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(Review Article)

Microbiology of Milk-An updated review

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Abstract

Milk is a very nutritious food in nature, playing an important role in human diets, but at the same time, it serves as an ideal medium for microbial growth, negatively affecting the health of consumers. Raw milk can contain a variety of disease-causing pathogens, as demonstrated by numerous scientific studies. Milk microbiology in India focuses on assessing bacterial contamination in raw and processed dairy products, the presence of food borne pathogens like *Salmonella* and *Staphylococcus aureus*, and understanding the microbial communities in spontaneously fermented milk products, with regulatory efforts by the Food Safety and Standards Authority of India (FSSAI), Government of India, New Delhi. The microbiological quality of milk plays an important role in ensuring the safety of consumers and producers. Milk microbiological quality is of public health concern due to zoonotic diseases that are milk-borne, such as tuberculosis, brucellosis, shigellosis, and salmonellosis. Raw (unpasteurized) milk can harbour a variety of pathogenic microbes that have been well documented for their harmful effect on human health. *Campylobacter jejuni* and *Listeria monocytogenes* account for almost 13% of pathogens found in raw milk batches around the world. *Staphylococcus aureus, Mycobacterium bovis*, *Brucella* and *Salmonella* are also common sources of raw milk contamination. Pasteurized milk is safe for direct consumption without boiling, preserving nutritional value. Pasteurization effectively kills raw milk pathogens without any significant impact on milk nutritional quality. Donkey milk has proved to be a potential alternative to bovine milk. Donkey milk has anti-allergic, antimicrobial, antifungal, hypoglycemic, anti-oxidative, and immuno-modulating properties, which are beneficial to human health

Keywords: Cattle milk; Donkey milk; Non-cattle milk; India; Pasteurized milk; Raw milk; *Salmonella*; *Staphylococcus aureus*

1. Introduction

India is the world's greatest producer of milk. India accounts for around 13% of global milk output and consumes nearly all of it. Milk is an important source of protein for many Indian households. However, milk intake is very low. Hence, it is necessary to examine production–consumption linkages of milk within the paradigm of accessibility, availability, and affordability [2, 3, 37, 38], In India, the dairy industry plays a very important role in the country's socio-economic, culture development and constitutes an important segment of the rural economy [2, 3-37, 38]. Dairy industry in India provides subsistence to millions of houses in villages, ensuring supply of quality milk and milk products to people in both urban and rural areas. Milk is a rich source of nutrients, including high-quality protein, fats, vitamins and carbohydrates [1, 2, 3, 37, 38]. Milk, in its natural form, is a unique food that provides nourishment to human beings since ages. It provides immunogenic protection and supplies nutrients, such as proteins, fat, carbohydrates, vitamins, and minerals, in an easily digestible form than any other single food [1-3-25-38]. There has been an increase in milk consumption in the developing countries, including India and the trend is likely to continue as the demand for animal-source food has shown a rising trend due to population growth, changing lifestyles, increasing wealth, and increasing

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dependence of large vegetarian population on dairy products for proteins and other micronutrients[1-20]. Milk is a very nutritious food in nature, playing an important role in human diets, but at the same time, it serves as an ideal medium for microbial growth, negatively affecting the health of consumers [1-20]. Raw milk, as it leaves the udders of healthy animals, contains very low numbers of microorganisms and is expected to be safe for human consumption [4, 5]. After secretion from udder, milk can be immediately contaminated by spoilage bacteria and pathogens from various sources, including animal faeces, soil, air, feed, water, bedding material, animal hide, infected udder, the exterior of udder and teats, milk containers and people[1-20].

Pasteurized milk is safe for direct consumption without boiling, preserving nutritional value[18]. Understanding the benefits of pasteurization is crucial for consuming milk safely. An immense nature of milk and its nutritional characteristics attract microbes, which contribute predominantly to the perish ability of milk [2-20, 26, 27, 35-36]. The quality and safety of milk is one of the dairy sector's main issues [1-30]. However, milk contamination may occur at any point, but the majority of contamination is generally associated with an unhygienic environment and practices[1-20, 26, 27, 35]. Contamination of milk after leaving the farm shed and processing unit is largely due to poor milk handling practices which lowers the keeping quality of milk[1-30]. The microbiological quality of milk plays an important role in ensuring the safety of consumers and producers. Milk microbiological quality is of public health concern due to zoonotic diseases that are milk-borne, such as tuberculosis, brucellosis, shigellosis, and salmonellosis. Due to the fact that milk-borne diseases are of public health importance, there is a serious need to analyze the milk for the sake of consumer health protection[1-36].

Microorganisms in dairy products play a dual role: while some contribute beneficially to fermentation and probiotic applications, others act as spoilage agents or pathogens, leading to economic losses and public health concerns[1-10]. Microbial communities in milk can be very complex and include numerous bacterial, yeast and fungal species originating from various sources of contamination, such as the external udder surface, milking equipment, air, water, feed, grass and soil[1-22]. Some of these microorganisms play an important role in the dairy industry, especially in fermented foods, while others can cause spoilage or compromise the safety of dairy products[1-20]. In fermented foods such as cheese, microbial communities contribute to the transformation of milk through their growth and metabolic activities. These collective genomes within microbial communities are essential for the production of many dairy products that are recognized and appreciated by consumers worldwide [1-20].

Milk microbiology in India focuses on assessing bacterial contamination in raw and processed dairy products, the presence of food borne pathogens like *Salmonella* and *Staphylococcus aureus*, and understanding the microbial communities in spontaneously fermented milk products, with regulatory efforts by the Food Safety and Standards Authority of India (FSSAI), Government of India, New Delhi [2-20, 26, 27, 35]. Furthermore, FSSAI aiming to improve quality and safety. Key issues include widespread contamination in raw milk and small-scale dairy products due to varying hygiene practices, though research also explores beneficial microbes and antimicrobial peptides from milk for use in food preservation[2-20, 26, 27, 35]. Indian research on milk microbiology highlights widespread contamination in both raw and pasteurized milk, exceeding hygienic standards for total aerobic counts. Studies often identify pathogenic bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella spp.*, and E. coli, linked to poor hygiene during milking, handling, and the cold chain [2-20, 26, 27, 35]. Recommendations frequently focused on improving sanitation, implementing good manufacturing practices, and educating consumers on safe handling practices, like boiling milk before consumption, to mitigate public health risks [2-20, 26, 27, 35, 36]. The safety and quality of milk and its derivatives are influenced by multiple factors, including hygiene and sanitation protocols, farm management practices, milking and milk handling procedures, storage conditions, transportation methods, the health status of lactating animals, and the quality of water used for cleaning milking equipment, udders, and teats [2-20, 26, 27, 35]. These elements represent critical control points for minimizing microbial contamination and safeguarding public health against milk-borne diseases [2-20, 26, 27, 35, 36].

Milk being a perishable commodity is highly vulnerable to bacterial contamination as it provides a favourable medium for its growth [2-20, 26, 27, 35, 36]. Today's consumers need clean, wholesome, and nutritious food that is produced and processed in a sound, sanitary condition and are free from pathogens. Milk passes through different handlers from the farm to the table of consumers and failure to maintain cold chain may result in poor quality of milk [2-20, 26, 27, 35, 36]. Raw, poorly processed and inefficiently handled milk and milk products can lead to severe food-borne illness in humans [2-20, 26, 27, 35, 35, 36]. Particularly in developing countries, the shelf-life of milk and milk products tends to be shorter as the production of milk and its processing into different milk products takes place under unsanitary conditions and poor production practices [5]. *Coliforms* are invariably found in raw milk but with good manufacturing practices, their number can be kept very low [2-20, 26, 27, 35, 36].

The hygienic quality of milk can be determined using several microbiological count methods, including total bacterial count (TBC), coliform count (CC) and coagulase-positive *Staphylococci* count (CPS) [2-20, 26, 27, 35]. TBC provides an estimate of the total number of aerobic bacteria present in raw milk [9] and is the most common method to evaluate the hygienic quality of raw milk at the farm level [10]. TBC is a useful indicator of the hygienic condition of the farm environment, the cow and the milking equipment [2-20, 26, 27, 35]. An increase in the TBC of bulk tanks can be related to environmental contaminants, unsanitary milk equipment, dirty udders and teats, and mastitis causing organisms [2-20, 26, 27, 35]. Temperature of storage and time since milking are also important in determining milk quality, as these factors influence the rate at which bacteria increase in number [2-20, 26, 27, 35]. A high level of TBC (> 106 cfu/ml) is associated with increased enzyme activity that can result in textural and flavor defects in raw milk and processed dairy product. *Staphylococcus aureus* is a major cause of bovine mastitis, resulting in large economic losses due to reduced milk production and quality [21]. *S. aureus* is frequently isolated from cows with mastitis [22–25] and bulk milk [2-20, 26, 27, 35].

2. Cattle and Non-Cattle Milk

Among micronutrient-rich foods, dairy products are consumed at relatively low levels in low-income countries [1-20, 36]. Dairy products are significant sources of animal-sourced proteins, essential amino acids, and other micronutrients, including zinc, vitamin A, and calcium. Due to its rich content of highly digestible proteins, minerals, and insulin-like growth factor-I [2-20, 36]. Milk has been identified as a significant food component for stimulating linear growth in children [2, 3-36]. Milk is thus regarded as a high-quality food, nutritionally. Humans are known to have consumed cattle (Bos taurus, cow) and non-cattle (such as goat and sheep) milks as part of their diet since prehistoric times [2-20, 26, 27- 35]. As a convenient source of nutrition, cattle milk is the most-consumed milk worldwide because of its widespread availability and large production volumes[2-20, 26, 27, 35].

Non-cattle milks are of nutritional importance to people in developing countries as well as in geographical areas in which the natural climate is unsuitable for the survival of dairy cattle[2-20, 26, 27, 35]. For example, buffalo milk in Asia, sheep milk in Europe and the Mediterranean basin (including the Middle East), camel milk ("the white gold of the desert") in Africa, goat milk ("the cattle of the poor") in Africa and southern Asia, horse milk in the steppe areas of central Asia, yak milk on the Tibetan plateau, reindeer milk in northern Scandinavia, musk ox milk in the Arctic, and mithun milk in the hilly regions of the Indian subcontinent[2-20, 26, 27, 35]. Of the total global non-cattle milk production, buffalo milk has nearly tripled, camel milk has nearly doubled, and goat milk has slightly increased during this period [2-20, 26, 27, 35]. No world statistics on the amounts of milk produced from other dairy species, such as yak, horse, donkey, deer, musk ox, and llama, are available [2-20, 26, 27, 35]. Much of the non-cattle milk production remains officially unreported because of the unknown amounts that are consumed locally at a farmer's home or are sold directly by farmers to local people, especially in developing countries [2-20, 26, 27, 35]. The buffalo, goat, sheep, and camel milking industry is well set-up in many parts of the world, is gaining popularity, and is proving to be a profitable business for those who have already implemented [2-20, 26, 27, 35]. For example, goat, sheep, camel, horse, and donkey milk are considered to be relatively more easily digestible, less allergenic, and more similar to human milk than cattle milk [2-20, 26, 27, 35]. The traditional dairy-cattle-based industry is becoming increasingly diversified with milk and milk products from non-cattle dairy species [2-20, 26, 27, 35]. The interest in non-cattle milks has increased because there have been several anecdotal reports about the nutritional benefits of these milks and reports both of individuals tolerating and digesting some non-cattle milks better than cattle milk and of certain characteristics that non-cattle milks are thought to share in common with human milk [2-20, 26, 27, 35]. Thus, non-cattle milks are considered to have potential applications in infant, children, and elderly nutrition for the development of specialized products with better nutritional profiles [2-20, 26, 27, 35]. However, there is very little scientific information and understanding about the digestion behavior of non-cattle milks[2-20, 26-35].

3. Donkey milk: Nutritional value

Donkey milk has received increasing attention in recent years due to its unique nutritional composition and potential biological activities. T**he global donkey milk market size was estimated at USD 23.2 million in 2021 and is projected to reach USD 45.0 million by 2028, growing at a CAGR of 9.9% from 2022 to 2028**[53]. This can be credited to the growing adoption of the product from the European economy, such as the U.K. and Germany [53-55]. Moreover, the rising awareness for skincare products and growing demand for powder-based jenny milk across the globe are anticipated to boost the market growth during the forecast period [53]. Donkey milk is mainly utilized in the manufacture of cheese, chocolates, as well as cheddar [53]. During the COVID-19 period, the use of donkey's milk increased owing to its benefits like high vitamin content as well as its ability to increase the immune system, which in turn has driven the market growth [53]. China has the highest donkey milk production globally, with an estimated annual output of approximately 270,000 tons [39-48-52-55]. This is driven by a large donkey population and a growing industry focused on milk, meat, and hides. While Europe holds the largest market share for consumption[53]. China leads in production volume [39-48-52]. The highest number of donkeys can be found in Ethiopia, followed by China and Pakistan[53]. It is especially well liked in the pharmaceutical and cosmetic sectors because of its capacity to revitalize and hydrate the skin [39-48-55]. The donkey milk powder origin is natural, and it is an important element that is largely utilized in the cosmetics industry [53,55]. In addition, the donkey milk powder is mainly a rich source of minerals, essential fats, protein, bioactive enzymes, and several growth factors like riboflavin, and vitamin D, which offer natural growth to the skin and tone it. Cosmetics & personal care contributed a share of around 60% of the global market revenue in 2021[53-55]. The growing adoption of jenny milk in the U.K. to maintain sustainable health is propelling the market growth[53, 55]. Europe is the main consumer of donkey's milk, and important nations such as Spain, Belgium, Portugal, Italy, Bulgaria, France, and Greece are the major consumer of donkey's milk in Europe[53, 55]. The rising consumption of refreshing milk products, the high imports of powder-based jenny milk, and the increasing health concern of consumers are expected to boost the market[53]. This is owing to the increase in requirements by the personal care industry, and escalating demand for innovative natural ingredients[53, 55]. In addition, the growing demand for herbal cosmetics and personal care products, such as peels and face masks. Jenny milk consists of naturally-derived cosmetic ingredients with unique cosmetic properties. The increased adoption of fairness cream to maintain the lightweight texture of the skin will propel the market demand[53, 55]. Rising investment from private firms to procure powder milk is projected to continue to grow in the market in the upcoming years[53]. Additionally, it is becoming more well-known for its ability to improve immunity, treat lactose intolerance, and support digestive health [39-48-53]. Donkeys are highly social animals. They enjoy bonding with other donkeys and often form lifelong friendships. They also seek connection with trusted humans and can recognise individual voices and faces[53]. The demand for donkey milk is steadily rising in India, especially in the country's cities [39-48-52]. As people's health concerns grow, they are looking for dairy alternatives. Donkey milk is being investigated as a potential commercial product for export as a result of this rising demand [39-48-52-55]. .In recent years, donkey milk attracts more attentions because of its similarities to human milk and unique advantage to be a supplementary of human milk [39-48-52]. For example whey protein of donkey milk is high. Moreover Donkey milk has a lot of unsaturated fatty acids specially linoleic acid, and low content of fat and cholesterol, is rich in calcium and selenium [39-48-52]. Donkey milk also has strong antioxidant activity, retarding aging process, and is rich in kinds of immune-boosting substances to human immune system [39-48]. Donkey milk is expensive due to its low production volume, as a single jenny (female donkey) produces only about 1 to 1.5 liters of milk per day, compared to a cow's 20+ liters [39-48]. The milk production process is also labor-intensive and costly, requiring manual milking three times a day, with a portion of the milk always reserved for the foal [39-48]. Furthermore, donkey milk is in high demand for its perceived nutritional and cosmetic benefits, which drives up its price. Donkey milk production is often associated with specialized farms dedicated exclusively to raising and milking donkeys [39-48]. The infrastructure and expertise required for such focused farming practices contribute to increased production costs. These specialized farms, with their unique set-up, play a crucial role in determining the market price of donkey milk [39-48-52]. The sustainable development of the donkey milk industry requires comprehensive approaches to resource protection, technological innovation, brand building, and supportive policies to realize its full potential in contributing to human health and economic development [39-48-52].

In India, there are three registered breeds of donkey: Spiti, Halari, and Kachchhi [49]. The Halari donkey breed is a recently registered one (Accession No: INDIA_DONKEY_0400_HALARI_05002) that is primarily found in the state of Gujarat, India. They may migrate across great distances and have a predominance of white colouring (National Bureau of Animal Genetic Resources, Karnal) [49]. This breed is primarily found in the Gujarat's Jamnagar, Dwarika, and Rajkot districts of India[49].

Gujarat is the Indian state that produces donkey milk, with entrepreneurs like Dhiren Solanki establishing farms in the state and supplying it to other states with higher demand, such as Karnataka and Kerala. Another entrepreneur in Tamil Nadu has also established what is described as the largest donkey farm in India[39-49]. The top donkey milk-producing states in India are Gujarat, Rajasthan, Tamil Nadu, and Haryana**.** Success Story for establishing India's largest donkey farm "The Donkey Palace" Shri U. Babu, a thriving entrepreneur from Vannarpet, Chennai, Tamilnadu is known for establishing India's largest donkey farm and becoming a reliable supplier of donkey milk to a couple of cosmetic manufacturing companies [39-49]. At present, Shri Babu has established India's largest donkey farm, managing approximately 5000 donkeys through a franchise model with more than 75 franchisee farms. He has also established "The Donkey Palace," One Health - One Solution - A Conservation, Recreation, and Awareness Center, which aims to promote donkeys' value and contribution to society and the economy. Beauty Cave Cosmetics stands out among the top Donkey milk Product manufacturer companies in India that engaged in world-class manufacturing, developing, and delivering different types of skincare products with the donkey milk [39-49]. The major donkey milk exporters in India are Joyful Donkey Farm, Asino Donkey Farm, Mishree Enterprises, Trilochan Donkey Farms and Mjm Donkey Farm[39-49]. Donkey milk production in India is a niche, but emerging, sector driven by demand for its use in cosmetics and traditional remedies, with farmers often maintaining small herds and selling directly to consumers[49-52]. While there is no official oversight, the market is growing, with high prices reflecting the low milk yield of donkeys, which is around 0.5 to 1.3 liters per day. Production is concentrated in smaller-scale operations, sometimes adapting to urban logistics for sales [39-49]. Compared to the milk of the major dairying animals such as cow, buffalo, goat, camel, and sheep, donkey milk has a significantly different composition. It has a higher proportion of lactose and less fat and protein content than bovine milk [15]. The low protein content in donkey milk offers real benefits because a high protein intake in formula-fed infants is mostly associated with the cases of early adiposity rebound [49-52, 55].

Donkey milk exhibits peculiar nutritional qualities similar to those found in human milk [49,55]. Therefore, it can be considered as an excellent substitute. In the last decade, extensive research has been undertaken on the milk of several donkey breeds due to its distinctive qualities. According to the study conducted by Garhwal et al., (2023) [49] Halari breed milk has good nutritional value and is nearly identical in composition to human milk[49, 55]. The high lactose content in Halari donkey milk makes it palatable and acceptable in terms of its taste attribute. Halari donkey milk offers an excellent opportunity to be employed in the production of low-fat dairy products due to its lower fat content[49]. The gross vitamin, mineral, and amino acid composition of Halari donkey milk are consistent with those reported for other breeds with slight differences[39-49]. The low microbial load in Halari donkey milk makes it microbiologically safe and contributes to its longer shelf life[49]. More extensive research can be conducted for **Halari** donkey milk to provide more conclusive insights about its nutritional qualities and possible derivatives[49].

Renowned for its nutritional richness, donkey milk boasts abundant proteins, vitamins, and minerals [39-48]. This exceptional nutritional profile not only contributes to its perceived value but also justifies the premium price associated with its consumption [39-48]. Consumers are willing to pay more for a product that offers enhanced nutritional benefits of donkey milk [39-48]. In Peru, donkey milk is used to treat diseases like asthma, bronchitis, diabetes, anabrosis, gastritis, and even ease climacteric discomfort [39-48]. There are also many people treating tuberculosis and gastric ulcer by drinking donkey milk in Xinjiang, China [39-48-52]. In some countries in Europe and America, donkey milk is not only the component of many biological products, a kind of health care products, accepted by more and more people[39-48]. Therefore, donkey milk has high nutrition value and wide medicinal value. As an insoluble protein casein is hard to be digested and can form larger and harder clots in baby's stomach[39-48]. However whey protein, belonging to soluble protein, only forms small and soft clots in infant's stomach, being easy to be digested and absorbed. Furthermore whey protein contains a variety of biological activity proteins, enzymes, peptides, immune factors and growth factors, which plays an important role in growth and mental development of human[39-48]. The nutritional value and biological potency of whey protein are very high, therefore it is praised as "the king of protein"[39-48]. The proportion of whey protein and casein in milk relates to the nutrition and absorption for body. In the body, lactose can be converted to glucose which provides energy and participates in the composition of tissues and organs, and galactose which can synthesize glycolipid, one of basic materials for neural tissue, and has a vital role in the development of infant nervous systems[39-48]. In addition, lactose can promote the proliferation of intestinal probiotics, inhibit the growth of spoilage bacteria, improve the absorption of calcium, phosphorus and other mineral elements, and boost the quality and strength of skeleton[39-48]. Therefore, the development and utilization of donkey milk has great economic value and social value[39-48].

The lactose content of donkey milk is high and it can promote the beneficial microbial proliferation of intestinal tract. Because of high ratio of whey protein, donkey milk's protein in infant stomach can form small and soft clot, which is easy to digest and absorb[39-48-52]. The fat and cholesterol content of donkey milk and donkey milk powder is low and the essential fatty acids content of human body is high, which is suitable for cardiovascular disease, gallstone disease and obesity patients, and the elderly[39-48]. In addition, the calcium phosphorus ratio of donkey milk is suitable[39-48]. After drinking, the burden to kidney is lighter, which is suitable for the imperfection of renal function in infant[39-48]. In general, the difference between donkey milk and human milk is small. The nutritive value and digestibility of donkey milk powder are better than cow milk powder [39-48-52]. The antioxidant capacity of donkey milk and powder is high. For these reasons, so far donkey milk and powder are considered as the best mammalian milk for infant especially in case of cow milk allergy, and diet for cerebrovascular diseases and diabetes mellitus patient[39-48-52].

The donkey milk has a remarkable similarity to human milk, in addition to its valuable nutritional composition and content of numerous immune factors [39-48, 55]. However, some basic characteristics of donkey milk have been established: low contents of fat and cholesterol, total proteins and casein and high contents of lactose, whey proteins, calcium, selenium, and Vitamin D3 [39-48-52-55]. The donkey milk is rich in various protective proteins (α-lactalbumin, lysozyme, lactoferrin, lactoperoxidase, and immunoglobulins), and shows strong antioxidant, antibacterial, antiviral, antifungal, hypoglycemic, antiparasitic, and antitumor activity [39-48]. Donkey milk can be considered functional food, having in mind that its fatty-acid profile and content of essential fatty acids are beneficial for cardiac health[39-48-55].

The total fat content in donkey milk is low compared to human milk, and for this reason it is necessary to enrich donkey milk with other fat in order to provide enough calories in a diet for children[39-48]. Commercialization of donkey milk and dairy products is still limited due to low production levels, that is lack of products on the market, and lack of product information [39-48-52]. Considering the research data from the literature, there is a need for human clinical trials in order to obtain a stronger evidence of the therapeutic properties of donkey milk[39-48-52].

Donkey milk represents a unique and valuable food resource with an exceptional nutritional composition and biological activities that more closely resemble human milk than cow milk. Its high proportion of whey proteins (55–65%), favourable fatty acid profile, abundant vitamins (particularly vitamin C at levels 4.75 times higher than in cow milk), and essential minerals contribute significantly to its potential applications in infant nutrition, medical treatments, and cosmetic formulations[39-48]. The documented antioxidant, antimicrobial, immunomodulatory, and anti-inflammatory properties further enhance its value as a functional food, offering considerable benefits for various demographic groups including infants with cow milk allergies, elderly individuals experiencing immunosenescence, and people with specific health conditions[39-48]. Despite these promising attributes, the donkey milk industry faces significant challenges that require comprehensive solutions for sustainable development[39-48-52].

The main nutritional components of donkey milk, including proteins, lipids, carbohydrates, vitamins, and minerals, while also examining its significant biological activities such as antioxidant, antimicrobial, immunomodulatory, and anti-inflammatory properties[39-48]. The protein profile of donkey milk is notable for its high proportion of whey proteins (55–65%), resembling human milk more closely than cow milk. Its relatively low-fat content (approximately 1.29%) with higher proportions of unsaturated fatty acids provides nutritional advantages for specific dietary needs[39-48-52]. The carbohydrate content, primarily lactose, contributes to energy provision and calcium absorption[39-48]. Donkey milk is also distinguished by its rich vitamin profile, particularly vitamin C (about 4.75 times higher than cow milk), and essential minerals including calcium, phosphorus, and zinc[39-48]. The biological activities of donkey milk extend to various applications in infant nutrition, particularly for children with cow milk protein allergies, potential medical treatments for infections and inflammatory conditions, and cosmetic formulations[39-48-52]. Despite these promising attributes, the donkey milk industry faces challenges including low milk yield, lack of standardized production methods, and quality control measures[39-48-52].

Donkey milk contains a relatively high protein content, ranging from 1.4 to 1.8 g/100 mL, primarily consisting of whey proteins and caseins [39-48]. A distinctive characteristic of donkey milk protein is the high proportion of whey protein, which accounts for approximately 55–65% of the total protein content[39-48-52]. The principal whey proteins in donkey milk include α-lactalbumin, β-lactoglobulin, and lactoferrin [39-48]. α-Lactalbumin serves as an essential component that not only provides a rich source of essential amino acids such as leucine and isoleucine, but also plays a crucial role in lactose synthesis[39-48]. It demonstrates high biological value and digestibility [39-48]. Although present in lower concentrations compared with other milk sources, β-lactoglobulin still contributes significantly to the overall protein profile [39-48]. Lactoferrin represents a multifunctional protein with antimicrobial, antiviral, and immunomodulatory activities [39-48]. ts iron-binding capacity inhibits the growth of iron-dependent bacteria and fungi[39-48]. Traditional classification categorizes milk as either casein milk or whey protein milk based on the protein composition [39-48]. Casein constitutes approximately 80% of cow milk protein, 50% of donkey milk protein, and less than 50% of human breast milk protein[39-48-52]. Whey proteins demonstrate both antibacterial and antiinflammatory properties [39-48]. Donkey milk contains a diverse array of essential amino acids similar to human breast milk, such as lysine, methionine, threonine, etc., facilitating efficient protein absorption and utilization [39-48]. These characteristics establish donkey milk as an ideal protein source, particularly appropriate for populations with increased protein requirements including children, pregnant women, and lactating mothers[39-48-52].

Donkey milk is characterized by its relatively low-fat content, averaging about 1.29%, which is only about 40% of the fat content in regular cow's milk [39-48]. The primary component of its lipid profile is triglycerides (TAGs), which make up over 95% of the total fat content [39-48]. This high proportion of triglycerides allows for the rapid release of fatty acids during digestion, making it easier for the human body to absorb [30]. Additionally, donkey milk contains a small amount of diacylglycerol (DAGs), which has health benefits, such as reducing fat accumulation and regulating blood lipids, thereby enhancing the nutritional value of donkey milk[39-48-52].

Although donkey milk contains less phospholipids than cow's milk, it still plays a significant role in its lipid system [39-48]. Phospholipids are essential components of cell membranes and are involved in various physiological activities in the human body, promoting fat metabolism and supporting the normal functioning of the nervous system [39-48]. Additionally, donkey milk has a lower cholesterol content, which aligns with its overall low-fat profile, further reducing the impact on blood lipids after consumption [39-48]. Furthermore, donkey milk contains comparatively lower levels of saturated fatty acids. Since the excessive consumption of saturated fatty acids has been associated with increased cardiovascular disease risk, the reduced saturated fatty acid content in donkey milk represents a significant nutritional advantage[39-48-52].

In contrast, donkey milk features a higher proportion of unsaturated fatty acids, particularly polyunsaturated fatty acids, which are essential for human health [39-48]. These include important compounds such as linoleic acid and linolenic acid. Linoleic acid, which cannot be synthesized by the human body and must be obtained through dietary sources, is relatively abundant in donkey milk and constitutes a higher percentage of total fat compared with other animal milks [39-48]. Research has demonstrated that linoleic acid can effectively regulate blood lipids, reduce cholesterol levels, and help prevent cardiovascular diseases [39-48]. Additionally, donkey milk contains measurable amounts of conjugated linoleic acid, which performs various physiological functions including lipid metabolism regulation, serum cholesterol reduction, blood lipid lowering, blood vessel elasticity improvement, blood pressure reduction, microcirculation enhancement, and atherosclerosis inhibition[39-48-52].

Due to its unique lipid composition, donkey milk offers numerous health benefits [39-48]. Although donkey milk contains fewer fatty acids than human breast milk, indicating a lower energy content, this reduced fat profile makes it an appropriate choice for individuals needing to monitor their fat intake [39-48-52]. The combination of low-fat content and high unsaturated fatty acid levels contributes to reduced cardiovascular disease risk by minimizing cholesterol deposition in the blood vessel walls, thereby supporting cardiovascular health [39-48-52]. For infants and young children, donkey milk lipids provide essential energy and nutrients required for growth and development while promoting brain and nervous system development[39-48-52]. Moreover, the unsaturated fatty acids in donkey milk help maintain intestinal flora balance by stimulating beneficial bacterial growth and supporting normal intestinal function[39-48-52].

4. Milk Pasteurization

Milk pasteurization is a process of heating raw milk to a specific temperature for a set time to kill harmful bacteria and other microorganisms that can cause illness, with the most common methods in Canada involving heating to at least 72°C for 16 seconds or 63°C for 30 minutes, followed by rapid cooling [4, 5,18]. This process makes milk safe for consumption by reducing the risk of diseases from pathogens like *E. coli, Salmonella*, and *Listeria* [4, 5,18]. In Canada, all milk sold to the public is required by law to be pasteurized[4, 5,18]. It is named for the French scientist Louis Pasteur, who in the 1860s demonstrated that abnormal fermentation of wine and beer could be prevented by heating the beverages to about 57 °C (135 °F) for a few minutes[4, 5,18]. Pasteurization of milk, widely practiced in several countries, notably the, requires temperatures of about 63 °C (145 °F) maintained for 30 minutes or, alternatively, heating to a higher temperature, 72 °C (162 °F), and holding for 15 seconds (and yet higher temperatures for shorter periods of time) [4, 5,18]. The times and temperatures are those determined to be necessary to destroy Mycobacterium tuberculosis and other, more heat-resistant, non-spore-forming, disease-causing microorganisms found in milk [4, 5,18]. The treatment also destroys most of the microorganisms that cause spoilage and so prolongs the storage time of food. Ultra-High-temperature (UHT) pasteurization involves heating milk or cream to 138–150 °C (280–302 °F) for one or two seconds. Packaged in sterile, hermetically sealed containers, UHT milk may be stored without refrigeration for months[4, 5,18]. Ultrapasterurized milk and cream are heated to at least 138 °C for at least two seconds, but, because of less stringent packaging, they must be refrigerated. Shelf life is extended to 60–90 days[4, 5,18]. After opening, spoilage times for both UHT and ultrapasteurized products are similar to those of conventionally pasteurized products. Pasteurized milk is widely available in India through brands like Amul, Mother Dairy, and Verka, but many consumers boil it to address concerns about potential recontamination during transport or post-pasteurization, and to ensure safety despite the milk being already treated to remove bacteria and prolong shelf life[4, 5,18]. In India, milk pasteurization typically employs high-temperature short-time (HTST) methods, heating milk to 72–75°C for 15–20 seconds, or ultra-high-temperature (UHT) for significantly longer shelf life [4, 5,18]. Other processes include low-temperature long-time (LTLT) and continuous or batch pasteurization. Regardless of the method, the process involves heating milk to kill pathogens and then rapidly cooling it to prevent bacterial regrowth, thereby extending its shelf life and ensuring safety[4, 5,18]. In general, almost all Gram-negative organisms in milk are destroyed with pasteurization at 63 °C for 30 min, and although some thermophilic and mesophilic bacteria, for example, *Micrococci* and *Streptococcus* spp., which are thermoduric, may survive pasteurization, they grow very slowly once the pasteurized milk is done [4, 5,18].

5. Raw animal milk: Disease-causing pathogens

Raw milk can contain a variety of disease-causing pathogens, as demonstrated by numerous scientific studies[4, 5,18]. These studies, along with numerous food-borne outbreaks, clearly demonstrated the risk associated with drinking raw milk[4, 5,18]. Pasteurization effectively kills raw milk pathogens without any significant impact on milk nutritional quality [4, 5,18]. Raw milk consumption is broadly understood to pose a significant public health risk as it introduces the consumer to harmful and potentially life threatening bacterial and viral pathogens[4, 5,18]. This fundamental risk far outweighs any alleged health benefits, many of which are only anecdotally supported and not backed by scientific evidence [4, 5,18]. In fact, some raw milk health claims are outright misleading, not true or taken out of context, and can put people in danger[4, 5,18]. Science is clear that the implementation of milk pasteurization has been a critical tool in keeping us safe from harmful microbes while utilizing helpful microbes to better our gut health[4, 5,18]. The manipulation of microbes on and in our food sources, including milk, has allowed us to harness a positive relationship with the microbial world to benefit human health[4, 5,18]. It is important to note that raw milk contamination can and does occur even when the milk comes from healthy animals and is handled with care[4, 5,18]. This contamination risk has existed as long as humans have consumed animal milk. Common contamination can occur at the udder, via direct transmission from blood of cow into the milk (systemic infection), fecal contamination during milking or through dirty equipment[4, 5,18]. Populations at highest risk of serious health complications from raw milk contamination include elderly persons, children under 5 years old and pregnant women[4, 5,18]. Additionally, immunocompromised populations, or persons without a fully intact immune response, are at extreme risk of contracting milk-borne diseases from raw milk ingestion[4, 5,18].

There has been an increased consumer demand for drinking raw milk [4, 5,18] and dairy products made from unpasteurised milk (including raw and thermised milk) such as cheese, butter, cream, ice-cream and kefir [1, 4, 5,18]. There has also been an increase in the popularity of consuming raw milk and associated products produced from non-bovine species such as goat, sheep, horse, donkey and camel [1, 3, 4]. The consumption of raw milk and unpasteurized dairy products can present health risks from contamination by a variety of pathogenic micro-organisms, with more risks being reported in association with cows' milk as compared to other species such as goats and sheep[1, 4, 5,18]. Microbes that may be present in milk can include pathogens, spoilage organisms, organisms that may be conditionally beneficial (e.g., lactic acid bacteria), and those that have not been linked to either beneficial or detrimental effects on product quality or human health. Although milk can contain a full range of organisms classified as microbes (i.e., bacteria, viruses, fungi, and protozoans), with few exceptions (e.g., phages that affect fermentations, fungal spoilage organisms, and, to a lesser extent, the protozoan pathogens *Cryptosporidium* and *Giardia*) dairy microbiology to date has focused predominantly on bacteria[4, 5,18].

Raw (unpasteurized) milk can harbor a variety of pathogenic microbes that have been well documented for their harmful effect on human health[4, 5,18]. *Campylobacter jejuni* and *Listeria monocytogenes* account for almost 13% of pathogens found in raw milk batches around the world. *Mycobacterium bovis*, *Brucella* and *Salmonella* are also common sources of raw milk contamination[4, 5,18]. These bacterial pathogens can cause serious illnesses, such as tuberculosis, brucellosis, listeriosis and typhoid fever. Bovine tuberculosis infection can lead to a persistent cough, chest pain, fever, night sweats, weight loss, fatigue and can become life-threatening without treatment. Brucellosis causes undulating fevers, chills, joint and muscle pain, headaches and profound fatigue[4, 5,18]. Infections can last weeks or even months and may relapse if not adequately treated[4, 5,18]. Meanwhile, *Listeriosis* poses a particular risk to pregnant individuals, newborns, the elderly and those with weakened immune systems. Symptoms include fever, muscle aches, nausea and diarrhea [4, 5,18]. Severe complications, such as meningitis or sepsis, can occur in vulnerable populations[4, 5,18]. Similarly, typhoid fever leads to sustained high fever, abdominal pain, headache and fatigue. Left untreated, typhoid fever can result in serious intestinal complications[4, 5,18].

Probiotic microorganisms must be of human origin in order to have an impact on human health. Bacteria present in raw milk are from infected udder tissues (e.g., mastitis causing bacteria), the dairy environment (e.g., soil, water, and cow manure), and milking equipment [2, 3, 4, 5,18-35]. High bacteria counts in raw milk only indicate poor animal health and poor farm hygiene. Bacteria in raw milk are typically not of human origin. An exception is *Streptococcus pyogenes*. *S. pyogenes* that has adapted to humans can be transmitted to animals. Once *S. pyogenes* is colonized in animals, it can be re-transmitted to humans as a **human pathogen** that causes strep throat. For example, *S. pyogenes* can infect a cow udder to cause mastitis [2, 3, 4, 5-18-36]. The infected cow udder can subsequently shed *S. pyogenes*, a pathogen, into raw milk. *Bifidobacteria* have been mentioned by raw milk advocates as the "good bugs" in raw milk [2, 3, 4, 5-18-35]. *Bifidobacteria* are bacteria commonly found in human and animal gastrointestinal track and they are bacteria that make up the gut flora. Since *bifidobacteria* are found in cow's GI track, they are present in cow's fecal matter. Raw milk collected with proper hygiene should not contain *bifidobacteria*. In fact, the presence of *bifidobacteria* in raw milk indicates fecal contamination and poor farm hygiene[2, 3, 4, 5-18-36]. Bacteria found in raw milk are not probiotic. Probiotic microorganisms must be non-pathogenic. In contrast, raw milk can host various human pathogens, including *E. coli* O157:H7, *Salmonella*, *Streptococcus spp*. *Yersinia enterocolitica*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Listeria monocytogenes, Mycobacterium tuberculosis*, and *Coxiella burnetti* [2, 3, 4, 5-18-35]. Pasteurization is the only method to achieve complete elimination of vegetative pathogens. Contrary to raw milk advocates' claims, pasteurization does not completely inactive these indigenous antimicrobial components in milk [2, 3, 4, 5-18-35].

6. Phytochemistry of Bovine milk

Research papers on milk's chemical composition highlight variations based on species, but generally, all milk contains water, fat (SFAs, MUFAs, PUFAs), proteins (caseins and whey), carbohydrates (primarily lactose), and minerals (ash). Key components like alpha-lactalbumin, lactoferrin, and immunoglobulin IgA are prominent in human milk, while bovine milk is complex with many fatty acids and phospholipids [1, 2, 3, 4, 5-18-35]. Normal bovine milk contains about 3 to 3.5% total protein. The two major groups of milk protein are casein (about 80%) and whey proteins (about 20%). The protein quality of pasteurized milk is not different from that of raw milk [1, 2, 3, 4, 5-18-35]. Fermented dairy products, especially yogurt, have been reported to ease lactose mal-absorption in lactose intolerant subjects. This enhanced digestion of lactose has been attributed to the intra-intestinal hydrolysis of lactose by lactase secreted by yogurt fermentation microorganisms. However, raw milk does not contain the same types of microorganisms at the similar levels that are found in yogurt. Yogurt that showed a benefit towards lactose intolerance typically contained 10^7cfu/ml or higher levels of *Streptococcus hermophilus* and *Lactobacillus bulgaricus*, and these microorganisms were **purposely** inoculated during yogurt manufacturing[1, 2, 3, 4, 5-18-35]. Milk contains both fat soluble and water soluble vitamins. Fat soluble vitamins include A, D, E, and K. Water soluble vitamins included B1 (thiamin), B2 (riboflavin), niacin, pantothenic acid, B6, biotin, folic acid, B12, and vitamin C. In general, pasteurization has a little effect on milk vitamin levels. Vitamins that are present at high levels in milk, such as riboflavin, B6 and B12, are relatively heat stable[1, 2, 3, 4, 5-18-35]. Other factors, such as storage temperature, dissolved oxygen, light exposure, packaging, and length of storage can have a much greater impact on milk vitamin stability. The only vitamin that is significantly heat labile is vitamin C but milk is an insignificant source for vitamin C. A cup of milk (240 ml) only provides about 5 mg of vitamin C [1, 2, 3, 4, 5-18-35].

Lipids are one of the most abundant classes of milk constituents. In general, they account for 6% of the total mass content of milk and mainly include triacylglycerides [15]. Indeed, 98% of milk fatty acids are found in the form of triesters of glycerol, while the remaining 2% comprises other glycerol esters (mono- and di-), phospholipids, glycolipids and free fatty acids [1, 2, 3, 4, 5-18-35]. About half of the fatty acids occurring in milk have a full saturated chain made of 18, 16 or 14 carbon atoms, i.e., C18:0, C16:0 and C14:0 [17], while unsaturated fatty acids are generally minor components compared to the total[1, 2, 3, 4, 5-18-35].

Milk contains various indigenous proteases, including plasmin and somatic cell proteases. The major proteolytic activity in milk is from plasmin[1, 2, 3, 4, 5-18-35]. Plasmin is part of a complex enzyme system consisting of plasmin, plasminogen, plasminogen activator, plasmin inhibitor, and plasminogen activator inhibitor. The plasmin system plays important roles in milk quality and cheese ripening [1, 2, 3, 4, 5-18-35]. Increase in plasmin activity is often reported in low quality milk with high somatic cell counts[1, 2, 3, 4, 5-18-35]. High plasmin activity in fresh milk reduces milk shelf-life due to the hydrolysis of milk casein and the production of bitter peptides. High residual plasmin activity in shelf-stable UHT milk has also been associated with age gelation, a product defect[1, 2, 3, 4, 5-18-35].

Lactose is a unique disaccharide found in milk. Lactose concentration in bovine milk is about 4.8%. People with lactose intolerance lack the enzyme, beta-galactosidase or lactase, to break down lactose into glucose and galactose during digestion[1, 2, 3, 4, 5-18-35]. All milk, raw or pasteurized, contains lactose and can cause lactose intolerance in sensitive individuals. There is no indigenous lactase in milk. Raw milk advocates claim that raw milk does not cause lactose intolerance because it contains lactase secreted by "beneficial" or probiotic bacteria present in raw milk[1, 2, 3, 4, 5-18-35]. As discussed in a later section (claim 4), raw milk does not contain probiotic organisms. Typical bovine milk contains about 3 to 4% milk fat, with 97.5% of the fat existing as triglycerides[1, 2, 3, 4, 5-18-35]. Pasteurization has essentially no effect on milk fat composition ; and for that reason, research on this topic is minimal. The microflora in raw milk is complex and unpredictable [1, 2, 3, 4, 5-18-35]. The antimicrobial components in milk can have either bactericidal, bacteriostatic, or no effect at all depending on the specific pathogenic species and strains involved [1, 2, 3, 4, 5-18-365]. The major antimicrobial compounds naturally present in milk include lactoferrin, lactoperoxidase, lysozyme, and xanthine oxidase [1, 2, 3, 4, 5-18-35]. There is no scientific evidence to support the claim that the indigenous antimicrobial compounds in raw milk kill pathogens and ensure raw milk safety [1, 2, 3, 4, 5-18-35]. Raw milk does not contain high enough concentration of these antimicrobial compounds to exert such an effect [1, 2, 3, 4, 5-18-36]. In the case of lysozyme and lactoferrin, if high concentrations of these components are observed in raw milk, it is often an indication of cow's compromised health condition (e.g. mastitis), simply due to cow's elevated natural defense system[1, 2, 3, 4, 5-18-36].

Compared with cattle milk and other ruminant milks, horse and donkey milk have a low casein-to-whey-protein ratio, similar to that in human milk[1, 2, 3, 4, 5-18-36]. Among the ruminant milks, goat, sheep, and camel milk have a lower casein-to-whey-protein ratio as well as a relatively higher β-casein-to-αs-casein ratio compared with cattle milk [1, 2, 3, 4, 5-18-35]. Thus, these non-cattle milks are an attractive alternative as a potential natural ingredient for infant formula (13); a lower casein-to-whey-protein ratio (i.e., a higher proportion of whey proteins) has been shown to be more desirable for faster digestion of the milk proteins in infant formula than a casein-dominant protein composition [1, 2, 3, 4, 5-18-35]. β-Lactoglobulin is considered to be one of the major allergens that is responsible for cattle milk allergy in children [1, 2, 3, 4, 5-18-35]. Thus, milk from species that lack β-lactoglobulin or have lower β-lactoglobulin-to-α-lactalbumin ratios are of interest for human consumption. Camel milk, like human milk, does not contain β-lactoglobulin or it may be present in trace amounts in different forms[1, 2, 3, 4, 5-18-35].

As non-cattle milk and milk products are highly regarded as a potential source of human nutrition, they can be utilized to develop specialized dairy products for people in all age groups[1, 2, 3, 4, 5-18-36]. Non-cattle milks are of great interest to people as well as industries, because of their perceived better nutritional properties compared with cattle milk[1, 2, 3, 4, 5-18-36]. However, most of these presumptions are based on anecdotal reports and only little scientific research has been conducted to understand the nutritional and physicochemical properties of non-cattle milks [1, 2, 3, 4, 5-18-36]. One widely perceived notion is the formation of soft curds in the human stomach for some non-cattle milks (such as goat, camel, horse, and donkey milk) [1, 2, 3, 4, 5-18-36]. Because of this, these milks are considered to be better digested and tolerated by people of different age groups[1, 2, 3, 4, 5-18-36]. However, to date, no direct scientific studies have been reported and there is a knowledge gap[1, 2, 3, 4, 5-18-36]. As cattle and non-cattle milks vary in composition and structure of the casein micelles and fat globules, they are likely to behave differently in the gastrointestinal tract, possibly affecting the kinetics of digestion and the bioavailability of nutrients[1, 2, 3, 4, 5-18-36].

7. Conclusion

Milk, a complex and nutrient-rich biological fluid, serves as a critical source of nourishment for humans and animals. It comprises a wide array of macro- and micro-constituents, including proteins, fats, carbohydrates, vitamins, minerals and bioactive compounds such as polyphenols and peptides. Milk is a very nutritious food in nature, playing an important role in human diets, but at the same time, it serves as an ideal medium for microbial growth, negatively affecting the health of consumers. Normal bovine milk contains about 3 to 3.5% total protein. The two major groups of milk protein are casein (about 80%) and whey proteins (about 20%). The protein quality of pasteurized milk is not different from that of raw milk. Milk contains various indigenous proteases, including plasmin and somatic cell proteases. The major proteolytic activity in milk is from plasmin. Lactose is a unique disaccharide found in milk. Lactose concentration in bovine milk is about 4.8%. People with lactose intolerance lack the enzyme, beta-galactosidase or lactase, to break down lactose into glucose and galactose during digestion. All milk, raw or pasteurized, contains lactose and can cause lactose intolerance in sensitive individuals.

The microflora in raw milk is complex and unpredictable. Milk contains both fat soluble and water soluble vitamins. Fat soluble vitamins include A, D, E, and K. Water soluble vitamins included B1 (thiamin), B2 (riboflavin), niacin, pantothenic acid, B6, biotin, folic acid, B12, and vitamin C. In contrast, raw milk can host various human pathogens, including *E. coli* O157:H7, *Salmonella*, *Streptococcus spp*. *Yersinia enterocolitica*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Listeria monocytogenes, Mycobacterium tuberculosis*, and *Coxiella burnetti*. These bacterial pathogens can cause serious illnesses, such as tuberculosis, brucellosis, listeriosis and typhoid fever. Bovine tuberculosis infection can lead to a persistent cough, chest pain, fever, night sweats, weight loss, fatigue and can become life-threatening without treatment. Brucellosis causes undulating fevers, chills, joint and muscle pain, headaches and profound fatigue. Microbial communities in milk can be very complex and include numerous bacterial, yeast and fungal species originating from various sources of contamination, such as the external udder surface, milking equipment, air, water, feed, grass and soil. Pasteurization is the only method to achieve complete elimination of vegetative pathogens. Pasteurized milk is safe for direct consumption without boiling, preserving nutritional value. Understanding the benefits of pasteurization is crucial for consuming milk safely.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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