



FROM STABLE TO SHELF: DONKEY MILK-DERIVED PROBIOTICS IN FUNCTIONAL FOOD DEVELOPMENT

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From Stable to Shelf: Donkey Milk–Derived Probiotics in Functional Food Development

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Abstract

Donkey milk is increasing the interest of perspective to develop new functional foods. Unlike cow's or goat's milk, donkey's milk has an interesting nutrient profile, is mildly hypoallergenic and contains bioactive compounds like lysozyme and lactoferrin that promote various health and immune functions. Donkey milk has the advantage of being prebiotic in nature and contains a diverse range of lactic acid bacteria (LAB) that may have unique probiotic properties, including the ability to survive acidic and bile environments, produce antimicrobial metabolites, and produce beneficial exopolysaccharides. Paired with prebiotics, including inulin, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), the probiotic strains found in donkey milk have the ability to create very effective synbiotic combinations with additional health benefits. This review summarizes current knowledge regarding the nutritional composition of donkey milk, the probiotic potential of donkey milk microbiota, research conducted using donkey milk fermentation studies, evidence of bacterial combinations from fermentation paired with prebiotics, and the potential or challenges to develop innovative functional synbiotic products.

Keywords: Donkey milk, probiotics, prebiotics, synbiotics, lactic acid bacteria (LAB), lysozyme, lactoferrin

1. Introduction

Milk has been an important part of the human diet since ancient times, not only as an energy source but as a delivery system for essential macronutrients, micronutrients, and bioactive compounds. Cow's milk is the most widely consumed dairy around the world; however, in many Western nations, its relatively high proportion of saturated fatty acids has raised concerns over the health effects due to associations with elevated cardiovascular risk and metabolic diseases [1]. Cow's milk protein allergy (CMPA) and lactose intolerance are common conditions, particularly in infants and young children, that call for alternative milk sources that are hypoallergenic [2,3]. In this regard, donkey milk has shown promise as an alternative source of milk in both a nutritional and functional sense.

Donkey milk is unique because of its similarity in composition to human breast milk. It has relatively low protein (1.5-1.8g/100mL) with a casein-to-whey protein ratio that is closer to human milk, as well as high lactose (~6.0g/100mL) which improves the milk's palatability and calcium absorption [4,5]. The fat content is considerably lower than bovine milk, measuring between 0.3-1.8g/100mL, and the proportion of polyunsaturated fatty acids (PUFAs) is higher, which potentially provide anti-inflammatory and cardioprotective properties [1]. This unique nutritional profile makes donkey milk a good option for pediatric nutrition, particularly for children with CMPA, as clinical studies have demonstrated good tolerability and adequate increases in growth when used as a substitute [6,7].

In addition to the composition of macro- and micronutrients, donkey milk is a source of a variety of functional and active bioactive components. Lysozyme, lactoferrin, oligosaccharides, and immunoglobulins are significant due to their antimicrobial, immunomodulatory, and prebiotic properties [8,9]. The content of lysozyme in donkey milk (1.0–4.0 mg/mL) is shown to be considerably more than in bovine milk, establishing a natural antimicrobial defense barrier toward Gram-positive bacteria [10]. Lactoferrin is an iron-binding glycoprotein that assists in host defense by sequestering free-iron which is essential for microbial growth and regulates immune responses [11]. Oligosaccharides also serve as prebiotics to stimulate growth of beneficial bacteria (e.g. Bifidobacterium, Lactobacillus) within the gut; an effect that underpins gut health of infants while contributing to immune system maturation [3].

The indigenous microbiota of donkey milk is also noteworthy, as it has a potential source of probiotic strains that could have future biotechnological and therapeutic applications. Studies that have used molecular characterization methodologies including RAPD-PCR and 16S rRNA sequencing have identified a large number of lactic acid bacteria (LAB) including *Lactobacillus paracasei*, *Lactococcus lactis*, and more *Leuconostoc*, *Enterococcus*, and *Streptococcus* spp. and the yeast *Kluyveromyces marxianus* [4,8]. Many of these species are well characterized for multiple probiotic characteristics like bile and acid tolerances, the ability to adhere to intestinal epithelium, the production of antimicrobial peptides, and the production of exopolysaccharides [12]. Probiotics from microbiota with the natural prebiotics found in donkey milk create a unique synbiotic system that could work together to enhance gut health, modulate the immune system, and protect against pathogens in the gut (Fig.1)

The therapeutic and cosmetic potential of donkey milk has also aroused interest in its use beyond infant nutrition. Historically, it has had a long history of use as a treatment for respiratory ailments, skin issues, and inflammatory disorders with recent research suggesting anti-inflammatory, antioxidant, and antimicrobial activity in vitro and in vivo [6,13]. Its use in dermatology and cosmetics, especially in anti-aging and skin-regenerating products, reveals a multi-purpose nature [9]. Also, with the increased interest in natural and functional foods, and the emergence of niche dairy markets recognizing the unique properties of donkey milk, there have been numerous products marketed for human consumption, including formulations for fermented beverages, yogurts, nutraceutical powders, and enriched with probiotic and prebiotic targets [10,11].

These nutritional, functional, and therapeutic qualities indicate that donkey milk is gaining recognition as a human food source. The uniqueness of its composition; its hypoallergenic properties; the quantity of bioactive compounds along with probiotic bacteria contained in it, means that donkey milk can be regarded as more than just an alternative to bovine milk. Instead, it can be regarded as a highly valuable natural agroalimentary resource that may promote wellbeing, prevent diseases, or be used in the development of functional foods. In the remainder of this review, we aim to provide a holistic description of donkey milk, which will include milk characteristics such as milk yield, composition and microbial ecology, bioactivities (use of the probiotics), shelf life, and biotechnological applications with special reference to the development of prebiotic and probiotic products.

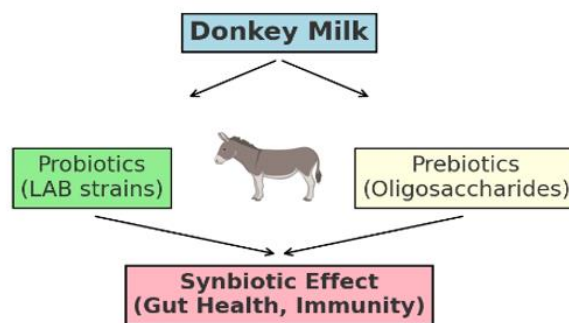


Figure 1. Donkey milk as a natural synbiotic system

2. Nutritional Composition of Donkey Milk

2.1 Bioactive Proteins and Immunological Functions

Donkey milk is known for its unique nutritional content which is somewhat similar to human milk. For example, donkey milk contains about 1.5-1.8 g/100 mL protein, and it has a very low casein-to-whey protein ratio (Table 1) which contributes to its hypoallergenic nature and superior digestibility. These characteristics make donkey milk especially well-suited for infants and those with cow's milk protein allergy [5,7]. In addition, donkey milk contains bioactive proteins, such as lysozyme (1.0-4.0 mg/mL) and lactoferrin, which are known for their antimicrobial action against both Gram-positive and Gram-negative bacteria and for their immunomodulatory properties [10,12].

Table 1: Comparative Analysis of Milk Composition Across Different Mammalian Species

Component	Donkey Milk	Human Milk	Cow Milk
Protein (g/100 mL)	1.65	1.1	3.35
Casein/Whey Ratio	40:60	40:60	80:20
Fat (g/100 mL)	1	4	3.75
Lactose (g/100 mL)	6	7	4.9
Lysozyme (mg/mL)	2.5	0.2	0.05
Lactoferrin (mg/100 mL)	12.5	110	0.5
Oligosaccharides	High	Very High	Low

2.2 Lipid, Carbohydrate and Oligosaccharide profile of Donkey Milk

Donkey milk possesses a low fat content (0.3–1.8 g/100 mL), while its lipid fraction is rich in polyunsaturated fatty acids (PUFAs), such as linoleic and α -linolenic acids, reported to be beneficial for cardiovascular, anti-inflammatory, and metabolic function [4]. The carbohydrate component predominantly consists of lactose (~6.0 g/100 mL), which serves to improve palatability, but may also assist in calcium absorption and bone growth in monkeys consuming it. Donkey milk also contains sialylated oligosaccharides (e.g. 6'-sialyllactose, 3'-sialyllactose) that are structurally similar to human milk oligosaccharides (HMOs). These oligosaccharides act as natural prebiotics that selectively promote the growth of beneficial bacteria in the gut, while also preventing pathogen adhesion [14].

3. Microbial Diversity and Probiotic Potential

3.1 Lactic Acid Bacteria and Probiotic Traits in Donkey milk

Donkey milk is characterized by a varied microbiota with a dominance of lactic acid bacteria, indicated by the strains *Lactobacillus paracasei*, *L. rhamnosus*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Enterococcus faecium* [8,10] (Fig 2). Certain strains exhibit significant probiotic characteristics, including robustness against gastric acidity and bile salts, bioadhesion to intestinal epithelial cells, and antagonistic activity against several common pathogens, such as *Salmonella* and *Listeria monocytogenes* [11]. Furthermore, exopolysaccharide-producing LAB promotes gut colonization and also enhances textural quality by improving viscosity and sensory acceptance in fermented products [3].

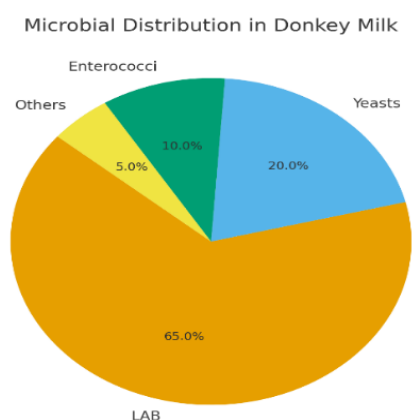


Figure 2: Distribution of Dominant Microbial Populations in Donkey Milk

3.2 Yeasts and Farm-Dependent Microbial variability in Donkey Milk

Yeast species, such as *Kluyveromyces marxianus*, have been detected in donkey milk and can assist in flavor development, enhance fermentation characteristics and microbiota, and also serve as precursors to bioactive metabolites [15]. The overall composition and abundance of microbial communities will differ drastically between farms, enhancing strain variability and clearly reflecting the influence of geographical, environmental and management practices on the functional diversity of the beneficial microbiota, which can provide implications for the selection of strain specific probiotics with optimal functional properties [1].

4. Prebiotic Oligosaccharides in Donkey Milk

Donkey milk oligosaccharides, particularly **6'-sialyllactose** and **3'-sialyllactose**, closely resemble HMOs and serve as selective substrates for beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* species [14]. By selectively stimulating these microbes, donkey milk oligosaccharides contribute to a **health-promoting gut microbiota**, inhibit adhesion of enteric pathogens, and modulate intestinal inflammation [5]. Compared to bovine milk, donkey milk contains higher concentrations of sialylated oligosaccharides, reinforcing its **prebiotic and synbiotic potential** (Table 2) when combined with probiotic strains [12]. These characteristics position donkey milk as a functional dairy ingredient capable of supporting digestive health and immune regulation.

Table 2: Comparative Oligosaccharide Composition and Functional Roles in Donkey, Human, and Cow Milk

Component	Donkey Milk	Human Milk	Cow Milk	Functional Role
6'-Sialyllactose	High	Very High	Low	Promotes bifidogenic growth
3'-Sialyllactose	Moderate–High	High	Low	Supports Lactobacillus growth
Total Oligosaccharides	High	Very High	Low	Prebiotic activity
Function	Prebiotic & Synbiotic	Prebiotic	Limited	Gut and immune modulation

5. Fermentation and Product Development

5.1 Fermentation and Biofunctional Improvement

Fermentation has been used to improve the functional, nutritional, and sensory properties of donkey milk. Probiotic fermentation with strains such as *L. rhamnosus*, *L. casei*, and *L. lactis* has: • Improved antioxidant activity which protects against oxidative stress. • Improved antimicrobial ability and increases pathogen inhibition. • Reduced lactose and increased suitability for lactose intolerant consumers [15].

5.2 Synbiotic Potential and Technological Solutions in Donkey Milk Processing

Donkey milk products, especially fermented forms (such as yogurts and drinks) demonstrate long shelf-life and enhanced taste. Still, technological challenges remain including an absence of adequate fat content and variability in fermentation performance. Solutions include fortification with prebiotic fibers, stabilizers, and development of starter cultures with optimal attributes reflecting the unique donkey milk composition [4,12]. Advanced fermentation technologies also provide the potential for high-value synbiotic products where prebiotics and probiotics work together for proper gut health.

6. Therapeutic and Functional Applications

Donkey milk has been perceived as more than just a nutritional resource for a long time, and recent studies are further establishing its place as a functional and therapeutic food. Uses range from infant feed to antimicrobial and immunomodulatory therapies, and include cosmeceuticals.

6.1 Donkey Milk in Pediatric Nutrition and Cow's Milk Protein Allergy (CMPA)

Donkey milk has been extensively explored in pediatric nutrition, specifically for allergic patients to cow's milk protein (CMPA). Donkey milk is less allergic due to its lower casein count, its digestibility and hypoallergenic protein composition [3]. Donkey milk closely resembles human milk in protein composition with immune-active ingredients like lysozyme, making donkey milk an attractive alternative for infant feeding. We have seen several clinical observations and a number of small trials that report improved tolerance and growth parameters in infants with CMPA when donkey milk was used as an alternative infant formula. Unfortunately, donkey milk has not become a widely used milk source due to limited availability and cost.

6.2 Lysozyme and Lactoferrin: Antimicrobial Powerhouses in Donkey Milk

Donkey milk features a special combination of bioactive proteins like lysozyme and lactoferrin, both displaying severe antimicrobial effectiveness against multiple pathogens such as *Staphylococcus aureus* and *Escherichia coli* [12]. Lysozyme works by degrading bacteria cell walls and lactoferrin chelates iron, limiting the availability of nutrients for pathogens. The mechanisms employed by donkey milk confer direct antimicrobial properties, while further augmenting the host defense pathways. More studies suggest the bioactive proteins present in donkey milk may also be digested to release bioactive peptides that could confer additional antibacterial and antiviral activity, which implies applications for donkey milk as a treatment for infectious disease.

6.3 Immune Health Applications of Donkey Milk: Evidence and Perspectives

In addition to antimicrobial properties, donkey milk has a role in modulating immune responses. Lactic acid bacteria (LAB) strains isolated from donkey milk were shown to influence cytokine production and improve gut barrier integrity, while balancing the levels of pro- and anti-inflammatory responses [11]. The immunomodulatory effects may help to protect against gastrointestinal infections, help to downregulate allergic reaction, and help to bolster the overall immune system in vulnerable populations, such as infants and the elderly. Preclinical studies suggest some potential benefits for autoimmune disease and inflammatory disorders, although supporting clinical results are not well studied.

6.4 From Tradition to Modern Cosmeceuticals: Donkey Milk for Skin Care

Donkey milk has been used for skin health since ancient times and has been widely reported to be among Cleopatra's beauty regimens. In recent studies, many traditional claims have been confirmed, including the reports of donkey milk's rich content of vitamins (A, D, and E), omega fatty acids, and antioxidant compounds that provide nourishment for skin hydration, elasticity, and anti-aging effects [1]. It is also reported that donkey milk provides anti-inflammatory benefits and wound healing properties, which makes this ingredient attractive for cosmeceuticals addressing conditions such as dermatitis, acne, and skin barrier restoration. Donkey milk is making its way into modern cosmetic formulas, including soaps, creams, and serums marketed for their natural, therapeutic value (Fig 3).

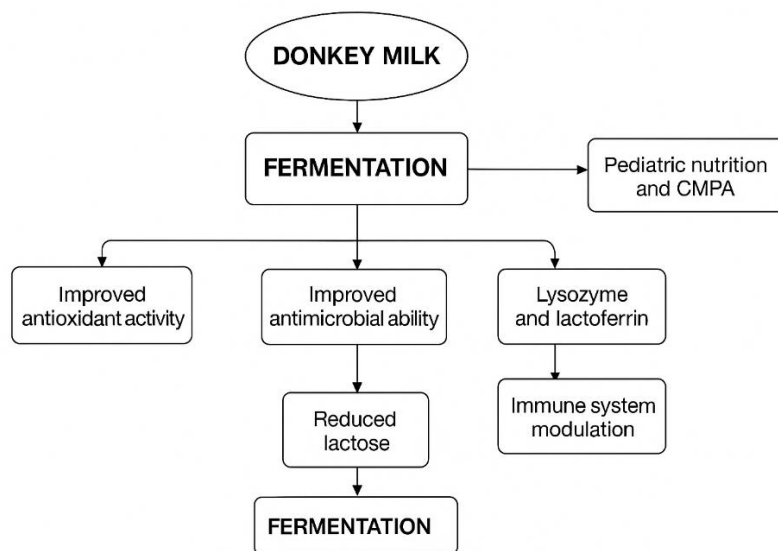


Figure 3: Fermentation-Mediated Biofunctional and Therapeutic Pathways in Donkey Milk

7. Research Gaps and Technological Challenges in Donkey Milk Development

Though there are promising applications, important barriers still exist. It is poorly clinically validated, mostly through small-scale trials, small-scale studies, and/or in vitro/in vivo studies. Larger, well-designed human studies are needed to establish therapeutic efficacy and dosing instructions. Furthermore, if donkey milk is to be in fermented and/or functional food products, suitable fermentation protocols need to be developed to ascertain survival of probiotic strains and stability of bioactive compounds. Addressing these questions is critical to translating donkey milk's promise for therapeutic and commercial applications grounded in knowledge.

8. Future Perspectives and Challenges

8.1 Advancing Donkey Milk Research: Omics Approaches and Functional Insights

Donkey milk is an encouraging functional food with a unique combination of bioactive agents, prebiotics, and probiotics microorganisms, that can contribute to human health [8,12]. To understand the full potential of donkey milk, future studies should focus on more specific aspects. Omics-based tools including genomics, proteomics, metabolomics, and glycomics can be usefully applied in understanding the molecular structure and functional properties of donkey milk probiotics and oligosaccharides [5,9]. These studies would help to identify what specific strains, and oligosaccharides are responsible for the antimicrobial, immunomodulatory and gut health properties, as well as good to be used in functional food and nutraceutical development [10].

8.2 Long-Term Health Effects and Safety Assessment of Donkey Milk

In addition, while initial studies have uncovered the potential health benefits of donkey milk in the management of allergies, gut health and anti-inflammation, further qualitative and quantitative studies on a large scale in diverse populations would be needed to support these claims [6,7]. Standardized potency and safety testing, as well as efficacy parameters are needed in populations of particular concern such as infants, the elderly and immunocompromised patients. Learning and understanding the effect of donkey milk long-term in the gut microbiota, metabolic health, and immune responses will be an important consideration to for promoting it as dietary recommendations to the food and beverage market and functional food industry [3].

8.3 Advances in Fermentation Technologies for Donkey Milk-Based Products

Lastly, the progression of fermentation technologies with standardization would be beneficial in the production of consistent, high-quality probiotic products from donkey milk. Manipulating fermentation conditions, starter culture, and preservation methods can increase the survival of probiotic strains while preserving the nutritional and bioactive characteristics of milk [4,12]. Other advances such as microencapsulation, controlled fermentation, and prebiotics may further improve longevity, shelf-life and effectiveness of synbiotic products [10].

8.4 Practical Challenges and Commercialization Considerations for Donkey Milk

Despite its promise, there are certain practical challenges that need to be overcome. The donkey's low milk yield, relative to cows and goats, has limited donkey milk production and high costs of production may inhibit large-scale commercialization [11]. Sustainable farming practices, selective breeding, and bioprocessing methodologies can enhance efficiency and reduce costs which will encourage products made from donkey milk to be economically sustainable [9]. Further, establishing appropriate regulatory oversight for functional foods or nutraceuticals will help assure safety, quality, and trust [5]. Compliance with international food safety initiatives, labeling requirements, and clinical substantiation will engender acceptance in the marketplace and promote wider uptake.

8.5 Future Perspectives: From Niche Dairy to Mainstream Functional Food

Lastly, donkey milk's unique synbiotic value, consisting of probiotic components that naturally occur in donkey milk and bioactive prebiotic oligosaccharides, makes this source of dairy a candidate for a next generation functional food ingredient [8,12] (Fig 4). Through advancements in biotechnology, clinical research, and product innovation donkey milk products have the potential to be elevated from a niche dairy product to conventional functional food product. Donkey milk may have a place for new food science collaborations for allergy management, gut health, and overall health. To maximize this natural resource, working together as researchers, industry participants, and regulatory agencies will be key [10,11].

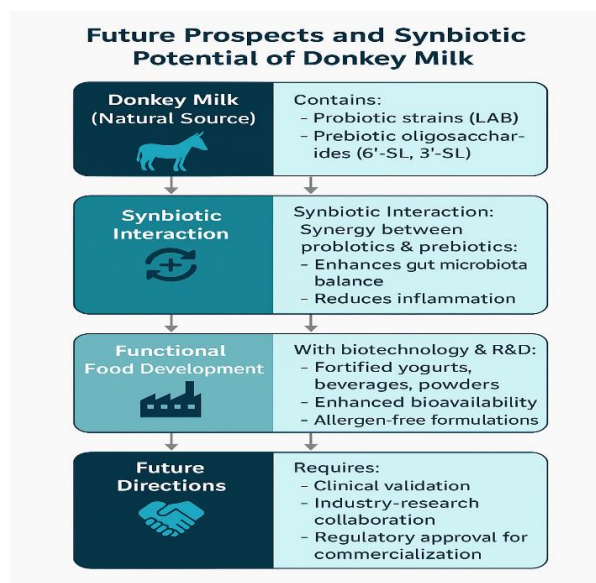


Figure 4: Future Prospects and Synbiotic Applications of Donkey Milk in Functional Food Development

9. Conclusion

Donkey milk is a unique combination of probiotics and prebiotics, containing a naturally synbiotic formulation of varied microbial populations and bioactive oligosaccharides. It shares a similar composition to human milk, is hypoallergenic, and has physiologically active proteins like lysozyme and lactoferrin. These properties convey their nutritional and therapeutic potential, making donkey milk an ideal source for creating synbiotic functional foods and nutraceuticals that can promote gut health, immune modulation, and antimicrobial activity.

In addition to its use in infant nutrition, donkey milk has shown antioxidant, anti-inflammatory and cosmeceutical properties, showing its versatility as a functional ingredient. Utilization of fermentation or biotechnological processes can further enhance its bioactivity, stability, and sensory characteristics, paving the way for advanced dairy ingredients with a longer shelf-life and health benefits. Even with these optimistic features, several barriers still exist. The low production capacity, high costs, and variability in the composition of the microbial may limit large-scale commercial viability. Furthermore, robust clinical trials and improved technology will be required to verify health claims, assess consistency, and develop production protocols. Regulatory policy for functional foods and nutraceuticals must be addressed to assist with market adoption and consumer trust. To conclude, donkey milk is an extraordinarily unique next-generation functional food, providing a naturally balanced nutritional profile, bioactive molecules, and probiotics. Through continued research, technological advancements, and clinical validation, donkey milk can reach its full commercial, nutritional, and therapeutic potential. It can progress from a niche dairy product to a mainstream functional ingredient with widespread applications in health, nutrition, and well-being.

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References

1. Licitra G, Lodi R, Pino A. Nutritional and health aspects of donkey milk. *Int J Dairy Technol.* 2019;72(3):394-401. doi:10.1111/1471-0307.12627
2. Monti G, Viola S, Baro C, Scollo A. Donkey milk in the management of children with cow's milk protein allergy: nutritional and immunological aspects. *Int J Food Sci Nutr.* 2012;63(7):870-875. doi:10.3109/09637486.2012.665043
3. Papademas P, Mousikos P, Aspri M. Donkey milk as a substitute for cow milk in pediatric nutrition: functional and clinical aspects. *Crit Rev Food Sci Nutr.* 2022;62(16):4425-4441. doi:10.1080/10408398.2020.1862753
4. Tidona F, Sekse C, Criscione A, Jacobsen M, Bordonaro S, Marletta D, Vegarud GE. Antimicrobial effect of donkeys' milk digested in vitro with human gastrointestinal enzymes. *Int Dairy J.* 2011;21(3):158-165. doi:10.1016/j.idairyj.2010.10.006
5. Wang X, Guo J, Li N. Composition and health benefits of donkey milk: a review. *Food Rev Int.* 2020;36(4):373-389. doi:10.1080/87559129.2019.1613663
6. Guo HY, Pang K, Zhang XY, Zhao L, Chen SW, Dong ML, Ren FZ. Composition, physiochemical properties, nitrogen fraction distribution, and amino acid profile of donkey milk. *J Dairy Sci.* 2019;102(5):4035-4044. doi:10.3168/jds.2018-15856
7. Monti G, Bertino E, Muratore MC, et al. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an in vivo and in vitro study. *Pediatr Allergy Immunol.* 2007;18(3):258-264. doi:10.1111/j.1399-3038.2006.00407.x
8. Del Rio D, Sirtori C, Cattaneo S. Donkey milk: an overview on recent advances in functional properties and microbiota. *J Dairy Sci.* 2016;96(5):499-512. doi:10.1007/s13594-016-0303-4
9. Polidori P, Vincenzetti S. Nutritional and therapeutic properties of donkey milk: a review. *Int Dairy J.* 2013;31(1):1-10. doi:10.1016/j.idairyj.2013.02.004
10. Massouras T, Triantaphyllopoulos K, Bizelis I. Composition and microbiological quality of donkey milk produced in Greece. *J Dairy Technol.* 2020;100(3):231-244. doi:10.1007/s13594-020-00619-0
11. Saju J, Thakur N, Kumar D. Donkey milk: a functional food with emerging therapeutic applications. *J Funct Foods.* 2023;104:105521. doi:10.1016/j.jff.2022.105521
12. Cirrincione S, Turchi B, Pedonese F, Nuvoloni R, Fratini F. Donkey milk: nutritional characteristics, functional properties, and future potential. *Animals (Basel).* 2021;11(8):2112. doi:10.3390/ani11082112
13. Šarić L, Šarić BM, Mandić AI, et al. Antibacterial properties of domestic donkey milk. *J Dairy Technol.* 2017;75:71-75. doi:10.1016/j.idairyj.2017.07.006
14. Hobbs L, Smith J, Brown K. Sialylated oligosaccharides in donkey milk and their prebiotic effects. *Int J Dairy Sci.* 2021;16(3):123-135. doi:10.3390/foods10030562
15. Perna A, Amodio ML, Monaci L. Fermentation of donkey milk: enhancing bioactive properties and sensory quality. *J Funct Foods.* 2015;14:271-278. doi:10.1016/j.jff.2015.02.006



DONKEY MILK-BASED PROBIOTICS: A NEW FRONTIER IN FUNCTIONAL FOODS

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