

# Donkey milk as a non-bovine alternative: a review of its nutri-functional properties, applications, and challenges

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**Abstract** Elevation in incidences of cow milk protein allergies warrants the need to investigate the suitability of non-bovine milk alternatives for human consumption. Donkey milk has emerged as a potential alternative attributed to its benefits to human health. Evidently, it is a great option for infants as it closely resembles human milk. Researchers have also investigated its suitability in producing numerous dairy products. This review discusses the various nutri-functional aspects of donkey milk, its applications and challenges in the manufacturing of infant formula, yogurt, cheese, ice cream,

kefir, and fermented milk. Research updates on processing techniques (thermal and non-thermal) for donkey milk preservation are also delineated. Despite abundant nutrients and desirable functional properties, the growth of the donkey milk industry is not significant. This is due to the lower yield, scattered population, and lack of regulatory standards for both products and processing. Recommendation on research gaps and obstacles in its commercialization are also addressed.

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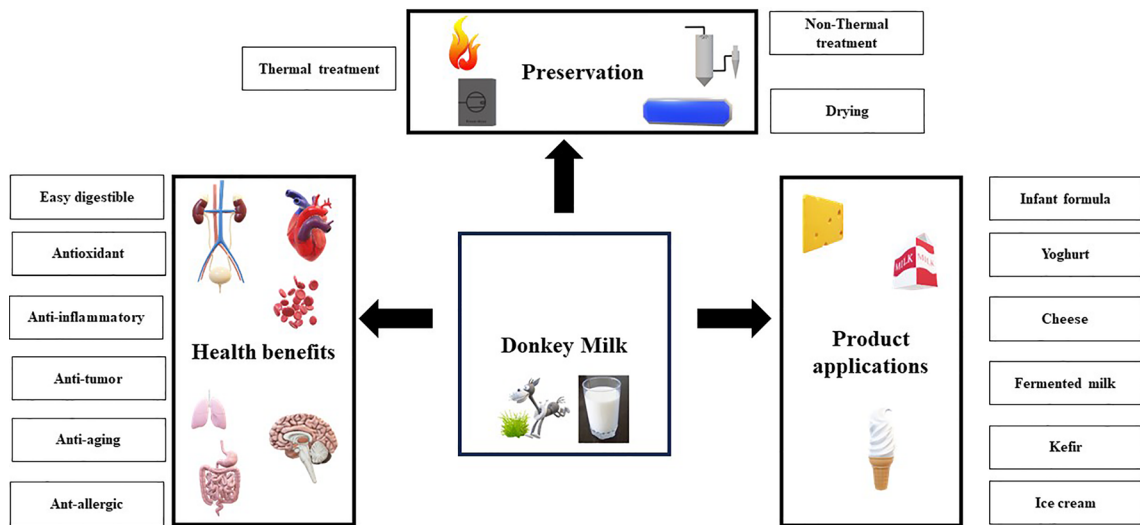
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Graphical abstract



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Introduction

Milk is a diverse and nutrient source of fat, sugar (carbs.), protein, vitamins, minerals, and enzymes (Munir et al. 2019). Milk from other species has always been used whenever breastfeeding is not possible, among which cow milk has gained significant attention (Martin et al. 2016). However, a rise in the incidences of cow milk’s protein allergy (CMPA) (due to casein,  $\beta$  lactoglobulin, and  $\alpha$  lactalbumin) drove the researchers to investigate non-bovine milk as a potential alternative (Lajnaf et al. 2023). As per reports, the production of non-bovine milks has risen by 165% between 1983 and 2013 against the 41% increase in cow milk (Ranadheera et al. 2018).

Interest in donkey milk (DM) is arising because of its similar composition and properties to human milk (Nayak et al. 2020). It is gaining popularity due to its potential to fulfill the supplemental needs of infants, neonates, and elderly people (Aspri et al. 2017a). Its higher lactose content can assist in producing soft curd inside the stomach, hence convenient to digest. It can also be given to infants suffering from cow milk enterocolitis syndrome (Mori et al. 2017). As per Nayak et al. (2022), DM was used by the French government to feed orphan infants in the nineteenth century. In addition to its positive impact on infants, DM has demonstrated beneficial effects in various other areas. These include promoting osteogenesis, assisting in the treatment

of patients with atherosclerosis, coronary disease, premature aging, and contributing to hypocholesterolemic diets (Baloš et al. 2023).

Lajnaf et al. (2023) said that DM is known for its therapeutic applications since ancient times. In several countries like Serbia, it has traditionally been employed as a natural remedy for treating asthma and bronchitis. It has significantly higher antimicrobial properties, contributed by its lactoferrin, immunoglobulin, lactoperoxidase, and lysozyme content. Literature has also reported its immunomodulation effectiveness against various bacteria, fungi, viruses, and diseases, including stress, tumors, staphylococcosis, listeriosis, heart diseases, and viral infections (Martini et al. 2021). Higher whey content and lower casein to whey protein ratio define its hypoallergenic properties and consumption suitability for people suffering from CMPA. Despite the higher lactose content in donkey milk, its fermented products have been found suitable for lactose intolerant population, attributed to their easy digestibility (Carminati and Tidona 2017). As per Nayak et al. (2022) it is a good source of polyunsaturated fatty acids, which assist in skin care.

The market for DM has experienced a significant growth, with a value of 23.2 million USD in 2021. It is projected to expand at a Compound Annual Growth Rate (CAGR) of 9.9% from 2022 to 2028. The rise might be due to the acceptance of DM products in economies like the U.K. and Germany. Additionally, there is a rising awareness of skin-care and nutritional benefits associated with DM products. These factors are significant contributors to future market growth. Furthermore, during COVID-19, DM utilization increased due to its perceived advantages, such as its high

**Table 1** Nutritional composition with macro and micronutrients milk of donkey and other species (percent composition g/100 g)

Component	Donkey milk	Human milk	Cow milk	Goat milk	Camel milk
Fat	1.21	3.38	3.46	4.62	1.80
Lactose	6.23	6.69	4.71	4.47	2.91
Ash	0.43	0.22	0.78	0.73	0.85
Energy (KJ/Kg)	1939.40	2855.60	2983.00	3399.50	2745.80
Total protein	1.74	1.64	3.43	3.41	1.80
Total casein	0.64–1.03	0.32–0.42	2.46–2.80	–	–
Total whey protein	0.49–0.80	0.68–0.83	0.55–0.70	–	–
pH	7.0–7.2	7.0–7.5	6.6–6.8		
Water	90.39	87.57	87.62	86.77	90.60

Table values adapted from Aspri et al. (2017b) and Vincenzetti et al. (2017)

vitamin content and potential immune-boosting properties (Consumer, F&B, 2023).

The donkey (*Equus africanus asinus*) belongs to the Equidae family. It has always been a supportive asset for the poor communities of the world (Norris et al. 2021). The worldwide donkey population was 50 million in 2019. They are scattered in different regions of the world, including South America, Africa, Southern Europe, and Asia, and there was a 19% rise in their population between 1997 and 2021 (Norris et al. 2021). DM is used in the dairy and cosmetics sector (D'Alessandro et al. 2018, 2019; Kriel et al. 2017). It is white, less viscous, and has a pleasant aroma and a

slightly sweet taste (Nayak et al. 2022). Consumption and utilization of DM can assist in fulfilling the dietary needs of highly populated as well as low-economy countries. In addition to this, it can assist the local farmers and small-scale milk production units. Researchers have also reported the manufacturing of numerous other products from DM, including yogurt, cheese, and fermented drinks like kefir (Akal et al. 2022; Aroua et al. 2023; Faccia et al. 2019; Gué-nard-Lampron et al. 2020; Salgado et al. 2021). It has natural lactobacilli probiotic bacteria, which makes it suitable for fermented products (Salgado et al. 2021). The world's costliest cheese (pule cheese) is made from DM (Carneiro et al.

**Table 2** Various studies summarizing the functional properties of donkey milk

Functional property	Results stated by study	References
Antimicrobial properties	Lysozyme inhibits the growth of LAB, food spoilage bacteria, and pathogens ( <i>Salmonella typhi</i> , <i>Pseudomonas aeruginosa</i> , <i>E. coli</i> , <i>Listeria monocytogenes</i> , <i>Enterococcus faecium</i> , and <i>Lactobacillus curvatus</i> ).	Aspri et al. (2017c); Hassan et al. (2022)
Antifungal	Disintegration or increased influx rate of H <sup>+</sup> across the plasma membrane of fungi due to presence of fatty acids i.e., capric and myristoleic acids etc.	Altomonte et al. (2019); Lajnaf et al. (2023)
Antioxidative and Hypoglycemic	Enhanced superoxide dismutase activity due to presence of bio-active peptides in DM, causes the breakdown of superoxide radical into H <sub>2</sub> O <sub>2</sub> and O <sub>2</sub> , and this is the cause of anti-diabetic effect of DM.	Martini et al. (2021)
Antiallergic	Rapid degradability and complete digestibility of low casein content is responsible for low allergenicity of DM.	Aspri et al. (2017b); Martini et al. (2021)
Anti-inflammatory	Improved functionality of paneth's cells by restoration of lysozyme and $\alpha$ -defensin, and a decrease in the dysbiosis connected with ileitis are responsible reasons for anti-inflammation properties of DM.	Hassan et al. (2022); Yvon et al. (2018)
Immunomodulating	DM produces cytokines (protein controls the inflammatory and immune response to infections) like interleukin 1 (IL-1), IL-6, and IL-2 etc., which improves innate immunity. As the lysozyme concentration of DM is similar to human milk, and more than cow milk, and lysozyme have whey protein fractions, which contribute to the immunomodulating effects of DM.	Martini et al. (2021)

2018). Researchers have explored numerous processing techniques such as pasteurization, drying, UV-C, High Pressure Processing (HPP) to ensure the safety of DM (Dambrosio et al. 2023; Matera et al. 2022; Papademas et al. 2020; Tedeschi et al. 2023; Vincenzetti et al. 2018). Despite the benefits of consuming DM, its industrialization is not up to a remarkable extent. It is due to the lack of awareness among the population which conveniently gets cow milk or plant-based milk. In addition to that donkey milk is not allowed to use in Islamic religion, which is 25% of the world population. Another possible reason is the lack of regulatory standards for products as well as processing. This review delves into the findings of the nutri-functional properties, applications, and challenges in the manufacturing of various donkey milk products. Updates on the investigation of various processing techniques for the preservation of DM are also addressed alongside the hurdles in the growth of the DM industry and the suitable recommendations for future research.

### Nutri-functional properties of DM

An overview of various components and their approximate concentrations in donkey, human, cow, goat, and camel milk is presented in Table 1. The composition of DM sets it apart from other milk varieties like cow's milk or goat's milk, giving it a distinctive profile. It has been found that DM has a similar composition of protein (casein and whey) and sugar (lactose) as human milk but significantly differs from cow, goat, and camel milk (Baloš et al. 2023). However, the most notable distinction between DM and human milk is the low-fat content in DM. Regarding the casein-to-whey protein ratio (70.3:100), DM falls between cow and human milk. It has lower fat and slightly higher lactose content than cow's milk. It is a good source of vitamins (A, B<sub>2</sub>, and C), major (calcium, phosphorus, magnesium, potassium, and sodium) and trace (iron, zinc, and copper) minerals (Massouras et al. 2017).

DM contains various immunological factors such as lysozyme, lactoferrin, omega-3 fatty acids, bioactive peptides and immunoglobulins (IgA, IgG, and IgM), (Papademas et al. 2021). These factors contributed toward the broad range of functional properties including antibacterial, antiviral, antifungal, antioxidative, antiallergic, anti-inflammatory, hypoglycemic, antiparasitic, and anti-tumor activities (Baloš et al. 2023). The various findings about functional properties are summarized in Table 2. DM is considered as hypoallergenic due to its unique protein profile, potentially reducing the risk of allergic reactions in infants (Aspri et al. 2017a). The low allergenicity and better digestibility of DM can be ascribed primarily to its low casein (particularly  $\alpha$ <sub>1</sub>-casein and k-casein) and high whey protein content (Baloš et al. 2023; Souroullas et al.

2018; Vințe and Coroian 2022). Immunochemical investigations also support the hypoallergenic nature of DM's as IgE from patients with cow's milk allergies weakly recognizes the DM caseins (Souroullas et al. 2018). Therefore, donkey milk can be a suitable substitute for the population suffering from CMPA. Further, the presence of lysozyme variants (~4000 mg/L) and a higher concentration of lactoferrin (~80 mg/L) in DM makes it superior in antimicrobial activity as compared to other milk-producing species (Altomonte et al. 2019; Aspri et al. 2017b, c; Hassan et al. 2022). Lysozyme inhibits bacterial growth by hydrolyzing the polysaccharides of bacterial cell walls (Hassan et al. 2022; Martini et al. 2019). Additionally, it stimulates the production of nitric oxide, which helps with blood circulation and lowers blood pressure (Hassan et al. 2022). According to Martini et al. (2021) non-bovine milks, including DM, are a rich source of lactoferrin (a protective protein) that arrests pathogenic growth and invasion by disrupting their cell walls. DM is also reported to have proteins which on hydrolysis produce antimicrobial bioactive peptides (Aspri et al. 2017b). This is attributed for the high antimicrobial activity of fermented DM (Aspri et al. 2018). Altomonte et al. (2019) reported the antifungal properties of DM. The studies showed sensitivity of *Microsporiumcanis*, *Microsporiumgypsum* and *Trichophyton mentagrophytes* towards four different pasteurized DM samples conforming to its antidermatophytic effect.

DM and products also demonstrate activities against reactive oxygen species, have antioxidative properties, improve the immune system, and preserve gut flora's balance (Li et al. 2022). High levels of bioactive peptides, high vitamin C,  $\alpha$ <sub>1</sub> casein,  $\beta$ - casein, and lactoperoxidase contribute to DM's antioxidant properties. At the same time, anti-inflammatory properties are due to the presence of high levels of bioactive peptides, lactoferrin, lysozyme, and omega-3 fatty acids. Yvon et al. (2018) demonstrated the anti-inflammatory characteristics of DM, where the concentration of antibacterial peptides (generated by Paneth cells) revived in mice suffering from Crohn's disease. Similarly, a study conducted by Jiang et al. (2018) supported DM's anti-inflammatory properties by administering DM lysozyme (DML) in mice suffering from dextran sulfate sodium (DSS) induced inflammation. This study demonstrated that DML not only assisted in reducing inflammation but also improved inflammatory bowel disease and positively impacted the gut microbiota. Studies by Li et al. (2020) have demonstrated the DM helps in lowering blood glucose levels by improving insulin resistance, increasing the body's ability to eliminate free radicals, and increasing antioxidant levels. Lower blood sugar and improved defense against insulin proved the hypoglycemic effect of DM hence it can be exploited for the treatment of type-2 diabetes.

**Table 3** Attributes of different dairy products manufactured from donkey milk

Product	Attributes	References
Infant formula	Suitable for infants with cow milk protein allergy. Provide similar attributes to human milk Abundant in amino acids, fatty acids, and taurine. Abundant growth hormones Reduce the risk of allergic reactions Easily digestible as compared to cow milk infant formula. Desirable protein to calcium ratio	Aspri et al. (2017b); Li et al. (2018)
Yogurt	Higher lactic acid bacteria compared to cow milk yogurts. Fibers were added to make up the viscosity, but it caused significant effect on sensory properties. Higher shelf life as compared to cow milk yogurt attributed to the presence of lactoferrin and lysozyme.	Akal et al. (2022); Salgado et al. (2021)
Fermented milk	Improved fatty acid profile. Lower saturated fatty acid. Rich in mono and polyunsaturated fatty acids. Shelf life of 21 days (refrigerated storage)	Cavalcanti et al. (2021)
Kefir	Superior microbiological quality compared to cow milk kefir. Higher antioxidant activity	Aroua et al. (2023)
Cheese	Lower yield. Gamy aroma and sweet flavor. Crumbly and soluble texture resembling feta cheese.	Faccia et al. (2018, 2019)
Ice cream	Two probiotics cultures were added ( <i>Bifidobacterium adolescentis</i> and <i>Lactobacillus plantarum</i> ). The population of both strains stayed at 9-log cfu/mL up to the storage of 4 months Lower fat. Storage affected the vitamin C content.	Tidona et al. (2017)

Kocic et al. (2020) has reported that DM can decrease the impact of aging. However, this effect is more pronounced by applying DM or DM's products directly on the body as it promotes deeper penetration of phospholipids and proteins inside the skin and keeps the skin's pH in the acidic range, preventing the appearance of inflammation and age-related changes to the skin. But there is no clear and detailed evidence of the antiaging effect associated with the consumption of DM and its phenomena.

## Applications of DM

Applications of DM involve its transformation into infant formula, cheese, ice cream and fermented products (yoghurt, kefir). It was observed that DM products have probiotic effects, and their consumption leads to favorable health traits (Salgado et al. 2021). The potential use of DM in different products is summarized in Table 3 and discussed below in detail.

### Infant nutrition and formula

Human milk is the most ideal source of nutrition and is crucial in promoting overall health and well-being throughout

infancy, childhood, and adulthood while supporting optimal growth and development in children (World Health Organization 2022). In addition to its nutrient composition, human milk comprises a diverse range of bioactive compounds that actively contribute to the shaping and modulation of the immune system, gut health, and brain development. Commercial infant formula, derived from cow's milk that mimics human milk closely are the suitable alternatives for human milk. Its purpose is to fulfill the nutritional requirements of preterm infants and ensure nutrition for infants in case of breast milk unavailability (Perrella et al. 2021). The nutritional needs of infants are specific and complex, and formulas are carefully formulated to meet those requirements. To utilize raw animal milk as a substitute for formula, it is crucial to comprehend the variations in oligosaccharides, lactose, and other nutritional content between human milk and animal milk. In a study by Wang et al. (2020), Chinese human milk's oligosaccharide and lactose content was compared to animal milk sources such as bovine, yak, goat, and donkey. The findings revealed that DM's lactose content was similar to human milk. However, the oligosaccharide profile varied regarding the type and quantity present in human and donkey milk. In addition to this, previous studies have reported that the digestibility of DM was found to be greater than that of cow's milk and comparable to that of human

milk (Martini et al. 2021). The  $\alpha$ -lactalbumin component of human breast milk forms a complex with oleic acid known as HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor Cells). This complex has demonstrated the ability to induce apoptosis selectively in tumor cells, making it a potential therapeutic agent against various types of tumors (Lerksuthirat et al. 2023).

Moreover,  $\alpha$ -lactalbumin is known to inhibit cyclooxygenase-2 (COX-2) and phospholipase A2 activities, resulting in enhanced anti-inflammatory attributes. In contrast,  $\beta$ -lactoglobulin, a major whey protein in cow milk and a common allergen in children, is absent in human milk. Both DM and human milk exhibit immune effects and can enhance the immune response in humans. Though, powdered infant formulas incorporating DM are regarded as promising alternatives to human milk for infants and newborn. However, there are certain challenges and gaps that need to be addressed. It is claimed to have potential antimicrobial and anti-inflammatory properties, although the extent of these effects in human infants needs to be better understood. Moreover, it has been discovered that DM contains growth factors and hormones, including human-like leptin, ghrelin, insulin-like growth factor 1, and triiodothyronine T3. These bioactive molecules directly impact metabolism, body composition, and the regulation of food intake (Brumini et al. 2013). Yet, infant formulas undergo extensive processing to enhance their digestibility and minimize potential digestive issues. However, extensive research is needed to establish its hypoallergenic properties and compare them to existing hypoallergenic formulas available on the market.

## Yogurt

DM yogurt represents a promising method for incorporating DM into human diets due to its widespread popularity as a dairy derivative. Akal et al. (2022) studied the antimicrobial properties of lysozyme and lactoferrin extracted from DM in yogurt. The outcomes of this study proved that lactoferrin and lysozyme from DM can assist in increasing the shelf life of yogurt by inhibiting undesirable microbial growth without affecting the product's sensory attributes.

DM's limited solid constituents (proteins and fats) pose challenges to attain optimal yield, consistency, and viscosity in the yogurt-making process. As viscosity perception plays a pivotal role in determining yogurts' quality and sensory approval, addressing this aspect becomes critical for consumer acceptance (Guénard-Lampron et al. 2020). Salgado et al. (2021) studied the influence of various fibers (inulin, apple fruit, and passion fruit) on the viscosity and sensory acceptance of DM yogurt compared to cow milk yogurt. Five yogurt formulations were developed: DM control, with inulin, with apple fiber, with passion fruit fiber, and cow milk control. The study conducted comprehensive analyses

of the physio-chemical and microbiological components of the yogurts. Notably, DM-based yogurts exhibited significantly higher counts of lactic acid bacteria (LAB) than cow milk yogurt. The pH and color of the yogurts were found to be significantly influenced by both the type of milk and the added fiber. The passion fruit fiber DM yogurt displayed the most favorable apparent viscosity (824.7 mPa s). Sensory evaluation results (specifically focused on the attributes of DM control, with passion fruit fiber, and cow milk control) revealed that despite the higher viscosity, the addition of passion fruit fiber resulted in a decline in overall acceptance. Therefore, while certain fibers may positively impact the apparent viscosity of DM yogurt, sensory acceptance remains crucial in determining the practical viability of such formulations.

## Fermented milk

The dairy industry produces numerous types of fermented milk with distinct physicochemical features since fermented milk is the most commercialized dairy product due to its suitable nutritional value (Miao et al. 2020). The fermentative process can improve sensory and functional properties of DM; therefore, the formulation of fermented DM products could be an interesting approach (Aspri et al. 2018).

The nutritional value and physicochemical characteristics of fresh DM and its fermented counterpart were studied by Cavalcanti et al. (2021). Additionally, the influence of refrigerated storage (4 °C, 21 days) on the characteristics of fermented DM was examined. Comparative analyses revealed that fermented milk exhibited higher acidity and reduced levels of lactose, minerals (calcium, phosphorous, magnesium, sodium, and zinc), and amino acids (particularly essential ones), compared to fresh milk. Conversely, it displayed an improved fatty acid profile, with lower saturated fatty acid content and higher monounsaturated and polyunsaturated fatty acid content than fresh milk. During the 21-days refrigerated storage period, fermented milk exhibited a decline in lactose content, while the amino acid profile showed improvement and the mineral content remained relatively stable. The storage period also negatively impacted the fatty acid profile of the fermented milk. Overall, this study confirms the significant nutritional value of both DM and fermented DM. Moreover, it suggests that fermented milk can be stored and refrigerated for up to 21 days while retaining its essential nutritional attributes.

## Kefir

Kefir is a type of fermented milk drink that originated in either Mongolia or the Caucasus Mountains of Russia (Ganatsios et al. 2021). It has a unique combination of healthy bacteria including yeasts (*Saccharomyces*, *Torula*,

*Kluyveromyces*, and *Candida spp.*), LAB (*Streptococcus*, *Leuconostoc*, *Lactobacillus*, and *Lactococcus*), and *Acetobacter* have (Vashisht et al. 2023). Kefiran, a water-soluble branched glucogalactan polysaccharide matrix produced by *Lactobacillus kefirifaciens*, may strengthen consumers' immune systems and promote resistance to pathogenic infections (Hamida et al. 2021). Kefir can be produced by inoculating milk with tiny clusters of certain microbe combinations contained in a particular polysaccharide matrix known as kefir grains. Fermentation can last up to 24 h (Vashisht et al. 2023). Aroua et al. (2023) assessed the traits of DM and cow milk kefir during storage for 28 days at 4 °C. During fermentation, the pH of cow's milk decreased significantly from  $6.75 \pm 0.045$  to  $4.22 \pm 0.062$ , while DM's decreased from  $7.01 \pm 0.011$  to  $4.28 \pm 0.030$ . During storage acidity values increased from  $63 \pm 2.08$  to  $170 \pm 2.80$  °D for cow milk and from  $92 \pm 1.0$  to  $163 \pm 1.30$  °D for DM kefir. Total solids displayed notable variation while protein content remained stable. Although lactose levels decreased significantly over time, fat content remained unchanged in kefir stored at 4 °C. Microbiologically, DM kefir exhibited significant differences compared to cow milk kefir, consistently displaying lower average bacterial counts, suggesting superior microbiological quality than cow's milk kefir samples. The samples also showed compelling antioxidant activity, as measured by the DPPH and ABTS assays, which improved during storage. The bacterium *Aeromonas hydrophila* showed the highest sensitivity to the action of kefir samples. Sensory tests indicated that participants preferred freshly prepared kefir over those stored for 28 days at 4 °C. Owing to its distinct bioactive activities and favorable microbiological properties, DM is a promising alternative as a source material for kefir fermentation.

## Cheese

Low casein concentration, large size of casein micelle, and poor colloidal stability are significant technical barriers to producing cheese out of DM (Faccia et al. 2019).  $\kappa$ -Casein, the protein that is responsible for gelation in cheese, is found to be absent or present in very scarce amounts (Cosenza et al. 2019; Luo et al. 2019). Knowing the fact that there are numerous technical challenges encountered during the preparation of cheese using DM milk as base material, attempts have been made to optimize the process parameters to produce fresh DM cheese with desired organoleptic characteristics. In contrast to cheese made from cow's milk, DM cheese has very peculiar sensory qualities. The aroma is referred to as "gamy", which comes from the DM's typical animal fragrance, crumbly and soluble texture like fresh feta cheese, and the flavor is noticeably sweet (Faccia et al. 2018, 2019).

Literature has reported the use of different coagulants for the preparation of fresh DM cheese. Faccia et al. (2018) used

calf rennet and reported that DM was not able to coagulate entirely without the aid of calcium chloride and lowering the pH with the addition of starter culture. The yield obtained was only 5.9%. Faccia et al. (2019) found that the microbial rennet and the starter culture can also be used to prepare fresh cheese from DM for around 42 min until molding. There was an increase in the yield of cheese to 7.21%. However, no supporting reason for the increased yield was given in the studies. The inclusion of microbial transglutaminase (MTGase) in a similar process as adopted by Faccia et al. (2019) for preparing DM cheese simultaneously with rennet addition resulted in the lowest coagulation time and highest curd viscosity due to intermolecular cross-linking of micellar caseins mediated by MTGase (D'Alessandro et al. 2019).

On the contrary, Giuseppe (2015) suggested that only camel chymosin can coagulate the DM; it took hours to complete the coagulation process. The PAGE analysis of DM cheese suggested that  $\beta$ -casein was the most prevalent fraction of protein followed by  $\alpha$ -casein; the bands of para  $\kappa$ -Casein were very light. Similar results were reported by Cosenza et al. (2019) and Luo et al. (2019).

## Ice-cream

The only study on ice cream prepared from DM was conducted by Tidona et al. (2017). Ice cream with strawberry flavor made from DM was combined with two LAB strains (*B. adolescentis* and *L. plantarum*) that exhibited probiotic characteristics. The fat and overrun of the product were 1.2 and 24.7%, respectively. The fat content was lower in DM ice cream as compared to the ice cream made from cow milk. The vitamin C level decreased after four months of  $-20$  °C storage. However, the ice cream's total phenol content and antioxidant capacity remained unaltered. The number of viable probiotic bacteria was greater than 9-log CFU/g for the duration of storage.

## Processing updates

Both thermal and non-thermal processing technologies, as well as drying, have been explored for the processing of DM. For the batch pasteurization treatment, similar processing parameters as for mild pasteurization of human milk ( $62.5$ – $65$  °C for 30 min) were used in the literature (Martini et al. 2018; Papademas et al. 2020; Tedeschi et al. 2023;) where decline in nutritive components like vitamin D<sub>3</sub>, ACE inhibitory activity, bioactivity and elevated protein lyophilization have been reported. However, continuous HTST treatments ( $75$ ,  $85$ , and  $92$  °C for 12, 6 and 3 s, respectively) had better nutrient retention where 20–60 and 2–22% decline in lysozyme and  $\beta$ -lactoglobulin was observed, respectively (Matera et al. 2022). Among drying techniques spray and

freeze drying were explored where spray dried powders had major impact on the lysozyme activity (58% residual activity) and  $\beta$ -lactoglobulin content (14% reduction), and volatile composition where inlet air temperature plays major role (Di-Renzo et al. 2013; Vincenzetti et al. 2018; Nayak et al. 2022). Whereas freeze dried powders had better nutrition retention and functional properties than spray dried (Di-Renzo et al. 2013; Vincenzetti et al. 2018). However, cost evaluation needs to be conducted for a better comparison.

Researchers have also explored non-thermal technologies for the processing of DM, aiming to retain its natural composition. Papademas et al. (2020) and Tedeschi et al. (2023) reported the use of Sure Pure UV system for the UV processing of DM where significant microbial reduction of pathogenic and spoilage organisms was observed with similar nutritive composition as of raw samples. High Pressure Processed DM also had a comparable shelf life to the thermally processed samples but with lesser effect on the quality parameters (lysozyme preservation). Hence these techniques proved to have better product than thermally treated ones, but they need advance scale up strategies and regulatory approvals to be applied at commercialized level. Further all these treatments used in these studies including thermal were conducted at different processing conditions which makes the comparison difficult. This warrants the need of standard parameters for each processing technique.

## Conclusion

Donkey milk has proved to be a potential alternative to bovine milk. It is suitable for the population suffering from cow milk protein allergy. Literature has reported that it has antiallergic, antimicrobial, antifungal, hypoglycemic, antioxidative, and immunomodulating properties, which are beneficial to human health. Other than that, its fermented products were found to be suitable for lactose intolerant population. Its similar composition to human milk provided the opportunity to feed infants where breastfeeding is unavailable. Infant formula made from donkey milk had better properties than cow milk formula but there is a need for rigorous research to solidify its health claims. Authors have also reported the manufacturing of cheese, yogurt, kefir, fermented milk, and ice cream using donkey milk with acceptable sensorial and nutritional attributes. However, lower yield and viscosity (due to lower total solids) and lower coagulation rate (due to the absence of k-casein) are the major challenges for these products. Numerous processing techniques have been explored for its preservation where non-thermal techniques like UV-C and High-Pressure Processing had better nutrient retention than the thermal techniques. However, there is a need for regulations and standard

processing parameters in both cases to evaluate and compare their efficiency and feasibility. Overall, there is a tremendous increase in the donkey milk market. However, industrialization is still facing numerous challenges. Lower yield, scattered population and the lack of regulations are the major issues. There is a need for further extensive research into the improvements in donkey milk production, marketing strategies and better manufacturing of products from it.

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