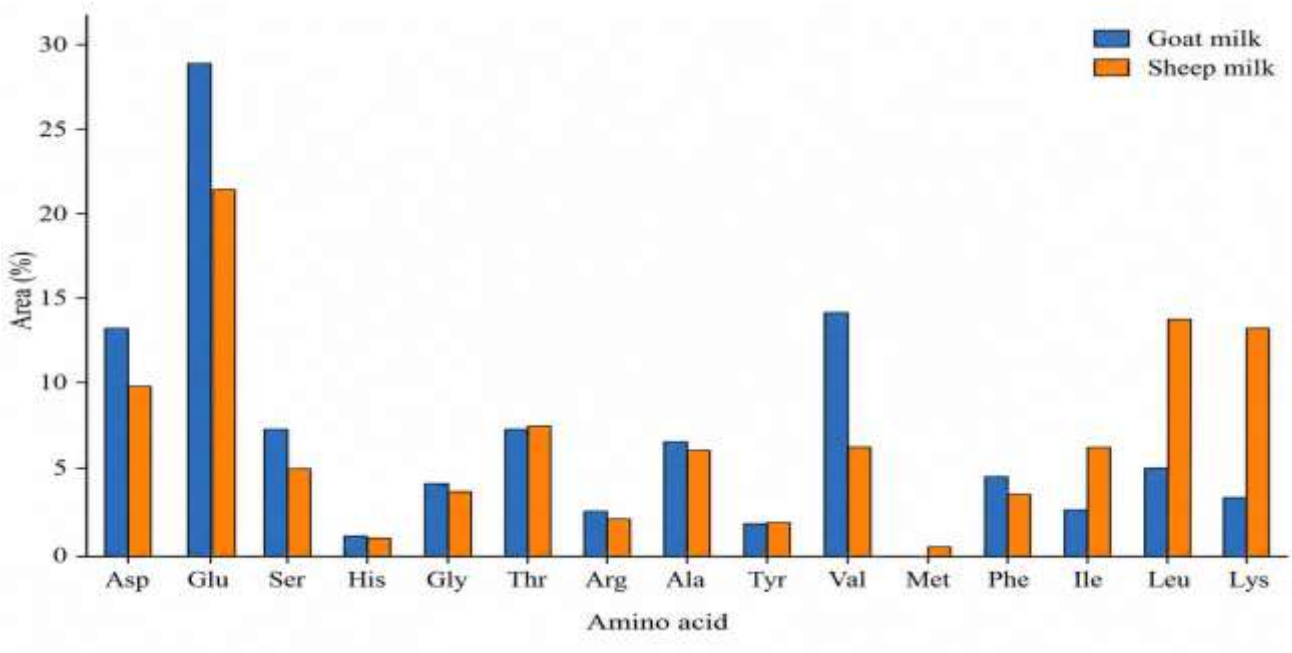

Figure 1 Comparative semi-quantitative amino acid profile of goat and sheep milk

demonstrated that the quantitative distribution of essential amino acids varies among species, breeds etc (Rafiq *et al.*, 2015; Landi *et al.*, 2021; Clark & Mora García, 2017).

From a nutritional perspective, the higher relative abundance of leucine and other BCAAs in sheep milk is noteworthy because these amino acids play central roles in skeletal muscle protein synthesis through activation of the mammalian target of rapamycin (mTOR) signalling pathway and contribute to maintenance of muscle protein turnover (Norton & Layman, 2006; Yoon, 2017; Neinast *et al.*, 2019). Likewise, lysine is indispensable for protein synthesis, collagen formation and calcium utilization, whereas glutamic acid serves as a major metabolic intermediate and contributes to the sensory characteristics of dairy products (WHO/FAO/UNU, 2007; Landi *et al.*, 2021).

The amino acid composition also has important technological implications for dairy processing. Variations in amino acid distribution influence protein hydration, intermolecular interactions, thermal stability, enzymatic hydrolysis and the generation of bioactive peptides during fermentation and gastrointestinal digestion (Foegeding *et al.*, 2002; O'Mahony & Fox, 2013; Tagliazucchi *et al.*, 2018). Tagliazucchi *et al.* (2018) demonstrated that digestion of goat and sheep milk proteins releases several peptides with reported antioxidant, antihypertensive and other biological activities. Although the present study did not evaluate peptide release or bioactivity directly, the higher relative abundance of essential amino acids and BCAAs in sheep milk suggests a protein composition that may favour the generation of nutritionally valuable peptide fractions following digestion (proteolytic enzyme) (Tagliazucchi *et al.*, 2018; Nielsen *et al.*, 2017; Nielsen *et al.*, 2024).

Implications for milk lolly development

The compositional, antioxidant and amino acid profiles demonstrate that goat and sheep milk should be considered distinct raw materials for milk-lolly formulation (Clark & Mora García, 2017; Balthazar *et al.*, 2017; Rafiq *et al.*, 2015). Sheep milk, with its higher total solids, protein, essential amino acids and DPPH radical-scavenging activity, provides a nutrient-dense matrix suitable for high-milk-solids products (Balthazar *et al.*, 2017; Rafiq *et al.*, 2015; Alvarez *et al.*, 2005). In contrast, goat milk offers lower fat content, comparable ABTS antioxidant activity and a distinctive amino acid profile, making it suitable for reduced-fat formulations (Clark & Mora García, 2017; Akshit *et al.*, 2024; Roland *et al.*, 1999). These differences indicate that formulation parameters, including fat standardization, milk solids and stabilizer levels, should be optimized separately for each milk type (Alvarez *et al.*, 2005; BahramParvar & Tehrani, 2011; Soukoulis & Fisk, 2016).

The present findings represent the properties of raw milk and should not be directly extrapolated to finished products. Processing and frozen storage may alter antioxidant activity, amino acid composition and functional properties (Khan *et al.*, 2019; Stobiecka *et al.*, 2022; van Lieshout *et al.*, 2020). Therefore, future studies should evaluate amino acid retention, antioxidant stability, physicochemical characteristics, melting behaviour, sensory quality and storage stability of the developed milk lollies before any functional or health-related claims are made (Muse & Hartel, 2004; Soukoulis & Fisk, 2016; Brodkorb *et al.*, 2019; Grundy *et al.*, 2025).

4. CONCLUSIONS

Goat and sheep milk exhibited distinct compositional, antioxidant and amino acid characteristics with important implications for non-bovine dairy product development. Sheep milk contained significantly higher fat, protein, lactose and total solids and demonstrated higher DPPH radical-scavenging activity, whereas ABTS activity was comparable between species. Semi-quantitative HPLC profiling revealed higher relative proportions of leucine, lysine and essential amino acids in sheep milk, while goat milk was enriched in glutamic acid, valine and aspartic acid. These findings support species-specific formulation strategies for milk lollies and other value-added dairy products. Future studies should quantify amino acids using calibration-based chromatographic methods and evaluate the effects of processing and frozen storage on antioxidant activity, amino acid retention, physicochemical properties, microstructure, sensory quality and storage stability. In addition, digestion and bio-accessibility studies are required to substantiate the nutritional and functional potential of goat- and sheep-milk-based products.

COMPETING INTEREST DECLARATION

The author declares that there are no known financial or personal competing interests that could have influenced the work reported in this manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are presented within the manuscript. Additional chromatographic records may be made available by the corresponding author upon reasonable request.

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