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Tibetan Plateau yak milk: A comprehensive review of nutritional values, health benefits, and processing technology

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ABSTRACT

Yak milk is a characteristic animal product of yaks in the Qinghai-Tibet Plateau. Although yak milk production is low, it is richer in nutrients such as protein, fat, and lactose, a more comprehensive range of bioactive components, and unique microbial resources than Holstein cow milk. The plateau environment makes yak milk resistant to hypoxia, anti-fatigue, antioxidant, antibacterial, and relieves chronic diseases. In this paper, based on the systematic analysis of yak milk research results in the past 20 years using CiteSpace 6.1.R2, we reviewed yak lactation performance and nutritional efficacy of yak milk. This paper summarizes the improvement of traditional yak dairy processing technology, and also focuses on the microbial diversity of yak milk sources and their beneficial effects. The purpose of this review is to provide scientific support for the development of a quality yak milk industry on the Tibetan plateau.

Introduction

Yak is the dominant animal living in the Qinghai-Tibet Plateau, with an average altitude above 4 m. It can adapt well to the harsh environment of high cold, high altitude, and severe hypoxia. Therefore, it is known as the "boat of the plateau"(Qiu et al., 2012). There are more than 17 million yaks globally, mainly distributed in China, Mongolia, Nepal, India, Pakistan, Afghanistan, Bhutan, and other countries along the Qinghai-Tibet Plateau. China has the largest number and breed groups of yaks, accounting for more than 95 % of the total yaks worldwide (Gao et al., 2021). In China, the yaks are mainly distributed in Tibet, Qinghai, Sichuan, and Gansu provinces (regions), with a small number in neighboring Yunnan and Xinjiang (Fan et al., 2020). Differences in geographic and climatic environments, ecological conditions, grass types, forage levels, selective breeding, and socioeconomic structures cause differences in body structure, appearance features, production performance, and utilization directions of yaks. At present, there are 12 local breeds of yak in China. Breeds such as the Plateau yak in Qinghai, the high mountain yak in Tibet, the Tianzhu white yak and the Gannan yak in Gannan, and the Maiwa and Jiulong yak in Sichuan are the main yak breeds in China (Fig. 1).

Yak is the core industry of highland livestock development, integrating meat, dairy, milk, wool, and service. Yak milk is an essential part of yak products, and its main nutrients are richer than ordinary cow's milk. Herders in Tibetan areas reportedly eat no vegetables or fruits all year round but show no apparent signs of vitamin or mineral deficiencies. Yak milk is suggested to be crucial in maintaining human health conditions (Ding et al., 2017a). The unique plateau environment also gives yak milk special physiological activities, and numerous studies have reported the health effects of yak milk in terms of hypoxia tolerance, anti-fatigue, antioxidant, and immunity improvement, which have great potential for development and application.

According to statistics, the annual production of yak milk in China increased from 705,000 tons in 2014 to 845,000 tons in 2019. It mainly originates from the four main production areas of Qinghai, Tibet, Sichuan, and Gansu (Fig. 2). In the past, 70 % of yak milk was used for feeding calves and drinking by herders. The milk purchased by enterprises only accounted for about 14 % of the total yak milk production. In recent years, the commercialization and development of yak milk have gradually become one of the critical channels to increase the income of herders in plateau regions. The main products include liquid milk, fermented milk, Qula(casein), milk powder, etc. Yak milk, as a unique natural, ecological, and economic resource directly affecting the level of development of plateau animal husbandry, urgently needs to be

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strengthened by using scientific and technological means. This paper uses CiteSpace 6.1.R2 bibliometric software with literature included in the Web of Science database from 2003 to 2022 as the data source. A comprehensive and systematic review and critique of research results on the topic of yak milk was conducted. The aims of this review are to (i) to improve yak milk production performance and secure the supply of high-quality raw milk; (ii) to investigate the types and roles of important bioactive components and microorganisms of yak milk sources and develop functional yak milk products; (iii) to improve the processing methods of traditional yak milk products to enhance the quality and flavor of the products, which will serve as a reference for promoting the innovation of yak milk products and upgrading of the market, as well as for the development of the yak milk industry.

Research progress in the field of yak milk

CiteSpace is a visual literature analysis software written based on the JAVA programming language, which can dynamically and intuitively display the relevant information of a specific research direction, to help scholars better analyze the current trend and the evolution of the frontiers of the field (Frigeri et al., 2023; Xu et al., 2022 & Zhong et al., 2022). Therefore, this paper takes 470 literature included in the Web of Science database from 2003 to 2022 as a sample and uses bibliometric analysis and content analysis to sort out the research background, hot issues, and future development of yak milk-related fields from the perspectives of time distribution and keywords. Fig. 1 shows the number of publications and growth trend of yak milk literature over the years. From 2003 to 2006, the number of published papers was less than 10 per year. The number of annual publications increased significantly after 2007. It exceeded 60 per year by 2020 and 2021 (Fig. 3a). The analysis of keyword co-occurrence patterns showed that the most frequent keywords were “expression” (95), “lactic acid bacteria” (72), “identification” (66), and “yak milk” (58) (Fig. 3b). To some extent, this reflects the research activity and interest in the field of yak milk.

We then examined the literature that generated a common interest and a surge in citations between 2003 and 2022 and found that the keywords that emerged across years shifted from “lactic acid bacteria (LAB)” or “lactobacillus” and “cheese” to “health,” “protein,” and “performance” (Table 1). Among them, “performance” is mainly reflected in yak feeding management-related contents, such as production performance, rumen fermentation, yak growth, and lactation. Thus, most previous studies have focused on analyzing the nutritional composition of yak milk and exploring the lactic acid bacteria resources and fermentation properties in yak milk. In recent years, due to the increased consumer demand for healthy dairy products, more attention has been paid to the bioactivity of yak dairy products, and fully exploring the potential benefits and functional properties of yak milk has become a persistent frontier hotspot.

The clustering analysis results of the keyword co-occurrence network presented nine clustering labels in descending order, including “lactic acid bacteria” (including high-frequency keywords such as traditional fermented milk, functional genes, and molecular identification), “growth performance” (including high-frequency keywords such as growth performance, nutrient digestibility, rumen fermentation, and weaned calf), and “antioxidant activity” (including high-frequency keywords such as LAB, adhesion capacity, antibacterial activity, mechanism of action, and fermented yak milk) (Table 2). The research content of each cluster is intertwined, and we summarize them into three hot topic areas: “feeding management,” “functional characteristics,” and “milk products,” and develop the subsequent review accordingly.

Table 1
Keyword emergence analysis of yak milk research in WOS database.

Key words	Strength	Begin year	End year
progesterone	2.60	2003	2006
DNA	2.55	2006	2009
lactobacillus	3.02	2011	2012
cheese	2.61	2014	2016
lactic acid bacteria	3.07	2015	2016
expression	4.29	2016	2019
yak	4.56	2019	2020
protein	3.79	2020	2022
health	3.01	2020	2022
performance	2.82	2020	2022

Feeding management of yaks

The yak is a crucial species indispensable for maintaining ecosystem function and developing grass grazing on the Tibetan Plateau. It has evolved a set of unique morphological and metabolic characteristics adapted to the stress of plateau adversity, including a compact body, thick coat, solid cardiopulmonary function, and high red blood cell count and hemoglobin levels. Yaks are late-maturing domestic animals. In the natural grazing state, breeding usually starts after 3–4 years of age. However, with better calf breeding and reserve cattle feeding, the age of the first mating of yaks can be advanced to two years old. Generally, one calf in two years or two calves in three years, with the most muscular reproductive capacity at 4.5–8.5 years, can be utilized for about 10 years.

Unlike the standardized feed feeding and intensive management of Holstein cows, yak feeding management is constrained and influenced by the ecological conditions of the distribution area, production methods, scientific and cultural level of the producers, religious beliefs, and other factors. Yaks graze in the alpine grassland all year round with little supplemental feeding and with the growth cycle of pasture grasses presenting the natural law of strong in summer, fat in autumn, thin in winter, and tired in spring. Yak milk production is seasonal, generally 150–180 d, with an annual milk yield of 230–400 kg, and the peak of milk production is July-August each year. Different regions and even the same region have differences in milk production. Based on the urgent need to strengthen the yak industry, the feeding management of yaks in China’s plateau region has gradually changed from “relying on nature” to “ecological, scientific, and standardized”. CiteSpace analysis shows

Table 2
Yak milk keyword co-occurrence network clustering table in WOS database.

Label	Cluster name	Key words	Size	Silhouette
0	lactic acid bacteria	lactic acid bacteria; traditional fermented milk; functional gene; molecular identification; qula cheese	72	0.776
1	polymorphism	polymorphism; PCR-sscp; variant; reconstituted skim milk	67	0.796
2	whey protein	whey protein; energy; absorption; acidophilus; lipid metabolism	58	0.674
3	fatty acid	fatty acid; chemical composition; rumen bacteria; yogurt; immunoglobulin	50	0.716
4	growth performance	growth performance; nutrient digestibility; immune response; rumen fermentation; weaned beef calf	37	0.731
5	seroprevalence	seroprevalence; altitude adaptation; gayal; genome analysis; gene	35	0.838
6	ace inhibitory peptide	ace inhibitory peptide; milk casein; spent grain; inhibitory peptides; molecular docking	32	0.816
7	antioxidant activity	lactic acid bacteria; adhesion ability; antimicrobial activity; underlying mechanism; fermented yak milk	29	0.805
8	yak	embryo; estrus; superovulation; milk composition	27	0.876

that research efforts have focused on improving the slow growth, long developmental cycle, and low milk and meat production performance in yaks.

Type of lactation

Unlike Holstein cows, yaks are mainly managed by milking and calf lactation. Depending on the season and pasture, there are two types of lactating yaks in actual production: fully lactating and semi-lactating yaks. The former refers to the same year of lactating yaks, to the summer lactation period for a few months. The latter refers to lactating yaks that are not pregnant in the year. In winter, milking is stopped only for calves. By the grass season of the following year, there will be a second lactation peak, about 2/3 of the amount of the first season, after which milking can be continued for consumption. Half lactation is a unique phenomenon of yak lactation; about 15 % of milk-producing yaks experience this lactation period for more than a year yearly. Unlike fully lactating yaks, semi-lactating yaks produced less milk and more milk components such as protein and fat (Liu et al., 2013). Cao et al. (2022) identified 202 differentially expressed proteins from skimmed milk of whole and half-lactating yaks. It was found that 109 proteins were increased, and 93 proteins were decreased in half-lactating yak milk compared to whole-lactating yak milk. Further experiments showed that the differential proteins were involved in catalytic and binding activities, and the main metabolic pathways were carbohydrate metabolism and amino acid biosynthesis.

The average cow will use the dry period of about two months to repair and renew her mammary tissue in preparation for the next lactation cycle. Mammary gland degeneration is a critical stage in the dry milk period of dairy cows. It is manifested as the atrophy of mammary gland epithelial cells and the decrease in the ability to synthesize milk components. At the same time, the concentration of related pro-apoptotic factors in mammary glands increased, and the physical tension of mammary epithelial cells also increased, initiating the apoptotic process of mammary epithelial cells. Studies have indicated that mammary gland atrophy and degeneration are also present in yaks in the semi-lactation stage. It was found that the expression of hemoglobin subunit-β, lactoferrin, and serum transferrin, all blood-derived proteins, was significantly increased in the milk of semi-lactating yaks compared to that of full-lactating yaks (Cao et al., 2022). Studies have reported a significant increase in lactoferrin content in mammary secretions during the dry milk phase, which can indicate the degree of mammary gland degeneration (Ollier et al., 2013). At the same time, somatic cell counts and mammary epithelial cell apoptosis rates were significantly increased in semi-lactating yaks (Jena et al., 2019), where levels of secretory protein 3 (CRISP-3), an essential protein in apoptosis, were upregulated, while levels of vinculin, which maintains cell growth and differentiation and promotes cell survival, were down-regulated. In addition, the keratin content in the milk of semi-lactating yaks was also significantly higher than that of full-lactating yaks, which may be related to the increased exfoliation of mammary epithelial cells in semi-lactating yaks (Dayton et al., 2022). The above evidence suggests that the mammary glands of semi-lactating yaks are degenerative.

As can be seen from the above, yak feeding management in pastoral areas is still dominated by traditional grazing methods. There is a lack of science and standardization in yak feed nutrition matching, seed selection and matching, disease prevention and control, and breeding environment hygiene, which is manifested in the imbalance of seasonal nutritional supply for grazing yaks, precise supplemental feeding methods for lactating yaks in cold and warm seasons, and lack of nutrition for lactating yaks and calves. Therefore, it remains a huge challenge to improve yak milk production and quality at the source.

Forage nutrition and milk composition

Ruminal fermentation and lactation performance of yaks fed a diet based on highland pasture are influenced by seasonal variations in pasture nutrition. The crude protein, crude ash, and short-chain fatty acid contents of warm-season forage grasses on the Qinghai-Tibet Plateau were significantly higher than in the cold season. In contrast, the natural fiber content was considerably lower than that of the cold season (Zhao et al., 2012). Fan et al. (2020) found that the crude protein content of forages in the flowering stage was higher than in the nutritional and senescence settings, and the neutral detergent fiber content of forages in the senescence stage was higher than in the healthy and flowering stages; for yak milk, the dry milk matter and fat content were higher in the nutritional and senescence stage groups than in the flowering stage, while the milk protein, total volatile fatty acids, acetic acid, and propionic acid content were higher in the nutritional and flowering stage groups than in the senescence group. It is worth noting that the availability of nutrients to livestock in purely pasture-fed systems varies by altitude. For example, Ma et al. (2021a) investigated the relationship between different altitudes and conventional nutritional indicators of natural forage and yak milk in Qinghai. They found that crude protein and fiber content of forage were higher at higher altitudes. In contrast, protein, lactose, and non-fat solids content and electrical conductivity of yak milk were more correlated with the acidic detergent fiber content of forage. The fat content, the most unstable component of yak milk, is also affected by fatty acids in seasonal pastures. Pan et al. (2021) analyzed the effect of the phenological stage on fatty acids in pasture, rumen fluid, and conjugated linoleic acid (CLA) in milk fat of grazing yaks and also found that CLA isomer c9t11, CLA isomer t10c12 and total CLA content in milk of grazing yaks were significantly higher in the peak grass stage than in the return and dry grass stage. In addition, the type and content of fatty acids in milk fat were also related to the rumen environment of yaks, and the biohydrogenation of yak rumen and the specific range of rumen microorganisms had a strong influence on the correlation between forage and milkfat fatty acids.

Feeding method

In addition, ration level and feeding practices can also regulate rumen fermentation and metabolism and thus affect the growth performance of yaks. For example, Pang et al. (2022b) found that adjustment of dietary forage to concentrate ratios (50:50, 65:35, and 80:20) altered not only the diversity of rumen microorganisms in yaks but also the content of rumen metabolites such as volatile fatty acids lipids, and lipid-like molecules. Pang et al. (2022a) found that compared to grazing, feeding and fattening in the whole mixed diet with housing increased the non-fiber degrading bacteria and hemicellulose degrading bacteria in the rumen of yaks, and also increased the content of total volatile fatty acids, acetic acid, propionic acid, and butyric acid, and upregulated energy metabolic pathways in yaks, which promoted efficient metabolism in yaks. Early refeeding of yak calves can also enhance the rumen fermentation performance. For example, Cui et al. (2020) found that preweaning supplementation with available feed and alfalfa hay can significantly enhance calves' body weight, height, length, and chest circumference and improve rumen fermentation and intestinal digestion and absorption. It significantly increased the concentration of volatile fatty acids, acetate, butyrate, and isovalerate in the rumen. At the same time, significant changes were also found in the relative abundance of rumen microorganisms, which reduced gastrointestinal inflammation and enhanced immune function. Wu et al. (2021) found that adding alfalfa hay and open feed significantly increased the levels of *Desulfobulbus*, *Olsenella*, *Pseudoflavonifractor*, and *Stomatobaculum* spp. It also significantly reduced the secretion of inflammation-related factors and promoted the growth and development of yaks. These studies provide some reference for more scientific prediction of yak performance, selection of rations, and regulation of yak milk quality in the future.

Nutritional composition and functional properties of yak milk

The particular plateau environment gives yak milk its unique nutritional value. Its protein, fat, lactose, ash, and total dry matter content are significantly higher than those of other animal milk, and it is also known as "natural milk concentrate." Compared with ordinary cow milk, the content of essential amino acids in yak milk protein is about 45 % higher. Yak milk fat is also rich in fatty acids. Many comparative analyses of the nutritional composition of milk from different species of yaks and other mammals have been reported, mainly focusing on the Maiwa and Gannan yaks (Table 3).

Nutritional composition of yak milk

Protein

Casein in yak milk accounts for about 60 % of the total protein, 1.5 times that of regular cow milk (Turkmen, 2017). The proportion of β-casein makes up about 45 % of the total casein. At the same time, yak casein micelle particles are more distributed below 100 nm in diameter, which is one of the reasons for the differences in structure and properties exhibited during the acidification of curd from different milk sources. β-casein has two genetic variants, A1 type, and A2 type, and the difference between them is mainly that A1 type β-casein produces the polypeptide fragment BCM-7 after digestion, while A2 type β-casein produces BCM-9. Compared to A1 β-casein, A2 β-casein has more beneficial effects on human health, especially in infants and children, including regulating gastrointestinal function, intestinal flora, type I diabetes, cardiovascular health, and inflammation. Chen et al. (2021b) found that yak milk only contains type A2 β-casein, while in most milk samples, type A1 and type A2 β-casein coexist.

Whey protein in yak milk accounts for about 20 % of milk protein, with β-lactoglobulin content much higher than Holstein cow milk, while α-lactalbumin content is lower. Yak milk is also richer in lactoferrin and bone bridge protein (Chen et al., 2021a), which have biological activities such as immune modulation, anti-cancer, and promoting self-metabolism (Lonnerdal et al., 2016).

Relying on the development of proteomics technology, the work on milk proteins' composition, molecular structure, and bioactive functions has gradually advanced. For example, Yang et al. (2015) compared whey samples from yak colostrum and mature milk by iTRAQ-labeled proteomics approach, from which a total of 183 proteins were identified, of which 86 differentially expressed proteins were associated with bioregulation and stress stimulation. Lu et al. (2018) identified respectively 169 and 128 proteins in the whey of regular cow milk and yak milk. Compared to cow milk, 26 proteins such as folate receptor α (FOLR1) and osteopontin isoform OPN-α precursor (SPP1) were significantly more in yak whey. In comparison, 45 proteins such as thymosin beta4 (TMSB4X), alpha-1-antitrypsin (SERPINA1), serum amyloid A1 (SAA1) and SAA3 were less abundant. Qiu et al. (2023) screened 268 differentially expressed proteins from yak and Holstein milk whey. The two whey differential proteins were mainly enriched in extracellular space, small molecule binding, and immune response and were associated with pathways such as *Staphylococcus aureus* infection, complement, and coagulation cascade, and glyceraldehyde-3-phosphate dehydrogenase, α2-HS glycoprotein, cluster protein, heme-binding protein and complement three may be the critical proteins in both whey. These findings not only deepened human understanding of yak milk protein composition but also further analyzed and identified the potential function of differentially expressed proteins. However, systematic information on the analysis of trace active protein content in yak milk is lacking, and further studies are needed.

Table 3
Comparative nutrient composition and content of Yak milk and Holstein milk. (Ma et al., 2017; Wu et al., 2018; Xi et al., 2017).

Nutrient composition	Maiwa yak milk	Gannan yak milk	Holstein milk
Protein/%	3.68 ~ 4.06	5.34 ~ 6.30	2.94 ~ 3.14
casein / (g/kg)	39.20 ± 1.04	37.30 ± 1.37	25.1
α_{s1}-casein /casein/%	29.23	30.38	~40
α_{s2}-casein/casein/%	11.15	12.41	~10
β-casein/casein/%	47.35	44.86	~35
κ-casein/casein/%	12.27	12.36	~15
whey protein/ (g/kg)	9.57 ± 0.64	10.08 ± 0.71	5.7
α-lactalbumin/whey protein/%	3.13	4.76	52.9 ~ 53.6
β-lactoglobulin/whey protein/%	67.12	58.83	18.4 ~ 20.1
serum albumin/whey protein/%	13.27	6.45	5.5 ~ 7.6
Fat/%	3.51 ~ 5.93	6.21 ~ 7.65	3.49 ~ 3.67
Type of fatty acid (g/100 g)			
Short chain fatty acid	1.11	0.93	2.21
Medium chain fatty acid	3.51	2.91	7.81
Long chain fatty acid	95.38	96.16	89.98
Saturated fatty acid	63.95	63.35	63.94
Monounsaturated fatty acids	31.54	32.97	27.23
Polyunsaturated fatty acids	4.51	3.68	3.24
Lactose/%	5.52 ~ 6.10	4.12 ~ 5.08	4.24 ~ 4.54
Total solids/%	17.51	17.27 ~ 19.57	11.60 ~ 11.82

Fat

Yak milk contains about 5.3 %-8.8 % of fat, almost twice as much as Holstein milk (Li et al., 2011). Compared to Holstein milk, yak milk has a lower content of short and medium-chain fatty acids while a higher content of long-chain fatty acids and unsaturated fatty acids (Table 3). In addition, the distribution of unsaturated and saturated fatty acid composition in the triglyceride backbone differed between yak and regular cow milk. Yang et al. (2022) found, compared to human milk, by comparison, that the stereospecific distribution of fatty acids was relatively similar between yak and cow milk. While human milk showed an absolute advantage in the distribution of long-chain saturated fatty acids at *sn*-2 and *sn*-1,3. Meanwhile, the *sn*-1 and *sn*-2 positions of yak milk triglycerides were mainly esterified by palmitic acid, followed by stearic acid, oleic acid, and myristic acid, and the proportion of unsaturated fatty acids such as linoleic acid and α-linolenic acid at *sn*-3 was higher than that of normal cow milk. Palmitic acid at the *sn*-2 position of triglycerides has been shown to increase the absorption of fatty acids and calcium (Liu et al., 2021). While short and medium-chain fatty acids esterified at the *sn*-3 position may also promote the complete digestion of milk fat in infants (Chen et al., 2020).

Many polyunsaturated fatty acids in fresh forage are hydrogenated under the action of yak rumen microorganisms and enzymes and finally absorbed by the intestine in the form of saturated fatty acids and trans fatty acids and deposited in tissues. Therefore, yak milk is mainly saturated fatty acids. The saturated fatty acids in yak milk mainly include myristic acid, palmitic acid, and stearic acid. Oleic acid is the highest content of monounsaturated fatty acid. In addition to arachidonic acid and α-linolenic acid, yak milk is also rich in functional fatty acids, which are not found in regular cow milk; eicosapentaenoic acid and docosahexaenoic acid are also high in content. These results suggest that yak milk has potential functional properties in anti-diabetes, anti-cancer, anti-oxidation, and immune regulation (den Hartigh, 2019). In addition to conventional fatty acids, yak milk fat contains more odd and branched chain fatty acids accounting for 3 %–6 % of the fatty acids. Further research found that it has a good anti-cancer effect and is related to colonizing neonatal intestinal flora (Fernandez, 2020). In addition, the content of conjugated linoleic acid in yak milk is also significantly higher than in cow milk, and the content of CLA increases with the elevation (Zongo et al., 2021).

Milk fat globules and fat globule membranes

Yak milk fat mainly exists in the form of milk fat globules. The average size of milk fat globules in yak milk (~4.39 μm) is more significant than in Holstein milk (~3.87 μm) (Luo et al., 2016). The large globules of milk fat make yak milk fat an ideal raw material for processing into butter, ghee, and other products. Luo et al. (2018) studied the lipid content of yak milk and the morphological properties of milk lipid globule membranes. They found that the cholesterol and sphingolipid contents in yak milk were high. It has been confirmed that higher levels of sphingomyelin can promote lipid metabolism and digestive health. Subsequently, Luo et al. (2020) used in vitro experiments to simulate the digestive process in infants and further confirmed that the lipolysis level of fat globules was significantly higher in yak milk and standardized yak milk than in cow milk. This may be related to the higher content of free fatty acids released by milk fat globules and the smaller particle size. The above evidence shows that yak milk has good digestive characteristics.

The large fat globules and specific surface area of yak milk make it rich in milk fat globule film. The lipids in yak milk fat globule membrane mainly include triglycerides, highly structured polar lipids, sterols, glycoproteins, and glycolipids (Luo et al., 2016). Yak milk liposphere membranes contain about 30 %-43 % protein, mainly composed of xanthine dehydrogenase/oxidase, electrophilic lipoprotein, mucin 1, a cluster of differentiation antigens 36, etc. Yak milk fat globule membranes have a wider variety of proteins than regular cow milk, and there are significant differences in the abundance of some specific proteins. For example, Ji et al. (2017) identified a total of 46 differential proteins from Maiwa yak and cow milk fat globule membranes. There were 20 high-abundance proteins in yak milk fat globule membranes, including glycosylation-dependent cell adhesion molecule 1, CD59 molecule, and milk adhesin. At the same time, histones, protein S100-A8, polymeric immunoglobulin receptor, and cathepsin-1 were in cow milk fat globule membranes. Zhao et al. (2019) identified and analyzed the differential expression of milk lipid globule membrane proteins in yak milk and ordinary cow milk by label-free quantitative proteomics techniques and found that 156 differentially expressed proteins were mainly involved in immune regulation, antioxidant, anticancer and antibacterial. In vitro HepG2 cell experiments also further confirmed that yak milk lipid globule membrane proteins were more effective in lipid metabolism and reduction. This may be related to the fact that 45 proteins with a high abundance in yak milk fat globule membranes are mainly involved in lipid metabolism.

Carbohydrates, minerals, and vitamins

Compared to cow milk, yak milk has a higher lactose content and is closer to human milk. Qu et al. (2016) found the structure of rockweed glycosylation in yak milk. Compared with human milk, the content of sialic acid in yak milk is higher. Sialic acid is an essential ingredient of gangliosides and glycoproteins. Singh et al. (2016) isolated two oligosaccharides from yak milk, Grunniose(Gal-$\alpha(1 \rightarrow 3)$GlcNAc-$\beta(1 \rightarrow 6)$Gal-$\beta(1 \rightarrow 4)$Glc $\leftarrow \alpha(3 \leftarrow 1)$GalNAc) and Vakose (Glc-$\beta(1 \rightarrow 3)$Gal-$\beta(1 \rightarrow 3)$ GlcNAc-$\beta(1 \rightarrow 6)$Gal-$\beta(1 \rightarrow 3)$GlcNAc-$\beta(1 \rightarrow 3)$Gal-$\beta(1 \rightarrow 4)$Glc), while optimizing their geometries. However, the functional effects and mechanism of yak milk oligosaccharides on human health still need further research and confirmation. The practical consequences of yak milk oligosaccharides on human health must be further explored.

Except for the similar phosphorus content, the main mineral content in yak milk is about 0.8 %, significantly higher than that of ordinary cow milk (Ma et al., 2017). The ratio of calcium and phosphorus in yak milk is closer to human milk than Holstein milk, which is more conducive to the absorption of calcium by the human body. Yak milk also has a higher iron content, which may be related to the particular living environment of yaks. Yaks have enough iron in their blood to adapt to the cold and oxygen-poor plateau environment. In addition, the mineral content of yak milk is also influenced by the breed, feeding conditions, and health status. For example, compared with Maiwa yak milk, Gannan yak milk has higher copper and iron content, while zinc content is lower (Ma et al., 2017). The vitamin content of yak milk is also higher than regular cow milk. The high content of VD may be related to the intense ultraviolet radiation at high altitudes, and the high content of VC and VE makes yak milk have a strong antioxidant capacity, which helps to reduce the oxidative damage caused by the high altitude environment (Dosek et al., 2007).

Immunologically active ingredients

Yak milk not only provides abundant energy and nutrients but also contains a variety of immune active substances such as immunoglobulin, insulin growth factor, and epidermal growth factor. Yak milk contains significantly higher levels of Immunoglobulin A (IgA), IgG, and IgM than other mammals, with IgA and IgG levels being about 1.5 times higher than those in human milk. The recent discovery of milk exosomes has further expanded the range of active ingredients in yak milk.

Milk exosomes are nanoscale extracellular vesicles secreted by mammary epithelial cells and loaded with “biological big data” derived from the highly conserved mammary genome, including proteins, ribonucleic acids, lipids, etc. Some researchers have confirmed by Western blot technique and flow cytometric analysis that yak milk contains higher levels of exosomes than regular cow milk, about 3.7 times more than regular cow milk. Furthermore, there is clear evidence that milk exosomes and the miRNAs can withstand the harsh environment of the gastrointestinal tract across the biological barrier to reach the blood circulation and peripheral tissues. Finally, by fusing with target cells to deliver signaling molecules from maternal cells to recipient cells, which critically participate in the growth and development, immune regulation, and inflammatory response of neonatal animals (Hock et al., 2017).

Some studies have reported that yak milk exosomal miRNAs also have some mitigating effects on intestinal inflammation and injury. Gao et al. (2021b) screened and identified 130 miRNA differentially expressed from cow and yak milk. It was found that bta-miR-34a was highly expressed in yak milk exosomes and may be the most effective regulator to alleviate intestinal epithelial 6 (IEC-6) hypoxia injury. Gao et al. (2021a) studied the effect of yak milk exosomes on IEC-6 barrier function in lipopolysaccharide-treated intestinal epithelial cells and its related mechanisms. They found that among the top 20 proteins expressed in yak milk exosomes, CD46 protein was an adequate protein in attenuating inflammatory damage in IEC-6 cells. Through further demonstration, yak milk exosomes were found to promote IEC-6 cell survival by activating the PI3K/AKT/C3 signaling pathway. Other studies on the interaction mechanism between bioactive components of yak milk and signaling pathways to promote body health are needed in the future.

Functional properties of yak milk and its products

Yaks are well adapted to the harsh environment of cold high altitude, thin air, and unpredictable terrain, which determines the unique biological function of yak milk. Numerous studies have confirmed that yak milk components and its products have antioxidant, anti-fatigue, hypoxia resistance, anti-inflammatory, immunity increasing, and anticancer effects, which play an essential role in maintaining the health of Tibetan herders.

Anti-oxidative effect

Yak milk and its dairy products are often considered to be beneficial in reducing oxidative stress caused by hypoxia and increased UV radiation in cold and high altitude herders, which is presumed to be closely related to its antioxidant components such as vitamins (including riboflavin, retinol, tocopherol, carotene, etc.), antioxidant enzymes, peptides, fatty acids, and some polar lipids (such as phospholipids, etc.). El-Salam and El-Shibiny (2013) found that both casein and its hydrolysis products from yak milk possess some antioxidant activity, and the hydrolysis products prepared by alkaline protease hydrolysis showed more significant scavenging of 2,2-diphenyl-1-pyridylhydrazine radical (DPPH), superoxide and hydrogen peroxide compared to intact yak casein. Also, the casein digest significantly reduced the production of NO and pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, and tumor necrosis factorz-α (TNF-α) in lipopolysaccharide-stimulated mouse peritoneal macrophages, consistent with the study of Mao et al. (2011). Liu et al. (2020) used alkaline protease and trypsin to hydrolyze yak casein to prepare a highly potent antioxidant peptide with the amino acid sequence Arg-Glu-Leu-Glu-Glu-Leu. While Qin et al. (2021) demonstrated the protective effect of yak casein peptide T8 against hydrogen peroxide-induced endothelial cell injury, probably through the upregulation of superoxide dismutase and glutathione reductase activities and reduction of malondialdehyde and reactive oxygen species content. Yang et al. (2021) also identified 3094 peptides from the hydrolysis products of yak Qula. The antioxidant peptide T10 attenuated H_2O_2-induced injury and increased cell survival in human umbilical vein endothelial cells.

Hypoxia tolerance and anti-fatigue effect

Yak milk also has both hypoxia tolerance and anti-fatigue potential. For example, Zhang et al. (2014) found that yak milk powder prolonged the survival time of hypoxic mice and the survival time of sodium nitrite intoxication, which may be related to the high content of CLA and iron in yak milk, presumably. Iron promotes the synthesis of erythrocytes and hemoglobin, and CLA inhibits the expression of prolyl hydroxylase, upregulates the expression of transcription factors related to hypoxia-inducible factors, and increases the level of erythrocytes and hemoglobin for improving hypoxia. Zhang et al. (2014) used a forced swimming experiment to test the anti-fatigue effect of yak milk powder. They found that yak milk powder could effectively promote gluconeogenesis and fat oxidation in male Kunming mice and reduce the accumulation of metabolites such as blood lactate and serum urea nitrogen during exercise, thereby slowing down the fatigue of the organism. Tong et al. (2015) also found through forced swimming experiments in mice that yak milk powder increased exhaustive swimming time, increased liver glycogen content, and decreased blood lactate level after exercise in mice compared with ordinary milk powder, which may be one of the mechanisms of its anti-fatigue effect. Some studies have found that yak milk exosome miRNAs can enhance the organism's hypoxia tolerance. For example, Gao et al. (2019) found that yak milk exosomal miRNAs improved the survival rate of IEC-6 cells in a hypoxic environment compared with bovine milk. It was further speculated that this might be due to miRNAs involvement in the regulation of the intracellular hypoxia-inducible factor pathway, increasing the expression of prolyl hydroxylase-1 and reducing the expression of hypoxia-inducible factor-α and its downstream vascular endothelial growth factor. Research on hypoxia tolerance and anti-fatigue effects of yak milk still needs to be completed, and research in this area needs to be strengthened.

Alleviating the effects of chronic diseases

It was found that Tibetan herders on a high-fat, high-protein diet were not more likely to suffer from chronic diseases such as hypertension, coronary heart disease, and high cholesterol than those on a low-fat diet. It is speculated that this may be related to the functional characteristics of yak milk and its products in the diet of Tibetan herders. Many studies have confirmed that milk protein is a significant source of angiotensin-converting enzyme (ACE) inhibitory peptide. For example, Jiang et al. (2007) and Lin et al. (2017) extracted and identified various ACE inhibitory peptides from yak milk casein and yak Qula casein, respectively. Lin et al. (2018) also confirmed that yak casein is a good potential precursor for producing ACE inhibitory peptides without cytotoxicity using in silico protein hydrolysis technique. ACE inhibitors activate endothelial nitric oxide synthase (eNOS) and increase NO production by increasing bradykinin levels (Persson et al., 2006). Lin et al. (2020) found that yak casein peptide KYIPIQ increased NO synthesis and phosphorylated eNOS expression in human umbilical vein endothelial cells and was also involved in the transmembrane transport mechanism in a Caco-2 cell model.

Numerous studies have shown that functional fatty acids have various functional characteristics, such as hypolipidemic, hypotensive, and anti-inflammatory. Luo et al. (2022) demonstrated that yak butter sphingolipids reduced total cholesterol, triglycerides, and low densith lipoprotein cholesterol levels in the serum of C57BL/6J mice induced by a high-fat diet, especially in the high-dose sphingolipid group. It is hypothesized that sphingomyelin is associated with significant upregulation of the lipid metabolism genes 3-hydroxy-3-methylglutaric acid coenzyme A reductase, stearoyl - CoA desaturase 1, and downregulation of pro-inflammatory factors such as TNF-α and IL-6 in liver tissue.

In addition, researchers have found that yak milk components have an essential role in cancer prevention. For example, Yuan et al. (2019) treated human breast cancer cells with purified branched-chain fatty acids from yak butter and performed transcriptomic analysis. It was found that differentially expressed genes FOS, fatty acid desaturase-2, and tumor protein TP53, which are associated with cancer and apoptosis, were down-regulated. Gu et al. (2022) identified three anticancer peptides from yak casein hydrolysis products, among which the novel TPVVVVPPFL peptide-induced apoptosis in cancer cells by inducing G2/M cycle block in MCF7 cells and S cycle block in MDA-MB-231 cells. The above evidence suggests that peptides derived from yak casein can potentially inhibit cancer cells.

Antibacterial effect

Many antimicrobial peptide fragments are also present in the amino acid sequence of yak milk proteins, and their activity is released when hydrolyzed in vitro with appropriate proteases or during gastrointestinal digestion and food processing (Khan et al., 2018). Pei et al. (2017) obtained two antimicrobial peptides from yak casein hydrolysate with the amino acid sequences Arg-Val-Met-Phe-Lys-Trp-Ala and Lys-Val-Ile-Ser-Met-Ile. Further antimicrobial experiments showed that the former effectively inhibited *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli* with inhibitory activity, while the latter inhibited the growth of fungi in addition to pathogenic bacteria. Lactoferrin in yak whey protein also has some antimicrobial activity, and its concentration is higher than that of ordinary cow's milk (Alichanidis et al., 2016). Dong and Zhang (2006) deduced amino acid sequence of yak lactoferrin contains four putative *N*-glycosylation sites: 300–303 (NKSQ), 387–340 (NVTC), 495–498 (NQTG) and 564–568 (NDTV). It is speculated that the reason for its antibacterial effect is that the *N*-terminal region lactoferrin-27 can bind to the microbial membrane, leading to the death of many bacterial cells.

Microbial composition and diversity in yak milk and its products

The unique geographical and climatic environment of Qinghai-Tibet Plateau, the high nutritional value of yak milk, and the traditional production process of nomads for thousands of years have given yak milk and its products unique microbial resources, and the study of its species and biodiversity is of great significance for product quality improvement and strain resource mining.

Microbial diversity of yak milk and its products

Some studies have found that there are abundant local strains in yak milk. For example, Ma et al. (2021b) found that the *Firmicutes*, *Proteobacteria,* and *Actinomycetes* were the dominant species common to yak, and the dominant genus is the unclassified cyanobacteria genus (unidentified-Cyanobacteria). The microbial diversity in yak milk is also influenced by various factors such as temperature, altitude, and rearing environment. Ma et al. (2021c) investigated the microbial diversity in yak milk at different altitudes. The results showed that *Firmicutes* and *Proteobacteria* were the dominant species in yak milk from Qinghai, while the dominant phyla in yak milk samples from high and low altitudes were *Acinetobacter*, and in the middle altitude yak milk samples were *Lactobacillus*. And the dominant genus in yak milk was unidentified Cyanobacteria, while the dominant bacteria in Pien Niu (offspring of a bull and a female yak) milk were *Bradyrhizobium*, *Bradyrhizobium elkanii, and Kosakonia oryzae*. Liu et al. (2020) found that the structure of the bacterial flora of raw milk originating from agricultural and pastoral areas of Tibet was significantly different. The *Bacteroidetes* and *Actinomyces* greatly enriched the farm area, while the pastoral area was dominated by the *Firmicutes.* And the eight genera of *Lactococcus*, *Bifidobacterium*, and *Enterococcus* were significantly increased in the agricultural area, while only *Streptococcus* and *Lactobacillus* genera were greatly enriched in the pastoral area. In addition, pathogenic and spoilage bacteria in raw milk can also reflect the health status of fresh dairy and storage time and other vital indicators.

The composition of microorganisms and their numbers in traditional fermented yak milk also showed certain characteristics. For example, Liu et al. (2015) identified 11 bacterial phyla and five fungal phyla from naturally fermented yak milk from two villages in the Tibetan region, in which the *Firmicutes* and *Ascomycetes* dominated, and *Lactobacillus* and *Saccharomyces cerevisiae* were the main bacteria and fungus. Zhu et al. (2021) characterized the diversity of bacterial flora in sour yak milk, Qula, and Tibetan mushroom yogurt in Tibetan areas of Gansu. They identified five bacterial species and 11 bacterial genera. Among them, *Firmicutes* and *Proteobacteria* were the dominant species, and *Lactobacillus* and *Streptococcus* were the high-abundance genera in various samples, followed by Coccus and Lactococcus.

The lactic acid bacteria in fermented products from different regional sources also showed different abundance at the genus level. Ding et al. (2022) collected traditional fermented yak yogurt samples in five major ecological geographic regions. Forty-seven kinds of lactic acid bacteria or subspecies and nine kinds of yeast were isolated and identified from the traditional fermented yak yogurt samples. It was found that the diversity of lactic acid bacteria in traditional fermented yak yogurt was significantly different from that in traditional fermented food at low altitude environmental conditions. In addition, the variety of lactic acid bacteria in fermented products from other regional sources showed different richness at the levels of species and genus. For example, Zhu et al. (2018) found that there were differences in microbial communities in the Qula samples from different provinces and regions, with the highest bacterial diversity and richness in the Sichuan sample, where the dominant genera were seven bacterial genera, including *Lactococcus*, *Pseudomonas*, and *Candida albicans*, while the Yunnan sample was the lowest, with the dominant genera being *Lactobacillus* and *Acetobacter*. Liu et al. (2020) compared and analyzed the Qula samples from seven regions in Tibet and found that the Lhasa samples had the most species of phylum and genus, in which the dominant genera were *Ralstonia*, *Pseudomonas*, and *Ochrobactrum*, while the dominant genera in pieces from the other six origins were mainly *Lactococcus* and *Lactobacillus*. Table 4 lists recent research results on microbial diversity in fermented yak dairy products. It provides a theoretical basis for their future development and utilization in functional dairy and beverage fermentation, health, and biomedicine.

The role of probiotics in yak milk and products

Different research teams have isolated and screened strains with special biological functions in yak milk and its products, such as lowering blood lipids, anti-aging, improving immunity, and relieving inflammation. For example, Ding et al. (2017b) found that *L. delbrueckii* subsp. *bulgaricus* F17 screened from fermented yak milk showed high free radical scavenging activity and survival. Further study showed that the actions of peroxidase, superoxide dismutase, and glutathione levels were significantly increased, and malondialdehyde levels were significantly decreased after administration to aging mice. Li et al. (2022) found that *Lactobacillus plantarum* As21, isolated from traditional fermented yak milk, exhibited high antioxidant capacity and survival rate when simulating the gastrointestinal tract. At the same time, the levels

Table 4
Microbial diversity of fermented yak dairy products and the isolation and identification of lactic acid bacteria.

Yak Dairy Products	Region	Separation and identification technique	Microbial composition	References
Fermented Yak Milk	Tibet	Pure culture method	The dominant flora are 3 species of Lactobacillus (*Lactobacillus fermentum*, *Lactobacillus helveticus* and *Lactobacillus curvatus*) and 5 species of yeast (*Saccharomyces cerevisiae*, *Candida kefyr*, *Candida lambica*, *Candida famat* and *Candida holmii*)	(Wu et al., 2009)
	Central Tibet	16S rRNA gene sequence analysis and denaturing gradient gel electrophoresis	The 211 isolated Lactobacillus strains were identified into 6 genera and 22 species and subspecies, among which 117 strains of Lactobacillus species, mainly *Lactobacillus* and *Bifidobacterium*	(Ren et al., 2017)
	Gansu	Pure culture method and 16S rRNA gene sequence analysis	The 195 isolated lactic acid bacteria were screened to obtain 5 strains, which were finally identified as *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*	(Qin et al., 2013)
	Qinghai	16S rRNA gene sequence analysis	The 148 *Lactobacillus* strains identified belonged to 5 genera and 13 species and subspecies, of which 52 strains were *Lactobacillus* and 96 strains were coccoid LAB	(Sun et al., 2010)
	Sichuan Qinghai-Tibet Plateau Pastoral Area	Pure culture method and 16S rDNA gene sequence analysis	The 56 isolated strains were further screened and 7 lactic acid bacteria were identified as *Lactobacillus paracei*, *Lactobacillus casei*, *Enterococcus faecium* and *Enterococcus stoica*	(Wu et al., 2013)
Qu la	Tibet	16S rRNA gene sequence analysis	Isolation of 14 strains of lactic acid bacteria, including *Leuconostoc mesenteroides* subsp. *dextranicum*, *Leuconostoc pseudomesenteroides*, *Enterococcus faecium* and *Lactobacillus plantarum*	(Duan et al., 2008)

(*continued on next page*)

Table 4 (*continued*)

Yak Dairy Products	Region	Separation and identification technique	Microbial composition	References
	Tibet	Pure culture method and ITS/5.8S rDNA gene sequence analysis	The 91 yeast colonies isolated were classified into 7 genera and 10 species, including *Kluyveromyces lactis*, *Pichia fermentans*, *Saccharomyces cerevisiae*, *Rhodotorula mucilaginosa*, *Kluyveromyces marxianus*	(Yang et al., 2014)
	three areas in Linzhi, Tibet	High-throughput sequencing technology	The taxonomic composition and abundance of the bacterial communities differed among the samples from the three regions, with the *Proteobacteria* and *Firmicutes* being the dominant phylum and the genus *Lactobacillus* being the dominant genus in all samples	(Chi et al., 2021)
	Tibet	High-throughput sequencing technology	Bacterial diversity and richness in all the samples of Qu la showed a decreasing trend with increasing altitude, and the dominant genera were *Lactococcus* and *Lactobacillus*	(Xue et al., 2022)
	Gannan, Gansu	High-throughput sequencing technology	The dominant bacterial phylum were *Firmicutes* and *Proteobacteria*, and the dominant genera are *Lactobacillus*, *Acetobacter* and *Lactococcus*	(Cao et al., 2019)
	Gannan, Gansu	Pure culture method and 16S rRNA gene sequence analysis	The 97 isolated lactic acid bacteria were further screened and identified to obtain 6 strains of lactic acid bacteria, including *Streptococcus thermophilus*, *Enterococcus durans* and *Lactobacillus rei* (strains G1, G2, G3 and G4)	(Wen et al., 2020)

of reactive oxygen species and malondialdehyde were decreased, promoting superoxide dismutase, catalase, and glutathione production.

Yak dairy products also contain some microbial strains that exert antagonistic effects on pathogens. Pei et al. (2018) found that *Lactobacillus plantarum* SLG1, isolated from yak cheese, produced the phycocyanin SLG1 with antibacterial activity against many spoilage organisms and pathogenic bacteria and certain fungi. Scanning electron microscopy showed that it caused bacterial death by destroying the integrity of the bacterial cell membrane. *Pseudomonas* Koreanus isolated from yak milk is also highly antimicrobial diverse and can also resist microorganisms by producing β-carboline (as1-acetyl-9H-*b*-carboline-3-carboxylic acid)(Kaur et al., 2019). Peng et al. (2021) purified the bacteriocin LP 21–2 created by *Lactobacillus plantarum* SHY 21–2 from fermented yak milk. It was found that when the bacteriocin LP 21–2 was exposed at 121 °C, it still had 96 % antimicrobial activity after 15 min and had high antibacterial activity against *Staphylococcus aureus* ATCC25923, *Salmonella typhi* CMCC50071, and *Saccharomyces cerevisiae* ATCC9763. It is presumed that the antibacterial mechanism is mainly by binding to the receptors on the surface of bacteria and penetrating the cell membrane to produce cytotoxicity, thus achieving the antibacterial purpose. In addition, some microorganisms isolated from yak milk and its products were found to have biological functions such as hypoglycemia, hypolipidemia, alleviation of liver damage, and anti-cancer (Table 5).

In summary, the biological activities of yak milk and its products have been extensively studied. This also provides a reference value for the development of functional products using yak milk. However, most of the existing studies are limited to cellular or animal models, and there are not enough clinical trials to confirm the therapeutic role of these functions in humans. The development of functional yak milk foods and their medicinal potential will depend on a vigorous advancement in the breadth and depth of research by scholars in the field.

Processing and production of yak dairy products

Types of yak dairy products and processing methods

Traditional yak dairy products have been made for thousands of years, with strong regional characteristics, and are an essential part of people's diet in highland areas. As shown in Fig. 4, fermented milk is made from yak milk by heating, cooling, inoculating, fermenting, and refrigerating after maturation. Ghee is usually produced in summer and autumn. Traditional ghee is made by pouring fresh yak milk into a barrel for making ghee, fermenting it slightly at 20℃, and milk fat is obtained by centrifugation, which is commonly wrapped and stored in Tibetan areas. Qula is also known as milk dregs; one is made from skimmed milk refined from ghee, while the other is made directly from raw yak milk. Generally, raw yak milk or skimmed milk is fermented by adding a small amount of finished products under natural conditions so that the casein is condensed and dried. In Tibetan areas, herders often mix ghee, brick tea, salt, etc., stir, and mix into the traditional ghee tea. Its flavor is rich and unique, loved by locals and tourists. And one of the traditional staple food of Tibetan herdsmen, "Zanba(Tsamba)," is made by frying barley and then grinding it into flour plus a small amount of ghee tea, Qula, and sugar. It is suitable for feeding hunger and cold, and is easy to carry and store.

Although Tibetan yaks have obvious advantages, they are widely distributed in the region, and their production is low. Yak milk is susceptible to seasonal influences, while transportation is difficult in production areas. Yak milk has many problems regarding acquisition, refrigeration, and transportation. For a long time, there have been many dairy processing enterprises in the yak production area with yak milk as raw material, but the scale is small. Moreover, the production process and equipment are backward, and there is no actual large-scale commodity. Some products are even in the traditional back home-based primary processing stage, resulting in yak dairy products still presenting more primary processed products, semi-finished products, and low value-added products with the maturity of yak milk processing technology and the improvement of dairy processing equipment and facilities in pastoral areas. Yak milk products are becoming more and more abundant, and now there are yak yogurt, milk powder, hard cheese, and casein in the market. Yak milk whey is a by-product of dairy products such as Qula and ghee. In recent years, research on yak milk whey protein has gradually increased. Besides using yak milk whey protein to produce beverages, it can also be made into powder by spray drying. Meanwhile, its safety and growth-promoting effects must be continuously explored during the subsequent development to make it a high-quality protein source for infant formula and middle-aged milk powder. At present, the yak milk industry in Qinghai, Gansu, Sichuan, and Tibet provinces is beginning to take shape through upgrading and new

Table 5
Related reports on the role of microorganisms in yak milk and products.

Disease	Strain	Experiment content	Experimental conclusion	Reference
Anti-fatigue	*Lactobacillus fermentum* HFY03 in fermented yak milk	Seventy-five ICR mice (6 weeks old) were divided into five groups: LF-HFY03 low-dose group (1.0 × 108 CFU/kg), high-dose group (1.0 × 109 CFU/kg), vitamin C group given 100 mg/kg vitamin C, running group and control group given 0.2 mL saline for oour weeks	Mice with prolonged swimming failure reduced urea nitrogen and lactate, increased fatty acid and liver glycogen, and reduced serum alanine aminotransferase, creatine kinase, and aspartate aminotransferase levels	(Zhang et al., 2021)
Type 2 Diabetes Mellitus	*Paenibacillus bovis* sp. *nov.* BD3526 in yak milk	Sixteen GK rats (18 weeks) were divided into two groups: BD3526 group rats were gavaged with 2 mL 50 mg/ml lyophilized BD3526 metabolite daily and control rats were gavaged with 2 mL 50 mg/ml skim milk powder daily for 4 weeks	*Paenibacillus bovis* sp. *nov.* BD3526 metabolite can improve diabetes by regulating intestinal flora through mucus enrichment and enhancing intestinal barrier function by stimulating intestinal epithelial cells to increase OCLN expression	(Qiao et al., 2020)
	Lactobacillus plantarum SHY130 in yak yogurt	Twenty-four male C57BL/6J mice (4 weeks old) were divided into three groups and treated with 1010 CFU kg^{-1} L of Lactobacillus plantarum SHY130 (SHY130) for 10 weeks in the diabetic group, and equal amounts of saline were administered by oral gavage daily in the normal control and diabetic groups	*Lactobacillus plantarum* SHY130 inhibits the proliferation of β and α cells in the pancreas of T2DM mice and increases the expression of short-chain fatty acid receptors GPR43 and GPR41 in the colon	(Wang et al., 2022)
Obesity	*Lactobacillus plantarum* HFY01 in fermented yak milk	60 C57BL/6 mice were randomly divided into 6 groups (normal group, model group, L-carnitine group, soymilk group, *Lactobacillus plantarum* HFY01 fermented soymilk group and *Lactobacillus bulgaricus* fermented soymilk group) for 12 weeks	*Lactobacillus plantarum* HFY01 significantly reduced body fat percentage and liver index, increased LDL cholesterol, triglycerides, alkaline phosphatase and transglutamic acid aminotransferase, and decreased HDL cholesterol in obese mice	(Li et al., 2020)
Hypercholesterolemia	*Lactobacillus casei* YBJ02 in yak yogurt	50 mice were divided into 5 groups: normal group, low concentration LC-YBJ02 treatment group, medium concentration LC-YBJ02 treatment group, high concentration LC-YBJ02 treatment and high fat model group	Different concentrations of LC-YBJ02 have inhibitory effects on elevated blood lipids in mice, especially high concentrations of LC-YBJ02 can reduce the content of cholesterol, triglycerides and low-density lipoprotein, and also effectively reduce the level of cholesterol in fecal excretion	(Qian et al., 2019)
	Lactobacillus plantarum LP3 in traditional yak fermented milk	Thirty male Sprague-Dawley rats (5 weeks old, weighing 120–130 g) were divided into normal diet, high-fat diet and high-fat diet + *Lactobacillus plantarum* LP3	The serum levels of total cholesterol, triglycerides and LDL cholesterol as well as the atherosclerotic index were reduced in the high-fat diet rats with *Lactobacillus plantarum* LP3	(Ding et al., 2020)
Alcoholic Liver Injury	*Lactobacillus plantarum* HFY05 in yak yogurt	Sixty male mice (6 weeks old) were divided into 6 groups: LP-HFY05-L and LP-HFY05-H groups were given 10^8 and 10^9 CFU/kg of LP-HFY05 per day, respectively; the LDSB group received 10^9 CFU/kg of LDSB per day; and the silymarin group received 100 mg/kg of silymarin per day for 8 weeks	The liver index of liver-injured mice and the levels of IL-6, IL-12, TNF-α and interferon-γ in the serum of mice were reduced, and pathological observations further indicated that *Lactobacillus plantarum* HFY05 reduced alcohol damage to hepatocytes	(Yi et al., 2019)
Constipation	*Lactobacillus fermentum* Lee in yak yogurt	120 mice were divided into six groups (20 mice in each group): regular and control groups were fed standard diet for 9 d; LF-Lee high-dose group (1x10^9 CFU/mL), low-dose group (1x10^8 CFU/mL) and Lactobacillus bulgaricus group (1x10^9 CFU/mL) were given 2 mL orally; mice in the drug-treated group were given 100 mg/kg dose of bixadil in water for 9 d	Increased gastrointestinal transit rate; increased levels of motilin, gastrin, endothelin, acetylcholinesterase, substance P, and vasoactive intestinal peptide, suggesting that *Lactobacillus fermentum* Lee has a beneficial effect on constipation	(Qian et al., 2015)
Cancer	*Lactic acid bacteria* Lan4 in yak milk	Hela (human cervical cancer cells) and HEK293 (human embryonic kidney) cells were used to perform anti-cancer activity assays. Cancer cell lines supplemented with 10 % heat inactivated (30 min, 56 °C) fetal bovine serum and 1 % penicillin–streptomycin mixture were grown in RPMI medium at 37 °C, 5 % CO_2 and 95 % relative humidity for the experiments	*Lactic acid bacteria* Lan4 exhibited significant anticancer activity and induced maximal apoptosis in Hela cells and was not toxic to non-cancerous HEK293 cells	(Kaur et al., 2017)
	Lactobacillus casei SB27 in yak milk	The human colon cancer cell line HT-29 was cultured in RPMI-1640 medium containing 10 % fetal bovine serum, and the cells were placed in 25 cm^2 flasks at 37 °C in an incubator with 95 % air and 5 % CO_2 humidified atmosphere	Extracellular polysaccharide produced by *Lactobacillus casei* SB27 significantly inhibits the proliferation of HT-29 colon cancer cells and increases the expression of genes such as B-lymphoma-2-related promoter, B-cell lymphoma/leukemia-2-related X protein, cysteinase-3 and cysteinase-8	(Di et al., 2017)
	Kluyveromyces marxianus PCH397 in yak milk	Human colorectal adenocarcinoma cells (SW480) were thawed and maintained in Leibovitz's/L^5 medium supplemented with fetal bovine serum and antibiotics and grown at 95 % relative humidity, CO_2-free, and 37 °C	Cell-free supernatant of *Kluyveromyces marxianus* PCH397 exhibits cytotoxic effects on SW480 colon cancer cells and induces cell cycle phase arrest after 24 h of treatment, suggesting that Saccharomyces marcescens PCH397 has potential preventive and palliative effects against colon cancer	(Nag et al., 2022)

enterprises, as well as the adoption of modern advanced production processes and technical equipment.

Improvement of traditional yak dairy processing

Traditional yak dairy products have unique flavors, rich nutrition, easy digestion, and absorption, but there are problems such as poor safety and unstable quality. This has hindered the process of yak milk industrialization. CiteSpace analysis shows that yak dairy processing cuts across several areas, including "isolation and identification" and "processing". The keywords "lactic acid bacteria", " milk casein", and "isolation" are closely related. The development of modern processing technology to form yak dairy products with unique flavors is an important research direction at present.

Yak dry cheese

The quality of raw milk has a significant impact on the curd formation, gel strength, and dehydration and shrinkage of the clot, which affects the composition and yield of the cheese. Tibetan herders in China are currently using low-temperature storage to alleviate the shortage of raw yak milk. Low temperature is an effective measure to control the growth of bacteria during the storage of raw milk. However, refrigeration can inhibit most thermophilic microorganisms. As storage time increases, proteases and lipases produced by cryophilic bacteria break down proteins and fats, resulting in changes in raw milk quality, leading to changes in processing characteristics. For example, Qiu et al. (2023) made hard cheese from yak milk refrigerated for 24 h, 48 h, and 72 h. They found that the moisture content of the cheese increased while the NaCl and fat content both showed a decreasing trend after maturation as the refrigerated time of raw milk increased. And the longer the refrigerated time, the deeper the hydrolysis of proteins in the cheese made from raw milk during maturation. Zhang et al. (2023) prepared hard cheese using yak milk in June and December in summer and winter, respectively, and found that the protein, fat, and dry matter contents of the cheese were higher in the winter group than in summer. And the stretchability, fat precipitation, and sensory quality of yak milk cheese were better in winter than summer. Shi et al. (2020) found that the fat content of raw milk affected the production of hard yak milk cheeses (full-fat > semi-fat > low-fat). With the increase in fat content in raw milk, the moisture content, hardness, elasticity, and chewability of the cheese decreased significantly. Compared to semi-fat and low-fat cheeses, full-fat cheeses had higher mucoadhesive properties and better flowability and sensory qualities.

Protein degradation and fat oxidation during cheese ripening are important for cheese texture and flavor formation. Liu et al. (2015) studied the effects of different fermentation agent on protein degradation during ripening of hard yak cheese. The results showed that the thermophilic fermentation agent had the strongest degradation ability resulting in heavier cheese bitterness; the mesophile fermentation agent had the weakest protein degradation making the cheese flavor lighter; the mixed fermentation agent had moderate protein degradation; and the finished cheese had a rich flavor and better tissue condition. Increasing the ripening temperature and extending the ripening time are common means of enhancing cheese ripening. For example, Song et al. (2015) found that ripening at 15 °C significantly increased the content of pH 4.6 soluble nitrogen, 12 % trichloroacetic acid nitrogen, and 5 % phosphotungstic acid nitrogen in yak milk hard cheese compared to ripening at 5 °C and 10 °C. At the same time, maturation at 15 °C made the cheese taste bitter gradually. Ma et al. (2019) found that the acidity value, carbonyl value, and thiobarbituric acid value of yak milk hard cheese increased with the ripening time, while the peroxide value first increased and then decreased.

The free amino acids cheese produces during ripening are susceptible to biogenic amines by microbial action. The need to limit the total amount of biogenic amines in cheese to less than 900 mg/kg has been suggested. However, regulations on limiting biogenic amines in yak milk cheese have not yet been implemented. Based on this, many research scholars have investigated the changes in biogenic amines during the maturation of yak milk hard cheese. For example, Song et al. (2021) found that although the levels of various biogenic amines in yak milk hard cheese tended to increase during the maturation period of 1–6 months, the levels of histamine, tyramine, and total biogenic amines were lower than the recommended safe doses of 50 mg/kg, 100 mg/kg and 1000 mg/kg. The relative abundance of each genus in yak milk hard cheese at different maturity stages varied, with *Streptococcus* being the dominant genus, followed by *Leuconostoc*. Yuan et al. (2022) found that yak milk heat-treated at 63 °C/30 min or 72 °C/30 s reduced biogenic amine production in hard yak cheese during 90 days of maturation. Song et al. (2022) analyzed the effects of different salt additions (1.0 %, 1.3 %, 1.8 %, and 2.3 %) on biogenic amine production during six months of maturation of yak milk hard cheese. It was found that when NaCl was added at 1.8 %-2.3 %, the overall trend of increasing cadaverine and putrescine content in cheese was observed with increasing salt content. The above research provides some reference for the formation mechanism of biogenic amines and the evaluation of the quality and safety of yak milk hard cheese. In the future, biogenic amine production can also be reduced in terms of fermenter type, quantity, and maturation conditions.

Yak yogurt

Yak traditional yogurt is mainly produced by hand in a family style. The synergistic action of the lactic acid bacteria and yeast from the raw milk and some latent, active amino acid converting enzymes make the fermented dairy products have a unique flavor and taste. However, natural fermentation often causes problems such as unstable product quality and short shelf life. Screening and preparing excellent fermentation agents is the fundamental way to solve the contamination of the fermentation process and poor product safety. Ao et al. (2012) screened and isolated *Lactobacillus fermentum* and *Lactobacillus paracasei* with excellent fermentation performance from traditional yak milk. The yak yogurt fermented with this strain was beautiful, with relatively high viscosity, medium acidity, good sensory quality, and high viable bacterial count. In this paper, the authors' group screened a strain of *Lactobacillus fermentum* HY01, which can effectively relieve enteritis and constipation, from homemade yak yogurt made by herders in Hongyuan County, Sichuan Province. It was then used as an auxiliary and yogurt fermenting agent for compound fermentation. By optimizing the preparation process of yak yogurt containing probiotic bacteria, the changes of volatile flavor substances during the post-ripening process of yogurt were analyzed, and the quality changes of yak yogurt containing probiotic bacteria during storage were observed. The above provides some references for developing LAB that can be applied to ferment yak milk with probiotic properties (Zhang et al., 2022, Yang et al., 2023). Our subject group also participates in the research and development of simple and standardized production equipment suitable for herdsmen to make high-quality yak yogurt. It explores the packaging and storage methods suitable for fermented milk in pastoral areas and forms some technical achievements with independent intellectual property rights.

Yak ghee and Qula

Both handmade and machine-made ghee are famous in Tibetan areas, but there are still some differences between them regarding quality and nutrition. Ma et al. (2020) compared and analyzed two types of yak ghee, handmade and machine-made. These results showed that the moisture content, fat content, drawing length, and viscosity of handmade ghee were higher than those of the mechanism ghee. The protein, unsaturated fatty acid content, and *sn*-2 palmitic acid in mechanism ghee were significantly higher than those of the hand-made ghee. Yak ghee also has problems such as incomplete removal of impurities during processing and decreased oxidative stability during storage, so it is necessary to continue to strengthen the research on the physical properties of yak ghee.

Qula, also known as casein, is a by-product of ghee production. The oxidative browning and poor flavor quality of the traditional way of making Qula is serious, and seriously affects its food value and the production and processing of downstream casein. Therefore, improving the conditions for the production of Qula is a matter of concern for researchers. For example, Ding et al. (2015) collected yak qula from different qula main production areas and measured and analyzed their absorbance, color, nutrient composition, and antioxidant index. By comparison, we found that the color and luster of Sichuan, Xinjiang, and Tibet qula were better. The fat content of Yunnan, Xinjiang, Qinghai, and Gansu yak qula was low, and the protein content was more than 70 %. The fat oxidation degree of Sichuan, Xinjiang, and Tibetan southern qula was lower.

At present, for the Tibetan Plateau pastoral areas of Qula, ghee, fermented milk, and other product processing methods are old, backward equipment. We have applied advanced theories and technologies such as food engineering, enzyme engineering, additives, vacuum technology, separation and drying technology, and quality control. After that, research and translational applications should be carried out in the following areas: (i) research and development of modern yak dairy product processing technology suitable for pasture areas; (ii) establishment of yak dairy product quality grade evaluation methods; (iii) research and development of new products and supporting equipment. In the future, we will gradually establish technical regulations for the production of high-quality yak dairy products, formulate product grading standards, and further carry out validation and revision as well as demonstration and popularization.

Concluding remarks and future outlook

The geographical and ecological characteristics of yak milk production areas and the biological characteristics of yaks themselves determine that yak milk is natural, green, highly concentrated, and functional. This paper summarizes and analyzes the research results in the field of yak milk in the last 20 years, and finds that great progress has been made regarding yak feeding management, yak milk nutrients, and the processing of their products. In particular, based on the inherent resource advantages and basic conditions of yak production area, China has built a national yak milk series products technology research and development center and yak milk industrial park after years of development, and developed and produced yak milk products such as yak yogurt, yak milk formula, casein, ghee, and bioactive peptide. Among them, the technical requirements of yak milk powder have been incorporated into national standards.

The following three aspects should be considered as to how to further improve the high value-added and industrialized level of yak milk products. First, although yak milk and its products have been found to have the efficacy of alleviating a variety of diseases (including diabetes, hypertension, enteritis, and tumors, etc.), there is still a lack of in-depth analysis of the specific mechanism of action of its bioactive components. Second, the unique microbial resources in yak milk also determine the probiotic function of dairy products to a certain extent, and their community activity, biological composition, and population structure should be further explored to promote the development of functional yak milk products. Lastly, yak milk has differences in nutritional composition and processing performance from ordinary cow milk, and we should devote ourselves to the organic combination of traditional methods and new technologies of yak milk production, and develop processing equipment and processes to support them. This will provide important technical support for the development of the yak industry in Tibetan Plateau pastoral areas.

CRediT authorship contribution statement

Aili Li: Writing – review & editing, Investigation, Visualization, Data curation. **Chuan Liu:** Writing – original draft, Resources. **Xueting Han:** Investigation, Resources. **Jie Zheng:** Resources, Data curation. **Guofang Zhang:** Resources. **Xiaoxi Qi:** Resources. **Peng Du:** Conceptualization, Supervision, Resources, Data curation. **Libo Liu:** Conceptualization, Supervision, Resources, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100919.

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