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Review Article

Nutritional Profile and Potential Health Benefits of Donkey Milk and Milk Products

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Abstract

Milk and milk products being rich in various nutrients are consumed globally as a part of human diet. Consumption of donkey milk and milk products is well-known since time immemorial. There appears to be an increasing awareness in using donkey's milk in human diet. Donkey milk contains a plethora of antimicrobial components as well as protective factors, which makes it unique milk from amongst milk of other species of animals. The chemical composition of donkey milk is comparable to human milk in terms of its low casein/whey protein ratio, low fat content, and high lactose content. In addition to similarity in nutrition composition, it has similar hypo-allergenicity, immune homeostasis conditions, and antimicrobial activity as human milk. Because of this donkey milk is considered as a functional food, and there is a growing interest in manufacturing of milk products from donkey's milk. Currently, donkey milk has been used in the preparation of a variety of dairy products viz. cheese, ice cream, milk powder and novel functional fermented beverages. As raw milk acts as a vehicle for pathogenic microbes that causes several diseases including brucellosis and tuberculosis, it is therefore, imperative that milk should be properly pasteurized to safeguard the health of the people.

Keywords: *Donkey Milk; Functional Food; Health Benefits; Milk Products; Nutritional Profile*

Introduction

Donkey milk (ass milk/jenny milk) is the lacteal secretion that is obtained from milking the healthy mammary gland of the domesticated donkey *(Equusa sinus)*. Donkey is a smaller member of the horse family whose taming began about 6000 B.C. in North Africa at valley of the Nile and since then donkeys have spread across Asia, India, South America and South Europe. According to Food and Agricultural Organization (FAO) three distinctive types of Indian donkey's viz., Indian, Indian wild and Kiang are prevalent in India. The two major breeds of Indian wild donkeys are prevalent in Rann of Kutch (Gujarat) whereas Kiang's is prevalent in Sikkim and Laddakh [1].

There has been an increasing interest in donkey milk due to health-promoting properties Donkey milk is now recognized as "pharma food" because of its nutritive, nutraceutical, and functional properties [2]. Donkey milk is a suitable alternative food for children suffering from cow milk allergy since its biochemistry closely resembles human milk [3]. In addition to similarity in nutrition composition, it has similar hypo-allergenicity, immune homeostasis conditions, and antimicrobial activity as human milk [4-6]. When an infant is suffering from cow milk protein allergy and in case of unavailability of breast milk, the use of donkey's milk has been suggested as a substitute [7].

Moreover, the organoleptic characteristics of donkey milk is similar to human milk making it more palatable and tolerable to infants [8-13]. Because of its anti-microbial, hypo-allergic and anti-aging properties, it is beneficial for the patients of inflammatory bowel diseases, Crohn's disease and ulcerative colitis [14].

In India, the price of donkey milk that is sold in the market varies from Rs 2000 to Rs 7000 per liter. The National Research Centre on Equines (NRCE) is planning to start marketing donkey milk of Halari breed in Hisar, Haryana [15]. Because of the interest in donkeys milk, in order to guarantee a safe product of high quality it is necessary to pasteurize the milk in order to safeguard the health of the people. Giribaldi and co-investigators [16] tested the efficacy of a new small-scale high-temperature short-time (HTST) pasteurizer (72°C for 15s) prototyped by them on donkey milk. The HTST apparatus was able to reduce the total bacteria count, and to completely eradicate *Enterobacteriaceae*. *Bacillus cereus*, when present, was decreased with low efficiency. They reported that the HTST device could be safely applied to pasteurize donkey milk. This communication is an attempt to present a brief review on the nutritional profile and potential health benefits of donkey milk and milk products.

Chemical composition

The chemical composition of donkey milk is comparable to human milk in terms of its low casein/whey proteins ratio, low fat content as well as high lactose content [5]. Because of its acceptable organoleptic properties as well as close resemblance to human milk [8] and low allergenic properties, donkey milk is has been suggested as an alternative for infants affected by cow milk protein allergy [10,41].

The totals solids (TS) content of donkey milk is lower than bovine milk as well as human milk. The average TS content of donkey milk is 9.5 g/100 mL which is lesser than that found in human milk (11.7 g/100 mL) and cow's milk (12.5 g/100 mL) [17,18]. The density of donkey milk ranged from 1.004 ± 0.002 and that of whole cow milk ranged from 1.043 ± 0.002 g mL^{-1} at 21°C [19]. Donkey milk has a lower calorific value in comparison to human and bovine milk. The calorific value of donkey milk is 40 kcal/100 g versus 62 kcal and 65 kcal/100g for human and bovine milk, respectively [20].

Protein

Donkey milk has a lower total protein content (about 1.63 to 1.7 g/100 mL) compared to cow milk [21]. The average total protein content of human is 1.42 g/100 mL. The casein content of donkey milk is 0.66 to 0.7 g/100 mL of milk, which is slightly higher than that of human milk which contains about 0.4g casein/100 ml [21,22]. The whey protein content in donkey milk is 0.64 g/100 ml which is very similar to human milk i.e. 0.76 g/100 ml [20,21]. The casein micelles in donkey milk have a higher diameter (295 ± 11 nm) and lower absolute zeta potential (-15.4 ± 0.5 mV) compared to that of bovine casein micelles. This is mainly attributed to the fact that donkey milk contains low levels of κ-casein. Donkey casein micelles contain a higher proportion of β-casein i.e. 51% that makes it less hydrated [23].

The casein to whey protein ratio in donkey milk was well as donkey milk powder is 70.3:100. The cholesterol content in donkey milk was 8.6 mg/100g [24]. The average casein/whey protein ratio of donkey milk is 1.3 with values ranging from 0.66 to 1.33 which is lower than that of bovine milk [14,25]. Because of its low casein: whey protein ratio consumption of donkey milk leads to formation of a softer clot which results in a better digestibility. The low casein/whey protein also plays an important role in the sensitization to cow milk protein fraction, reducing the allergenic capacity. It has been reported that the lower the value of casein/whey protein ratio, the lower the allergenic capacity [26]. The casein: whey protein ratio in donkey milk is 1.1, while that of cow milk is 4.6. The casein: whey protein ratio of donkey milk is around four times lower than that of cow milk [22].

The low allergenicity of donkey milk can mainly be ascribed to the presence of lower proportion of κ- and αS1-caseins. κ- and αS1-caseins are the major allergens of cow milk [27]. The essential amino acid content in donkey milk is higher in comparison to bovine milk

[14]. Beta-casein is the main fraction in donkey milk 0.37 g/100 mL, respectively), which is very similar to human milk i.e. about 0.27 g/100 ml [28,29]. Beta-lactoglobulin is absent in breast milk [30] but present donkey milk (about 0.27 g/100 mL [28,31].

Carbohydrates

Donkey milk has similar lactose content to human milk i.e. about 7 g/100g [18,32]. The high content of lactose in donkey milk is accountable for its good palatability. Lactose assists in intestinal absorption of calcium and phosphorus. These salts are important for bone mineralization in infants as well as in the prevention of osteoporosis [33]. Because of presence of high lactose content, donkey milk is a good growth medium for potentially probiotic strains of *L. rhamnosus* and wields a good influence on gut health [34]. Donkey milk contains sialylated oligosaccharides, which have antimicrobial and prebiotic effect, and capacity to stimulate the immune system [35].

Minerals

The average ash content in donkey milk is 0.36% [18], which is slightly higher than human milk (about 0.22%). The average ash content of cow's milk is about 0.76% [21]. The lower amount of minerals in donkey milk decreases the load of minerals in the kidneys of infants. The calcium, phosphorus, and magnesium content in donkey milk is higher than that present in human milk but lower than that present in cow's milk. The average sodium content of donkey milk is 118 mg/kg compared to 346 mg/kg in cow milk [19]. The sodium and potassium contents in donkey milk are comparable to those reported for human milk [18,36]. The average calcium, potassium, magnesium and phosphorus content in donkey milk is 329, 271, 37.3 and 104 mg/kg, respectively that is much lower compared to the average calcium, potassium, magnesium, and phosphorus content in cow milk, which is 1340, 1490, 111 and 481 mg/kg, respectively [19].

Fat

There is a wide variation in the reported average fat percentage in donkey milk, which ranges from 0.50% to 1.7%. However, these values are much lower compared to the average fat percentage of human and bovine milk, which is 3.1% and 3.7%, respectively [18,20].

Donkey milk and human milk fat have a higher proportion of unsaturated fatty acids (UFAs). It has been reported that the UFAs of donkey and human milk is about 43 and 55 g/100 g of fat, respectively. These values are way higher than that present in cow's milk which contains about 29 g UFAs/100 g of fat. The saturated fatty acid content in donkey milk is similar to that of human milk i.e. about 57 and 45 g/100 g fat, respectively which is much lower than that of cow milk at 71 g/100 g [22,37]. The ratio of unsaturated fatty acids to saturated fatty acids in donkey milk is 0.75, which is lower than that of human milk i.e. 1.22 but higher than that of cow's milk i. e. 0.41.

Donkey milk is a good source of essential fatty acids. Donkey milk fat contains a high level of both linoleic (C18:2) and linolenic (C18:3) acids, which is about 9.0 g 100 g^{-1} and 5.1 g 100 g^{-1} of total fatty acids, respectively. Donkey milk and donkey milk powder is rich in essential amino acids, fatty acids, and taurine content [24].

The average diameter of milk fat globules in donkey milk is 1.92 microns which is much lower than that present in bovine milk. The average diameter of bovine milk fat is more than double the average globule size of donkey milk fat globules. Because of the smaller size fat globules it contributes to a large surface area for lipase action, which could be one of the reasons for higher digestibility of donkey milk [38].

Enzymes

Enzymes in donkey milk have some distinctive features, such as bactericidal property that distinguishes donkey milk from milks of other mammals [39].

The microbial inhibitory activity in donkey milk is very high compared to milk of other mammals. This has been ascribed to presence of high level of lysozyme and lactoferrin in donkey's milk [40]. The high content of lysozyme (1.0 g/L) in donkey milk is responsible for the low bactericidal concentration [7]. The concentration of lysozyme in donkey milk has been reported to be as high as 4000 mg/ L [17,20]. The lactoferrin content in donkey's milk is about two times higher than that reported for bovine milk [39]. The microbial content of donkey milk was only 10^4 cfu/ml [34].

Vitamins

Donkey milk is rich in vitamin C whereas the vitamins A and E and other water-soluble vitamins is much lower in comparison to that reported for cow's milk [31]. The average vitamin C content is 5,700 mg/100 mL in donkey milk which comparable to that of human milk, which is about 5,600 mg/100 mL. The average vitamin C content in cow's milk is about 1,500 mg/100 mL [42,43]. A higher vitamin D content (2.3 mg/100 mL ± 0.86) (about 92 UI) was reported in Amiata donkey milk. This value is much higher than average values reported for bovine and human milk (0.03 mg/100 mL) [37,44].

Donkey milk products

There is an increased interest in donkey milk because of its healing properties as well as similarity to human milk. Because of this donkey milk is considered as a functional food and there is a growing interest in manufacturing milk products from donkey milk [34]. Donkey milk has been used in manufacture of a variety of dairy products viz. cheese, ice cream, milk powder, fermented milk products, etc.

Donkey milk exhibits poor thermal stability. Sedimentation was detected even at low temperatures i.e. on heating at 75°C for 10 minutes. Due to its poor colloidal stability and presence of high calcium environment; donkey casein micelles are more sensitive to heat treatment than whey protein [23]. A combination of heat treatment along with high pressure processing (HPP) has been suggested to improve the shelf life of donkey milk. Treating pasteurized milk with HPP extends the shelf life of donkey milk up to 30 d when stored at 4°C [45].

Cheese

Manufacture of cheese from donkey milk is very challenging, because of its poor clotting activity. This has been attributed to the low total solids content and lower proportion of casein content in donkey milk especially κ-casein, which produces a very weak gel and does not allow formation of a firm curd on renneting [12]. Presence of low fat content of donkey milk can negatively affect the texture and flavor of the cheese produced [8]. In order to improve the curd firmness, D'Alessandro and co-workers [46] fortified donkey milk with microbial transglutaminase (MTGase). MTGase was added simultaneously with rennet (42°C) in acidified milk (pH 6.3). It was found that the addition of MTGase with rennet resulted in better curd firmness and more retention of casein in cheese compared to control.

Iannella [47] manufactured donkey milk cheese using pure camel chymosinas clotting agent. The yield of fresh cheese was 3.32%. The total solids content of the fresh cheese after 12h in molds was 35.65%. The pH of cheese after 30 minutes from the production was low at 5.34) which provided a good protection against pathogens agents. Galassi and others [48] investigated the possibility of the use of ass's milk as substitute for lysozyme. Nine Italian hard cheeses made by incorporating lysozyme obtained from hen egg white (1.6g hens egg white/100 kg latte). These samples were compared to nine hard cheeses prepared by addition of asses milk (10L asses milk/500 kg di latte), as a lysozyme source. They reported a lower cheese yield at 24 hour in cheese prepared by addition of ass's milk but no difference were found in clotting time and curd-firming time between both the types of cheeses. There was no perceptible difference in sensory attributes between both the types of cheeses.

Sampaio [49] studied the clotting process using donkey milk and a plant aspartic protease-cyprosin, obtained from *Cynara cardunculus* flowers and compared it with clotting ability of microbial rennet. Clotting activity of cyprosin in combination with addition of 0.1%

(w/v) $CaCl_2$ was more effective compared to microbial rennet. Niro and co-investigators [50] tested the effectiveness of donkey milk as a substitute to hen's egg white lysozyme in averting cheese blowing in Italian Grana cheeses. Use of cow-donkey mixed milk showed good microbiological and sensory quality. The authors suggested the use of donkeys' milk as a new technological approach in hard cheese making, aimed at avoiding late-blowing defects.

A novel cheese manufactured from a blend of donkeys' and caprine milk (60: 40% v/v) was prepared. The fully ripened cheese was classified as a high-fat, extra-hard cheese, with high sodium (29.97 $g.kg^{-1}$), magnesium (3.07 $g.kg^{-1}$) and potassium (4.70 $g.kg^{-1}$) content. Medium-chain fatty acids accounted for 18.21% of the total fatty acid content. *Escherichia coli, Salmonella* spp., *Enterobacteriaceae*, coagulase-positive staphylococci, *Listeria monocytogenes, Bacillus cereus*, *Clostridium perfringens* as well as moulds were under the limit of detection in all analyzed samples. The mature cheese was very salty with strong pronounced creamy, fatty and acidic taste with a moderately hard and crumbly texture [51]. Donkey milk cheese from a Martina Franca ass was prepared from calf rennet and compared with cheese made from cow milk. The donkey cheese had a unique and pleasant flavour, which was different from that of control [52].

The effect of jenny milk as an inhibitor of late blowing defect in cheese which is caused by clostridia and coliforms in ewe cheese making was studied. Semi-hard cheese was manufactured by addition of 0.5L donkey milk to 45L bovine milk. After a ripening period of 60d a significant decreased in coliform bacteria count was observed in cheese containing jenny milk compared to cheese prepared without addition of donkey milk. The authors recommended that addition of jenny milk in cheese making is a novel method which may have a significant effect in preventing growth of spore-forming clostridia strains [53]. A procedure for cheese making from donkey milk was established for producing fresh cheese prototypes from donkey milk. The cheese yield was 5.9% yield with a pH of 6.12 pH. The dry matter, fat and protein content of the cheese was 32.4%, 2.1% and 18.5 % respectively. The donkey milk cheese had a high content of β-casein. The flavor of the cheese was found to acceptable [54].

Ice cream

Strawberry flavoured ice cream was prepared from donkey milk added with two strains of lactic acid bacteria which were reported to have probiotic properties viz. *Lactobacillus plantarum* 998 and *Bifidobacterium adolescentis* ATCC15703. The ice cream had very low fat levels with an overrun of around 24.7 ± 1.2%. During 4 months of storage at -20°C there was a decrease in vitamin C content. However, no changes were found in the total phenols and the antioxidant capacity of the ice cream. The viable probiotic bacteria count was over 9 log CFU/g throughout the period of storage [55].

Donkey milk powder

Since donkey milk has lower fat and cholesterol content and contains a suitable calcium phosphorus ratio and is resistant to oxidation donkey milk and donkey milk powder is good supplementary of cow milk and for infant milk powder [24].

Optimization of spray fluid bed dried donkey milk powder from dwarf grey breed was done using different combinations of concentrations of lecithin and temperatures. It was reported that the optimum values of lecithin concentration and inlet air temperature were 0.68% and 57°C, respectively. Freeze drying was carried out at -51°C and 0.043 milli bar pressure for 35.5h after freezing of milk at -20°C for 24h. The proximate composition of freeze dried donkey milk powder was almost similar to that of raw donkey milk. The handling and reconstitution properties of the freeze dried donkey milk powder were better compared to spray fluid bed dried donkey milk powder [56].

Donkey milk of two breeds namely dwarf grey and large white was characterized and it was found that dwarf grey breed donkey milk was more suitable for powder production. This was because of the higher amount of physico-chemical (particle size, protein) and nutritional composition (lysozyme, Ca, B3, cysteine, omega 3) present in such type of milk [57]. In order to obtain an "extra-grade" milk powder from donkey milk concentrate it was suggested that the maximum allowed inlet air temperature should be 173.5°C [58].

Donkey milk subjected to heat treatment at 85°C showed greater loss of activity of lysozyme and beta-lactoglobulin. The lysozyme and beta-lactoglobulin levels decreased by 60% and 87%, respectively. There was a 100% decrease in lactoferrin content heat treatments higher than 65°C. At temperatures lower than 85°C lysozyme was found to be present at comparable concentrations in raw and heat-treated milk [59]. Spray drying process resulted in a significant decrease in lysozyme activity (58% of residual activity) and β-lactoglobulin content (6.43 mg/mL in fresh milk vs 5.51 mg/mL in spry-dried milk). The decrease in lysozyme activity and beta-lactoglobulin content was due to the high temperature to which donkey milk is subjected [60].

The concentration or antimicrobial activity of lysozyme the in jenny (donkey) milk remained unaffected by condensation and pasteurization process. The antimicrobial activity of donkey milk lysozyme was as effective as the antimicrobial activity of synthetic antibiotics against some gram-positive strains and also against the gram-negative strain *X. campestris* [61].

Fermented milk products

Donkey milk is recently gaining attention due to its nutraceutical properties. Because of its low casein content it forms a very weak gel and difficult to convert into cheese, hence, the production of fermented milks from donkey milk could be another way to increase the shelf life of donkey milk.

Carminati and co-workers [62] identified and characterized culturable and cultivable acidifying and thermoduric LAB microbiota present in donkeys milk. It has been reported that *Lactobacillus plantarum* strains might be a good candidates in the production of a novel type of fermented milk from donkey milk not only because of its probiotic potential but also because of its ability in enhancing the growth of *Streptococcus thermophilus*. Both the cultures viz. *S. thermophilus and L. plantarum* had the ability to acidify donkey milk within24 h at 37°C. The fermented donkey milks had an average pH value of 2.91 ± 0.16 and 1.78 ± 0.66, respectively. Both *S. thermophilus* and *L. plantarum* were highly resistant to lysozyme. The minimum bactericidal concentration > 6.4 mg lysozyme/mL for 100% of *S. thermophilus* and 96% of *L. plantarum* was observed [63].

Coppola and co-workers [34] showed that ass's milk is an excellent choice for preparation of probiotic beverages. They examined the effectiveness of heat treatment on microbiological and biochemical quality of ass's milk (Martina Franca breed) and its effects on the growth of 4 different strains of *Lactobacillus rhamnosus*. They observed low bacterial counts (about 4 x 10^4 CFU/mL) in raw machine milk samples that were attributed to the high levels of lysozyme. The pH values remained stable during 15 days of storage. The ass's milk demonstrated to be a good growth medium for potentially probiotic strains of *L. rhamnosus*. Chaivari and others [64] investigated the possibility of producing a fermented beverage from donkey's milk using the probiotic bacterial strains *Lactobacillus rhamnosus* AT 194, CLT 2/2, and *Lactobacillus casei* LC 88, isolated from Parmigiano Reggiano cheese and recommended a technology for production of a fermented beverage from donkey's milk.

Yogurt prepared using a mixture of donkey-ovine milk (70:30) was most preferred based on its texture as well as acidity ($P < 0.05$) from amongst different ratios studied. However, there was no significant ($P < 0.05$) improvement in taste and colour of the product [65]. Different probiotic strains of *Lactobacillus* spp. (*L. acidophilus, L. bulgaricus, L. paraplantarum, L. plantarum, L. pentosus and L. rhamnosus*) were capable to grow in donkey milk, as confirmed by the high microbial count (> 9 log colony forming units/ml) and low pH values when donkey milk was inoculated with these strains of *Lactobacillus* for 48h signifying the prospect of using donkey milk as a growth medium for several probiotic strains [70]. Perna and co-workers [66] manufactured a probiotic yogurt (*Lactobacillus acidophilus, Lactobacillus casei*) from donkey milk (YP) and standard yogurt (YC) and investigated their functional features during the storage up to 30d at 4°C. The lactose content gradually decreased in both yogurts during storage. At 30 days of storage the lactose content was 2.36% and 2.10% in YC and YP, respectively. During storage of both yogurt types, the antioxidant activity increased, however, YP showed a higher antioxidant activity than YC.

Salakidou and co-investigators [67] manufactured a yogurt beverage from donkey milk using a symbiotic starter culture for Bulgarian yogurt from selected probiotic strains of *Lactobacillus delbrueckii ssp. Bulgaricus* BB18 and *Streptococcus thermophilus.* The beverage was stored for 15 days at +4°C – + 6 °C. During refrigerated storage, the content of free amino acids in the yogurt beverage increased to 632.50 mg kg^{-1} on day 15. In a study conducted by Tidona and others [68] incorporated sunflower oil to donkey milk to manufacture a fermented beverage with low energetic intake and improved textural and health characteristics. The milk-based emulsion was fermented using two strains of *Streptococcus thermophilus* showing distinctive abilities. One of the strains (St 907) was used to produce ropy exopolysaccharide (EPS), whereas the other strain (St 563) was used to produce folic acid. There was a 10 times (2.03 ± 0.17 µg/100 mL) increase in folic acid content in the fermented beverage in comparison to that present in donkey milk (0.16 ± 0.03 µg/100 mL).

Kefir prepared from donkey milk showed higher antioxidant activity, antibacterial activity and total phenolic content compared to cow milk kefir. However, the total flavonoid content of cow milk kefir was found to be higher than donkey milk kefir. The acceptability of cow milk kefir was found to be higher than donkey milk kefir. The authors suggested that donkey milk kefir be an alternative nutrient for consumers [69].

Conclusion

Donkey milk is a rich source of various vitamins, minerals, omega fatty acids, and immunoglobulins. It is easily digestible and well suited to nourish infants who are not breastfed. It is also very suitable for children who are allergic to cow milk protein. Donkey milk possesses several medicinal and cosmetic properties. It is used to manufacture several dairy products, such as ice cream, cheese, milk powder, yoghurt, and others. It is emphasized to undertake additional studies on the nutritional and medicinal properties of donkey milk. Attempts should also be made to reduce the cost of donkey milk so that it can be easily accessible to the low income group of the people. There is a need to increase awareness on the use of donkey milk in our diet.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the manuscript.

Authors Contribution

All authors have contributed equally during the manuscript preparation.

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