



Review Article

Research progress on chemical composition, microbial diversity and effects on human health of koumiss

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Abstract: Koumiss is an acidic dairy beverage made from the raw milk of mares fermented by lactic acid bacteria and yeast, exhibiting country- and region-dependent differences. Koumiss development history dates back over a thousand years and this beverage has a broad consumer market in China, Russia, Europe, and Central Asia. Koumiss is rich in nutrients such as proteins, unsaturated fatty acids, vitamins, and minerals, and it displays multiple health benefits, including blood pressure- and cholesterol-reducing, parasitic disease treatment, antioxidant, sleep-improving, and immune regulatory effects. Research on koumiss fermentation as well as on the related strains and components has recently become a hot topic. However, the microbial diversity in koumiss, direct-vat starter development, industrial production, and the koumiss-related health benefits and underlying effector mechanisms require further exploration. To contribute to koumiss product development, this study summarised the key areas and hotspots in koumiss research and focused on the research progress related to the nutritional components, microbial diversity, health benefits, and production process of koumiss. This overview could help to identify bacterial types in koumiss, provide a reference for direct-vat starter development, and shed light on the health benefits of koumiss. Finally, this study would serve as a useful reference for koumiss quality control and koumiss product stability improvement.

Keywords: koumiss; microbial species; nutritional components; fermentation process; health benefits

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1 Introduction

With improvements in living standards, consumers are increasingly prioritising health. Yoghurt, a nutritious fermented food, offers a rich taste profile and possesses numerous health benefits, rendering it popular among consumers of various age groups.

Koumiss, a fermented dairy product with a history spanning more than 1,000 years, enjoys a broad consumer market in China, Russia, Central Asia, and Eastern Europe. Some studies have suggested that it originated from the nomadic tribes of the Eurasian steppes. Ancient nomads, who required sustenance for herding or military campaigns, would carry skins of fresh mare milk on horseback or with them. Constant jostling during travel, along with temperature effects, would gradually ferment fresh mare's milk into a sour milk beverage due to microbial action.

Modern koumiss production utilises fresh mare milk as the primary ingredient, which is fermented with lactic acid bacteria

and yeast to create a low-alcohol beverage. Among a variety of microorganisms, koumiss possesses antibacterial and antioxidant properties and may have potential benefits for various conditions, enabling both nutritional and medicinal value. This study focused on five aspects of koumiss: nutritional components, microbial diversity, health benefits, production processes, and product development, thereby providing a basis for further research and development.

2 Research on koumiss bibliometrics

This study used the Web of Science (WOS) core database as the source of literature, with the search formula "TS = koumiss OR TS = kumiss OR TS = kumys OR TS = coomys OR TS = airag OR TS = arrag OR TS = chige OR TS = chegee OR TS = chigo OR TS = fermented mare's milk OR TS = qymyz". The search period is from the establishment of the database to 2023. The search and screening process yielded a total of 213 papers on koumiss, and

VOSviewer software was used to analyze countries, institutions, authors, and keywords. In the national/institutional cooperation network, each node represents a country/institution, the node size corresponds to the number of papers published by the country/institution, and the line indicates whether there is a cooperative relationship. In the author distribution map, each node represents an author, the size of the node indicates the number of papers published by the author, the line indicates whether there is a cooperative relationship between the authors, the number of connections represents the strength of cooperation between the authors, and different colors represent different clusters. In the keyword clustering map, each node represents a keyword, the size of the node indicates the frequency of the keyword, nodes of the same color form a theme together, and the line indicates the connection between keywords.

2.1 Distribution of countries and institutions

By 2023, a total of 36 countries/regions worldwide have WOS paper output in the field of koumiss research. China ranks first with 135 papers (63.38%), with an average citation frequency of 29.44, and China has close cooperation with other countries. Japan ranks second with 22 papers (10.33%) and an average citation frequency of 22.23. Turkey ranks third with 13 papers (6.1%) and an average citation frequency of 17. Kazakhstan ranks fourth with 10 papers (4.69%), and Mongolia ranks fifth with 9 papers (4.23%). The WOS papers in this field from China, Japan, Turkey, Kazakhstan, and Mongolia total 189, accounting for as much as 88.73%, indicating that Asian countries pay high attention to

research in this field. Table 1 lists the top 10 countries for the output of WOS papers on koumiss, Fig. 1 illustrates the research national collaborative network for koumiss.

By 2023, a total of 218 institutions worldwide have WOS paper output in this field. The top six institutions in terms of paper output are all from China, namely Inner Mongolia Agricultural University (80 papers, 37.56%), China Agricultural University (11 papers, 5.16%), Tianjin University of Science and Technology (9 papers, 4.23%), Shanghai Jiaotong University (7 papers, 3.29%), and Jiangnan University (7 papers, 3.29%). It can be seen that the institutions studying koumiss are mainly concentrated in China, and Chinese institutions studying koumiss have a high degree of cooperation, but there is less cooperation between research institutions in different countries. Table 2 lists the top 10 institutions in terms of the number of publications on koumiss, Fig. 2 illustrates the cooperative network of international institutions for koumiss research.

2.2 Author analysis

By 2023, a total of 917 authors worldwide have WOS paper output in the field of koumiss. The top 10 authors in terms of the number of papers published are Zhang Heping, Sun Zhihong, Liu Wenjun, Sun Tiansong, Kwok Lai-yu, Zhang Wenyi, Menghe Bilige, Chen Yongfu, Yu Jie, Chen Xia. These authors are all from Inner Mongolia Agricultural University and have close cooperation, indicating that the team of Professor Zhang Heping from Inner Mongolia Agricultural University is the main team studying koumiss. Table 3 lists the top 10 authors in the number

Table 1 Top 10 countries in terms of koumiss research paper output in WOS

Serial number	Country	Number of WOS papers	Percentage of papers (%)	Average citation frequency per paper
1	China	135	63.38	29.44
2	Japan	22	10.33	22.23
3	Turkey	13	6.1	17
4	Kazakhstan	10	4.69	8.1
5	Mongolia	9	4.23	31.67
6	France	8	3.76	25.5
7	USA	6	2.82	37
8	Poland	6	2.82	11.5
9	Korea	5	2.35	33
10	Kyrgyzstan	4	1.88	6.5

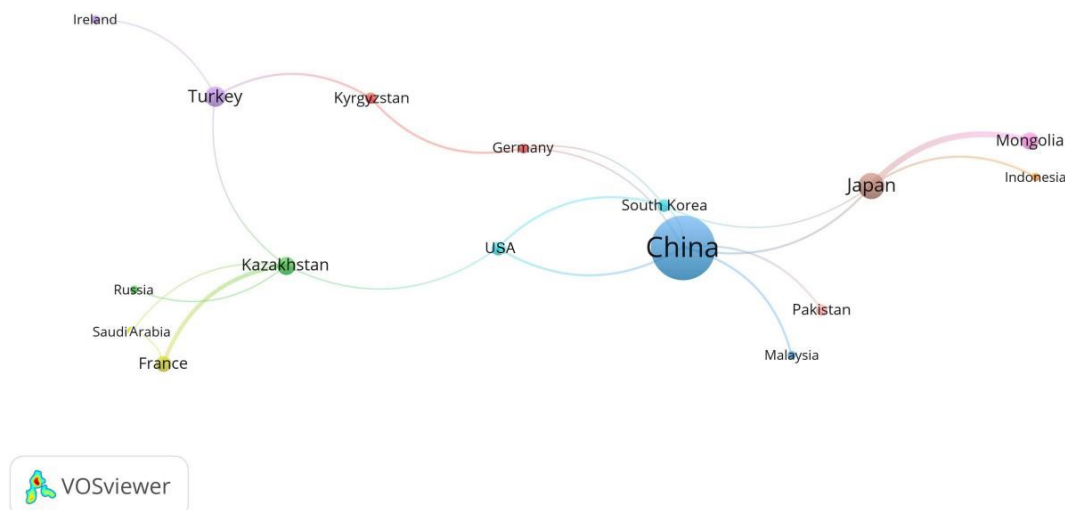


Figure 1 National cooperative network for koumiss research.

Table 2 Top 10 institutions in terms of koumiss research publication volume

Serial number	Organization	Country	Number of WOS papers	Average citation frequency per paper
1	Inner Mongolia Agricultural University	China	80	34.91
2	China Agricultural University	China	11	20.82
3	Tianjin University of Science and Technology	China	9	69.44
4	Shanghai Jiaotong University	China	7	37.29
5	Jiangnan University	China	7	55.71
6	Chinese Academy of Sciences	China	6	53.67
7	Okayama University	Japan	6	20.5
8	Al-Farabi Kazakh National University	Kazakhstan	5	7.4
9	Cirad	France	5	7.4
10	Kafkas University	Turkey	5	5.6

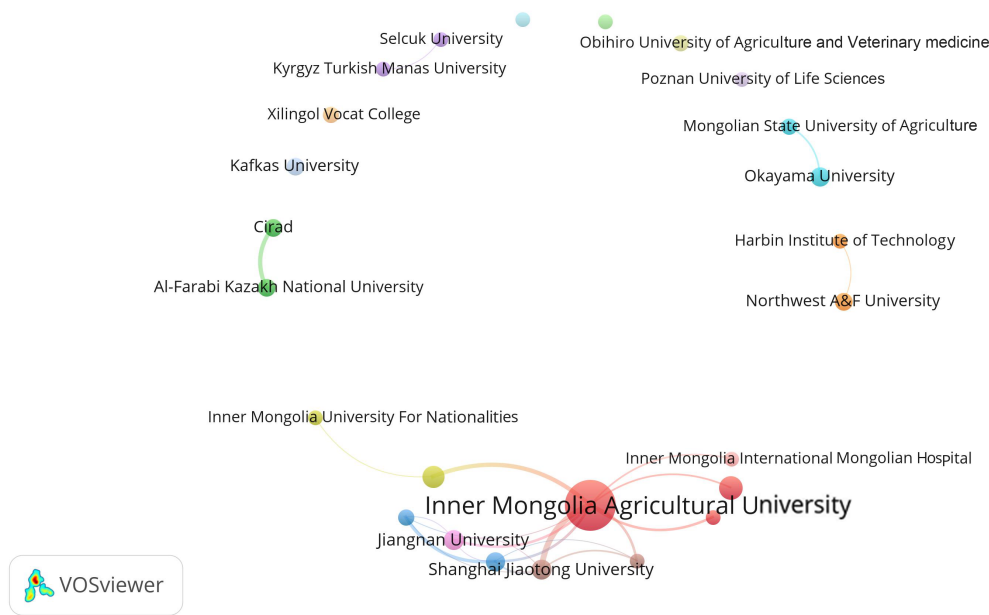


Figure 2 International collaboration network in koumiss research.

Table 3 Top 10 authors in terms of publication volume in koumiss research

Serial number	Author	Organization	Number of WOS papers
1	Zhang Heping	Inner Mongolia Agricultural University	51
2	Sun Zhihong	Inner Mongolia Agricultural University	19
3	Liu Wenjun	Inner Mongolia Agricultural University	19
4	Sun Tiansong	Inner Mongolia Agricultural University	15
5	Kwok Lai-yu	Inner Mongolia Agricultural University	13
6	Zhang Wenyi	Inner Mongolia Agricultural University	12
7	Menghe Bilige	Inner Mongolia Agricultural University	11
8	Chen Yongfu	Inner Mongolia Agricultural University	9
9	Yu Jie	Inner Mongolia Agricultural University	7
10	Chen Xia	Inner Mongolia Agricultural University	6

of published articles on koumiss, Fig. 3 illustrates the collaborative network among authors working on koumiss.

2.3 Keyword analysis

Keywords are the concentration and refinement of the central idea of the literature. The higher the frequency of keywords, the hotter the research. The VOSviewer software was used to co-occur analysis of keywords related to koumiss, with a co-occurrence

frequency ≥ 5 as the screening condition, and a total of 68 keywords were obtained. After analysis, it was found that Identification, Lactic acid bacteria, Strains, *Lactobacillus*, Yeasts, Probiotics, etc. appeared frequently, indicating that the research focus on koumiss is concentrated on the strain types of lactic acid bacteria and yeasts in koumiss, and the research on probiotic strains in koumiss. Because the current difficulty in the development of koumiss products is mainly concentrated on the

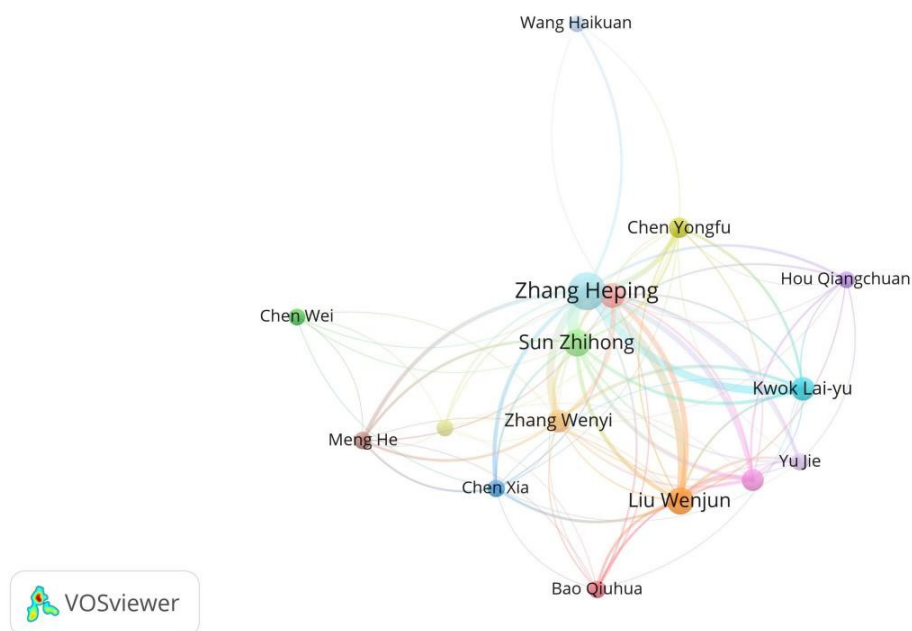


Figure 3 Author collaboration network in koumiss research.

unclear types of bacteria in koumiss, and the direct fermentation agent for koumiss has not yet been successfully developed, the current research focus on koumiss is mainly concentrated on identifying the dominant bacteria in koumiss. By drawing a keyword clustering view, it was found that there were five clusters. By drawing a superimposed time view, it was found that the early research on koumiss was mainly focused on traditional koumiss research, strain identification, and in recent years, single-molecule real-time sequencing technology to identify strain types, intestinal flora, anti-inflammatory effects of koumiss, and the mechanism of koumiss in treating diseases have become emerging hotspots. So this article also mainly discusses from these aspects. Table 4 lists the ranking of keyword frequency in the koumiss research literature, Fig. 4 illustrates the keyword co-occurrence cluster network in the research literature of koumiss, Fig. 5 presents the keyword co-occurrence time network in the research literature of koumiss.

Table 4 Frequency ranking of keywords in koumiss research literature

Serial number	Keyword	Occurrences	Total link strength
1	Koumiss	94	418
2	Identification	65	322
3	Lactic acid bacteria	56	298
4	Strains	30	172
5	<i>Lactobacillus</i>	29	134
6	Milk	25	130
7	Probiotics	24	138
8	Bacteria	24	93
9	Diversity	22	147
10	Airag	22	124
11	Cheese	20	111
12	Yeasts	18	121
13	Products	17	104
14	Lactobacilli	17	91
15	Tarag	16	104

3 Nutritional components of koumiss

Mare milk is rich in various nutrients, such as water, protein, fat, lactose, minerals, and vitamins. Its nutritional composition closely resembles that of human milk. The fat content in mare's milk is significantly lower than that in cow's milk and human milk, with a predominance of medium-chain fatty acids. Additionally, mare's milk has a relatively high polyunsaturated fatty acid content^[1,2]. The whey protein percentage in mare milk is 20% higher than in cow milk, thus it is more easily absorbable^[3]. Mare milk is also rich in various vitamins, notably vitamin C, which is significantly higher than in cow and sheep milk^[4].

Upon fermentation of fresh mare's milk, the activity of lactic acid bacteria increases the acidity and decreases the pH; yeast activity also produces small amounts of alcohol and carbon dioxide. Due to the fermentation action of lactic acid bacteria and yeast, the lactose content in mare's milk continually decreases, while the contents of lactic acid, amino acids, and fatty acids increase. This process also generates flavor compounds like acids and esters. Post-fermentation, the contents of total free amino acids, peptides, free fatty acids, and free calcium and phosphorus in mare's milk also increase progressively^[5]. Sun analyzed the nutritional components of 28 samples of koumiss from the Xinjiang region (China) and found a fat concentration of 1.31–2.54 g/100 mL, a protein concentration of 1.39–2.78 g/mL, a lactose concentration of 1.34–3.40 g/100 mL, an ash concentration of 0.21–0.43 g/100 mL, a calcium concentration of 54.84–119.92 mg/100 mL, and a phosphorus concentration of 41.36–96.30 mg/100 mL, validating the rich nutrition of koumiss^[6]. The yeast and lactic acid bacteria in koumiss can also synthesize vitamins B₁, B₂, B₁₂, niacin, and vitamin C, further enhancing its nutritional value. Mu *et al.*^[7] found that the vitamin C content in koumiss reached up to 78.4 mg/100 g. In 2022, at the Inner Mongolia Quality and Standardization Research Institute's review meeting on koumiss group standards, vitamin C was included in the nutritional indicators of koumiss for the first time, suggesting its potential as a natural vitamin C supplement. The changes in acidity and the increase in flavor compounds during fermentation provide koumiss with a spicy and refreshing taste, including a unique fermentation flavor^[8]. The flavor compounds

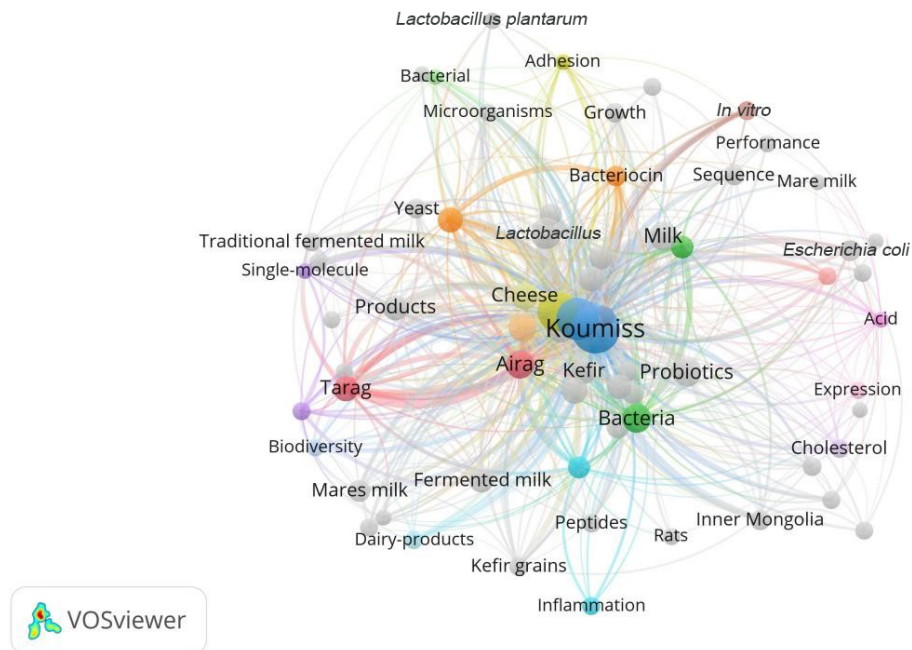


Figure 4 Co-occurrence clustering network of keywords in koumiss literature.

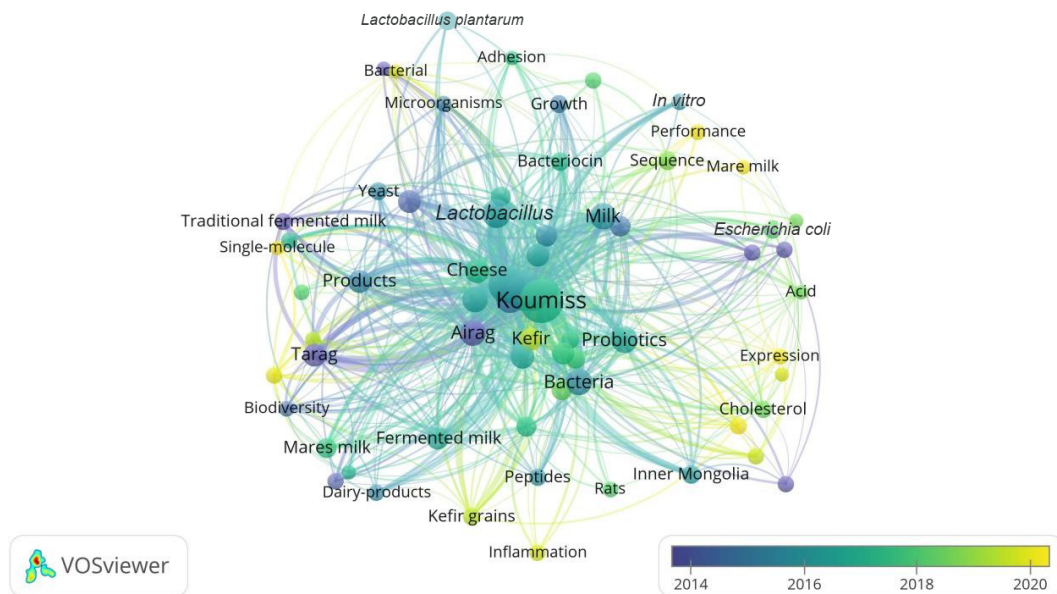


Figure 5 Co-occurrence temporal network of keywords in koumiss literature.

directly determine its taste, which serves as a primary factor for consumers when choosing koumiss. Therefore, the content of flavor compounds can operate as an indicator for assessing the quality of koumiss, providing important guidance to evaluate its taste and standardise its production. Meng *et al.*^[9] used Solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) methods to detect the flavor compounds in koumiss from Inner Mongolia, China, and identified 112 types of flavor compounds, mainly acids, esters, alcohol. Xia *et al.*^[10] found 52 types of flavor compounds in koumiss using the Liquid liquid extraction gas chromatography-mass spectrometry (LLE-GC/MS) method, including 18 acids, 17 esters, 8 alcohols, 3 ketones, and 3 aromatic compounds. The main flavor compounds were acids and esters. In contrast, fresh mare's milk contains only 22 types of flavor compounds, indicating significant differences in the types and contents of flavor compounds before and after fermentation. It is necessary to further analyse changes in flavor compounds

during the fermentation process and the impact of microbial growth in koumiss on its nutritional components using metabolomics and other research methods. Table 5 shows a portion of the nutrients in koumiss and fresh mares' milk^[11].

4 Microbial diversity in koumiss

Koumiss from both dairy enterprises and home workshops is produced primarily through natural fermentation. The variety of natural fermenting agents used in different regions, influenced by the local climate, geographical location, and microbial growth conditions, leads to diverse dominant microbial species in koumiss across various countries and regions.

4.1 Diversity of lactic acid bacteria in koumiss

Koumiss is rich in a variety of lactic acid bacteria. Robinson *et al.*^[12] isolated *Lactobacillus paracasei* subsp. *paracasei*,

Table 5 Comparison of nutrient composition between fresh mares' milk and koumiss

Nutrients	Fresh mares' milk	Koumiss
Lactose (mg/L)	45.75 ± 2.15	46.12 ± 1.93
Lactic acid (mg/L)	(8.20 ± 1.21)–(32.13 ± 1.54)	
Protein (%)	1.86 ± 0.07	2.22 ± 0.08
Fat (%)	(0.74 ± 0.16)–(1.48 ± 0.08)	
Vitamin B ₁ (µg/100 g)	(1.48 ± 0.12)–(4.86 ± 0.42)	
Vitamin B ₂ (µg/100 g)	(2.48 ± 0.12)–(4.86 ± 0.32)	
Vitamin E (µg/100 g)	(44.63 ± 0.63)–(99.90 ± 0.05)	

L. paracasei subsp. *tolerans*, and *Lactobacillus curvatus* from koumisses collected from Mongolia and Inner Mongolia, China. Ishii *et al.*^[13] isolated 43 strains of lactic from Inner Mongolia, China, including *Lactobacillus rhamnosus*, *L. paracasei* subsp. *paracasei*, *L. paracasei* subsp. *tolerans*, and *L. curvatus*. Zhang *et al.*^[14] collected 29 koumiss samples from the Ili region in Xinjiang, China, and identified *Lactobacillus helveticus*, *Lactococcus lactis*, *Lactobacillus kefiranoferiens*, and *Streptococcus thermophilus* as the dominant species through single-molecule real-time sequencing. Gesudu *et al.*^[15] collected 31 samples from Xilin

Gol in Inner Mongolia, China, and found *L. lactis*, *Streptococcus parauberis*, *Lactobacillus hamsteri*, and *L. helveticus* to be the dominant species. Kochetkova *et al.*^[16] collected samples from Buryatia, Russia, and identified the genera *Lactobacillus*, *Acetobacter*, *Lentilactobacillus*, *Streptococcus*, and *Lactococcus* as dominant in koumiss using 16S rRNA sequencing. Sun *et al.*^[17] collected 46 sterile samples from the Inner Mongolia Autonomous Region, Xinjiang Uygur Autonomous Region, and Qinghai Province in China and discovered 11 types of lactic acid bacteria, including *L. helveticus*, *Lactobacillus casei*, and *Lactobacillus plantarum*, *L. kefiranoferiens*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus diolivorans*, *Lactobacillus kefir*, *Lactobacillus reuteri*, *Lactobacillus pontis*, *Lactobacillus delbrueckii*. Li *et al.*^[18] collected six koumiss samples and six mare milk samples from Xilingol League, Inner Mongolia Autonomous Region, China, and studied the microbial changes before and after fermentation of mare milk by high-throughput sequencing technology. They found that the dominant genera in koumiss was *Lactobacillus*, *Acetobacter*, *Lelliottia*, *Acinetobacter*, *Streptococcus*, and *Lactococcus*. There were significant differences in the dominant species of lactic acid bacteria in koumiss across countries and regions. Other koumiss species included *L. plantarum*, and *L. fermentum*^[19–25]. Table 6 lists the lactic acid

Table 6 Lactic acid bacteria diversity in koumiss^[13–22, 24, 25]

Countries and regions	Production processes	Microbial detection methods	Lactic acid bacteria
Inner Mongolia Autonomous Region of China	Natural fermentation	Pure culture method	<i>L. rhamnosus</i> ; <i>L. paracasei</i> ssp. <i>paracasei</i> ; <i>L. paracasei</i> ssp. <i>tolerans</i> ; <i>L. curvatus</i> <i>L. helveticus</i> ; <i>L. lactis</i> ;
Xinjiang Uygur Autonomous Region of China	Natural fermentation	PacBio single molecule real-time sequencing technology	<i>L. kefiranoferiens</i> ; <i>L. kefir</i> <i>L. helveticus</i> ; <i>L. hamsteri</i> ; <i>L. lactis</i> ; <i>Lactococcus garvieae</i> ; <i>Lactococcus raffinolactis</i> ; <i>Enterococcus faecalis</i> ; <i>Enterococcus camelliae</i> ; <i>Enterococcus casseliflavus</i> ; <i>Enterococcus durans</i> ; <i>Enterococcus sulfureus</i> ; <i>Enterococcus asini</i> ; <i>Enterococcus italicus</i> ;
Inner Mongolia Autonomous Region of China	Natural fermentation	Single molecule real-time sequencing technology	<i>S. parauberis</i> ; <i>S. thermophilus</i> <i>Lactobacillus</i> ; <i>Streptococcus</i> ; <i>Lactococcus</i> <i>L. helveticus</i> ; <i>L. casei</i> ;
Buryatia of Russia	Natural fermentation	16S rRNA gene sequencing technology	<i>L. plantarum</i> ; <i>L. kefiranoferiens</i> ; <i>L. fermentum</i> ; <i>L. acidophilus</i> ; <i>L. pontis</i> <i>L. helveticus</i> ; <i>L. casei</i> ;
Xinjiang Uygur Autonomous Region of China	Natural fermentation	16S rRNA gene sequencing technology; phylogenetic analysis	<i>L. plantarum</i> ; <i>L. diolivorans</i> ; <i>L. kefir</i> ; <i>L. reuteri</i> <i>L. helveticus</i> ; <i>L. plantarum</i> ; <i>L. delbrueckii</i>
Inner Mongolia Autonomous Region of China	Natural fermentation	16S rRNA gene sequencing technology; phylogenetic analysis	
Qinghai Province of China	Natural fermentation	16S rRNA gene sequencing technology; phylogenetic analysis	

(Continued)

Countries and regions	Production processes	Microbial detection methods	Lactic acid bacteria
Inner Mongolia Autonomous Region of China	Natural fermentation	Illumina MiSeq sequencing technology	<i>Lactobacillus</i> ; <i>Streptococcus</i> ; <i>Lactococcus</i> <i>L. casei</i> ; <i>L. diolivorans</i> ; <i>L. farciminis</i> ; <i>L. helveticus</i> ; <i>L. hilgardii</i> ; <i>L. kefiranoformis</i> ; <i>L. kefir</i> ;
The Mongolian Provinces of Arhangai, Bulgan, Dundgobi, Tov, Uburhangai, and Umnugobi	Natural fermentation	16S rRNA gene sequencing technology; phylogenetic analysis	<i>Lactobacillus parafarranginis</i> ; <i>L. plantarum</i> ; <i>L. lactis</i> ssp. <i>lactis</i> ; <i>Leuconostoc mesenteroides</i> ; <i>Leuconostoc pseudomesenteroides</i> ; <i>Enterococcus faecium</i> ; <i>S. thermophilus</i> <i>L. plantarum</i> ; <i>Lactobacillus pentosus</i> ; <i>L. helveticus</i> ; <i>L. acidophilus</i> ; <i>L. paracasei</i> ; <i>L. fermentum</i> ; <i>L. mesenteroides</i> ; <i>L. curvatus</i> ; <i>L. lactis</i> ; <i>Lactobacillus jensenii</i> ; <i>L. kefiranoformis</i> ; <i>Lactobacillus kitasatonis</i> ; <i>L. kefir</i> ; <i>Lactobacillus buchneri</i> ; <i>S. thermophilus</i> ; <i>E. faecium</i> <i>L. plantarum</i> ; <i>L. pentosus</i> ; <i>L. lactis</i> ssp. <i>cremoris</i> <i>L. helveticus</i> ; <i>L. acidophilus</i> ; <i>L. casei</i> ssp. <i>pseudoplatanturum</i> ; <i>Lactobacillus gasseri</i> ; <i>L. casei</i> ssp. <i>casei</i> ; <i>L. curvatus</i> ; <i>Lactobacillus sanfrancisco</i> ; <i>Lactobacillus coryniformis</i> ssp. <i>Coryniformis</i> ; <i>Lactobacillus brevis</i> ; <i>L. plantarum</i> ; <i>Lactobacillus homohiechill</i> ; <i>L. fermentum</i> ; <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> ; <i>Lactobacillus ruminis</i> ; <i>Lactobacillus crispatus</i> ; <i>Lactobacillus farciminis</i> ; <i>Lactobacillus hilgardii</i> <i>L. plantarum</i> ; <i>L. paracasei</i> ssp. <i>Tolerans</i> ;
Xinjiang Uygur Autonomous Region of China	Natural fermentation	Denatured gradient gel electrophoresis	<i>L. paracasei</i> ; <i>L. casei</i> ; <i>L. rhamnosus</i> ; <i>L. brevis</i> ; <i>L. fermentum</i>
Inner Mongolia Autonomous Region of China	Natural fermentation	16S rRNA gene sequencing technology; phylogenetic analysis	
Xinjiang Uygur Autonomous Region of China	Natural fermentation	Pure culture method	
Kazakhstan	Natural fermentation	16S rRNA gene sequencing technology; phylogenetic analysis	

bacteria species in koumiss.

4.2 Diversity of yeasts in koumiss

Yeast is an essential fermenting microorganism in koumiss that

produces alcohol, carbon dioxide, and various flavour compounds that contribute to its unique taste. Watanabe *et al.*^[20] collected 22 koumiss samples from Mongolia and found that the dominant yeast species was *Candida pararugosa*, *Dekkera anomala*,

Issatchenkia orientalis, *Kazachstania unispora*, *Kluyveromyces marxianus*, *Pichia mandshurica*, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*. Ni *et al.*^[23] collected 28 koumiss samples from Xinjiang Uygur Autonomous Region in China and found that the dominant yeast species was *Saccharomyces unisporus*, *K. marxianus*, *Pichia membranaefaciens*, *S. cerevisiae*. Montanari *et al.*^[26] collected 94 koumiss samples from Kazakhstan and found that the dominant yeast species was *S. unisporus*, it also includes *S. cerevisiae*, *Candida buinensis*, *K. marxianus*. Miyamoto *et al.*^[27] collected six types of koumiss from Ulaanbaatar, Mongolia and identified *K. marxianus* as the dominant yeast. Zhang *et al.*^[28] collected five samples each from Inner Mongolia and Xinjiang, China, and found *K. marxianus*, *Pichia cactophila*, and *Pichia fermentans* to be dominant in Inner Mongolia, China, with *Candida zeylanoides* and *K. marxianus* being dominant in Xinjiang (China). Mu *et al.*^[29] collected 98 samples from the Xinjiang Uygur Autonomous Region, Qinghai Province, and the Inner Mongolia Autonomous Region in China, and isolated 655 yeast strains belonging to 12 different species across nine genera.

S. cerevisiae, *K. unispora*, and *K. marxianus* were the dominant yeasts. The types of yeasts used are listed in Table 7.

From these analyses, it is evident that koumiss contains a diverse range of lactic acid bacteria and yeasts that form its main microbial fermentation system. Cheirsilp *et al.*^[30] found that co-culturing lactic acid bacteria with yeast in milk allowed the lactic acid bacteria to break down lactose into galactose and glucose, providing a carbon source for yeast, which in turn promoted the growth of lactic acid bacteria by utilising lactate. Gobetti *et al.*^[31] added maltose, sucrose, glucose, and fructose as carbon sources to DeMan, Rogosa and Sharpe (MRS) culture medium to study the interaction between two yeast strains and two lactic acid bacteria strains. The results showed that adding sucrose increased the number of live lactic acid bacteria in co-cultures compared to that in monocultures, as yeast can decompose sucrose into glucose and fructose, thereby providing a stable carbon source for lactic acid bacteria. Thus, lactic acid bacteria and yeasts can symbiotically utilise metabolic products. However, antagonistic interactions exist between the two. Nielsen *et al.*^[32] found that the growth of lactic

Table 7 Yeast diversity in koumiss^[20,23,26-29]

Countries and regions	Production processes	Microbial detection methods	Yeast
Mongolia	Natural fermentation	Random amplified polymorphic DNA-polymerase chain reaction; phylogenetic analysis based on the sequences of 18S–26S ITS1 and ITS2	<i>C. pararugosa</i> ; <i>D. anomala</i> ; <i>I. orientalis</i> ; <i>K. unispora</i> ; <i>K. marxianus</i> ; <i>P. mandshurica</i> ; <i>S. cerevisiae</i> ; <i>T. delbrueckii</i>
Xinjiang Uygur Autonomous Region of China	Natural fermentation	Sequence analysis of the D1/D2 region of 26S rDNA; phylogenetic analysis	<i>Saccharomyces unisporus</i> ; <i>K. marxianus</i> ; <i>P. membranaefaciens</i> ; <i>S. cerevisiae</i> ; <i>S. cerevisiae</i> ; <i>S. unisporus</i> ; <i>K. marxianus</i> ; <i>C. buinensis</i> ; <i>K. marxianus</i> ;
Kazakhstan	Natural fermentation	Pure culture method	<i>Dekkera bruxellensis</i> <i>Candida parapsilosis</i> ; <i>C. zeylanoides</i> ; <i>Cryptococcus albidus</i> ; <i>Cryptococcus saitoi</i> ;
Mongolia	Natural fermentation	Denatured gradient gel electrophoresis	<i>K. marxianus</i> ; <i>P. cactophila</i> ; <i>P. fermentans</i> ; <i>S. cerevisiae</i> ;
Xinjiang Uygur Autonomous Region of China	Natural fermentation	26S rDNA D1/D2 area sequence analysis technology; ITS sequence analysis technique	<i>Saccharomycopsis crataegensis</i> <i>C. zeylanoides</i> ; <i>Clavispora lusitaniae</i> ; <i>C. saitoi</i> ; <i>Cryptococcus uzbekistanensis</i> ;
Inner Mongolia Autonomous Region of China	Natural fermentation	26S rDNA D1/D2 area sequence analysis technology; ITS sequence analysis technique	<i>K. marxianus</i> ; <i>P. cactophila</i> ; <i>P. fermentans</i> ; <i>Rhodotorula mucilaginosa</i> ; <i>Rhodotorula dairenensis</i> ; <i>S. cerevisiae</i> ; <i>S. crataegensis</i> <i>C. pararugosa</i> ; <i>D. anomala</i> ;
Qinghai Province of China	Natural fermentation	Denatured gradient gel electrophoresis	<i>Issatchenkia orientalis</i> ; <i>K. unispora</i> ; <i>K. marxianus</i> ; <i>Pichia deserticola</i> ; <i>Pichia manshurica</i> ; <i>P. membranaefaciens</i> ; <i>S. cerevisiae</i>

(Continued)

Countries and regions	Production processes	Microbial detection methods	Yeast
Xinjiang Uygur Autonomous Region of China	Natural fermentation	Denatured gradient gel electrophoresis	<i>C. pararugosa</i> ; <i>D. anomala</i> ; <i>Geotrichum</i> sp.; <i>I. orientalis</i> ;
			<i>K. unispora</i> ; <i>K. marxianus</i> ; <i>P. deserticola</i> ; <i>P. manshurica</i> ; <i>P. membranaefaciens</i> ; <i>S. cerevisiae</i> ; <i>D. anomala</i> ; <i>Geotrichum</i> sp.; <i>I. orientalis</i> ;
Inner Mongolia Autonomous Region of China	Natural fermentation	Denatured gradient gel electrophoresis	<i>K. unispora</i> ; <i>K. marxianus</i> ; <i>P. deserticola</i> ; <i>P. manshurica</i> ; <i>P. membranaefaciens</i> ; <i>S. cerevisiae</i> ;

acid bacteria was inhibited by fatty acids produced during the metabolism of lipolytic yeast when co-cultured. Therefore, it is necessary to further explore the symbiotic mechanisms between different species of lactic acid bacteria and yeast in koumiss to lay the foundation for the development and industrial production of direct vat-set fermenters.

Koumiss, as a beverage with a long history, is mainly produced through natural fermentation. Natural fermentation relies on complex local microbial communities, resulting in the rich and diverse lactic acid bacterial and yeast content in koumiss. Moreover, lactic acid bacterial and yeast types in koumiss from different countries and regions reportedly differ, potentially due to geographical and climatic differences, affecting the microbial population. Further large-scale, multicentre studies would be required to identify the microbial species in koumiss. In addition, different research teams used significantly different methods for identifying microbial species in koumiss, including pure culture, fingerprinting, second- and third-generation sequencing, and metagenomic sequencing techniques. The accuracy of these techniques varies, and the sequencing technique-related differences might also factor in the differences between the microbial species in the koumiss samples collected by different teams. With the development of sequencing technology, the accuracy of microbial diversity detection in koumiss would improve.

5 Health benefits of koumiss

In regions where koumisses are produced and consumed, labourers have discovered, through extensive experience, that they may have potential benefits for various conditions. According to historical records as far back as the Yuan Dynasty (A.D. 13th–14th), the use of koumiss to treat diseases enhances stomach heat, aids with digestion, regulates physical fitness, promotes blood circulation, resolves stasis, improves sleep, and detoxifies and nourishes the blood^[33]. In addition, in Inner Mongolia, China, and Mongolia, traditional Mongolian medicine includes a unique “koumiss (chige) therapy” and former Soviet regions have established treatment centres for it. Modern clinical and basic research has confirmed the efficacy of koumiss in the treatment of various diseases.

5.1 Lowering blood lipid and cholesterol levels

Cholesterol is an essential substance in the human body. High-

density cholesterol protects blood vessels, whereas increased low-density cholesterol increases the risk of atherosclerosis and coronary heart disease. Hou *et al.*^[34] conducted a clinical randomised controlled trial in which subjects consumed 750 g of koumiss daily. Blood lipid levels, gut microbiota, and visceral indices were measured on days 0, 30, and 60, revealing significant improvements in hyperlipidaemia symptoms and increased blood high-density lipoprotein levels. This improvement may be attributed to the modulation of gut microbiota by koumiss and its metabolic products, such as *S*-adenosyl-*L*-methionine, carnosine, lysophosphatidylinositol, and dipeptides. Pan *et al.*^[35] isolated 10 LAB strains of lactic acid bacteria from koumiss. *In vitro* experiments showed that *L. fermentum* SM-7 reduced cholesterol levels by 66.8%. In hyperlipidaemic mouse models that were fed *L. fermentum* SM-7, significant reductions in serum total cholesterol, triglycerides, low-density lipoprotein cholesterol, and the atherosclerosis index were observed, demonstrating the potential of koumiss in treating hyperlipidaemia. Menghe *et al.*^[36] isolated *L. acidophilus* MG2-1 from Mongolian koumiss. In a hyperlipidaemic Wistar rat model fed with both bioactive and inactivated *L. acidophilus* MG2-1, an inhibitory effect on the increase in serum cholesterol was ultimately observed, indicating its potential in preventing arteriosclerosis. However, the mechanisms of action of koumiss in the treatment of hyperlipidaemia and cholesterol regulation remain to be explored.

5.2 Immune regulation

When human immunity is compromised, susceptibility to the cold, flu, and other illnesses increases. With growing health consciousness, the demand for functional foods that enhance immunity is also increasing. Li *et al.*^[37] developed an immunosuppressed mouse model and found that koumiss increased the number of CD4⁺ and CD8⁺ cells and reduced the immunosuppressive and destructive effects of cyclophosphamide (CY) on the intestinal immunity of mice. Rong *et al.*^[38] isolated *L. helveticus* NS8 from koumiss, which showed protective effects against TNBS-induced colitis in mice and induced peripheral blood mononuclear cells to secrete anti-inflammatory cytokine interleukin-10 (IL-10). Additionally, *L. helveticus* NS8 elevated IL-10 levels, reducing the pro-inflammatory effects of lipopolysaccharide (LPS) on the mouse macrophage line RAW264.7, suggesting that it indirectly combats cancer and pathogenic bacteria by enhancing immunity.

5.3 Antihypertensive effects

Angiotensin I-converting enzyme (ACE) is a membrane-bound glycoprotein crucial for blood pressure regulation, and ACE inhibitors are effective against hypertension. Sun *et al.*^[39] isolated 21 *Lactobacillus* strains from koumiss, with 16 exhibiting ACE inhibitory activity above 50% and two producing γ -aminobutyric acid (GABA). Chen *et al.*^[40] isolated and purified four active peptides (PI, PK, PM, and PP) from koumiss using ultrafiltration and high-performance liquid chromatography. *In vitro* experiments revealed that these components inhibited ACE, necessitating further research on the antihypertensive efficacy and pharmacological basis of koumiss.

5.4 Antibacterial effects

In the former Soviet Union, koumiss was primarily used to treat tuberculosis, with several studies reporting its clinical efficacy^[41]. In China, Mongolian hospitals, such as the Inner Mongolia International Mongolian Hospital and Xilingol Mongolian Hospital, have used koumiss as an adjunct therapy for tuberculosis, with a long history and extensive clinical experience. Clinical trials in several hospitals in Inner Mongolia have confirmed that koumiss combined with anti-tuberculosis drugs can effectively kill *Mycobacterium tuberculosis*, enhance patient immunity, and restore pulmonary function. Hou *et al.*^[42] demonstrated *in vitro* that mare milk fat is effective against *M. tuberculosis*. Sun *et al.*^[43] found that koumiss had significant inhibitory effects on *Bacillus subtilis*, *Micrococcus luteus*, and *M. tuberculosis*, and identified lactic acid and mare milk fat as the main antibacterial substances. Ham *et al.*^[44] fed chickens a mixture of *Saccharomycopsis fibuligera* and *L. plantarum* isolated from koumiss and observed changes in the intestinal microbiota, resulting in faster growth and a significant decrease in *Escherichia coli* in the faeces. Clinical trials and *in vitro* experiments have shown that koumiss inhibits various harmful bacteria, suggesting its potential as a broad-spectrum antibiotic. However, research on the mechanisms of action of koumiss against harmful bacteria is still in its infancy, rendering it an aim of future research.

5.5 Treating toxoplasmosis

Toxoplasma gondii is a zoonotic parasite that infects all warm-blooded animals, including humans, and can cause significant losses in livestock and aquaculture. Current effective drugs for toxoplasmosis, such as sulfonamides, can damage liver function and cause a long-term rash. Therefore, the development of new drugs for the treatment of toxoplasmosis is necessary. Yan *et al.*^[45] constructed a *T. gondii*-infected mouse model and found that although koumiss did not increase survival rates, it reduced parasite burdens in the brain, heart, spleen, and liver tissues, alleviated clinical symptoms, increased IL-10 concentrations, and moderated reactivation of *T. gondii* infection. Yan *et al.*^[46] found that koumiss increased the abundance of Lachnospiraceae and *Akkermansia muciniphila* in the gut microbiota of infected mice, improving toxoplasmosis and reducing brain cyst numbers, indicating its efficacy against toxoplasmosis.

5.6 Improving sleep

Insomnia, characterised by the chronic inability to sleep normally, is becoming increasingly prevalent worldwide. The pathogenesis of insomnia is not fully understood; however, one

theory suggests that it results from the dysregulation of central nervous system neurotransmitters. GABA is a key inhibitory neurotransmitter in the central nervous system and is typically present at lower-than-normal levels in patients with insomnia. Byun *et al.*^[47] found that administering 300 mg of GABA extracted from fermented rice germ to patients with insomnia effectively improved sleep quality, reduced sleep latency, and increased sleep efficiency, with no significant adverse reactions. Zeng *et al.*^[48] screened *L. pentosus* S5 from Xinjiang (China) koumiss, a high GABA producer (1.6 g/L), suggesting the potential of developing dairy products using *L. pentosus* S5 to improve sleep. In addition, GABA supplementation can alleviate anxiety, prevent and treat epilepsy and other mental disorders, reduce blood sugar levels, and improve memory.

5.7 Antioxidant activity

The production of free radicals is necessary for the human body, as they regulate cell growth and intercellular signal transmission and inhibit bacteria and viruses. However, the excessive accumulation of free radicals can lead to high levels of reactive oxygen species (ROS), which are highly toxic to cells. They can damage DNA, lipids, and proteins and trigger various diseases, such as cancer, arteriosclerosis, diabetes, and cardiovascular diseases. Che *et al.*^[49] isolated two probiotic lactic acid bacterial strains, S3 and Y11, from Xinjiang (China) koumiss, which showed good tolerance to hydrogen peroxide solution and the ability to scavenge hydroxyl radicals and chelate iron ions (Fe^{2+}), thus exhibiting efficient reduction activity. However, the material basis of the antioxidant activity of these strains in koumiss remains unclear, necessitating further analysis to develop natural antioxidant products.

6 Production techniques for koumiss

6.1 Traditional techniques

Currently, koumiss production predominantly involves traditional fermentation methods. Mongolian herders on grasslands typically produce koumiss in summer when pastures are lush, providing abundant food for lactating mares and resulting in higher milk yields and better quality. On average, the mares were milked 6 times daily. Fresh mare's milk is first filtered through fine gauze, cooled naturally, and then added to a wooden barrel or clay pot containing fermented koumiss. Using a wooden stirrer, the mixture was churned 5–6 times daily for approximately 1,000–1,500 strokes per session. This process achieves homogenisation and aeration, allowing thorough mixing of yeast with air to accelerate fermentation and disperse milk fat, thereby resulting in a smoother koumiss^[50]. Milk is ready for consumption after 1–2 days of fermentation, or the fermentation time can be adjusted to suit personal taste preferences. Danova *et al.*^[51] categorised koumiss into three levels based on pH: strong (pH 3.3–3.6), moderate (pH 3.9–4.5), and mild (pH 4.5–5.0). However, there are currently no standardised norms for grading the koumiss. Figure 6 shows the process of milking horse milk and the tools used to ferment koumiss by grassland herdsman.

6.2 Industrial production

As consumers increasingly recognise the nutritional and health benefits of koumiss, the demand for this product is growing. Traditional home-based production methods cannot meet



Figure 6 Pictures of milking horses and traditional fermentation tools used to make koumiss. (A) Herdsmen on the grassland collect mare's milk; (B) A ceramic pot for fermenting koumiss.

consumer demand because of limited output, unspecified microbial strains, unstable product quality, and short shelf life. Consequently, dairy enterprises are exploring the industrial production of koumiss. They incorporate equipment such as fermentation tanks, homogenisers, and filling machines to create a cleaner and more hygienic fermentation environment. Temperature-controlled equipment allows precise control over the fermentation temperature and duration, aiding in the quality control of koumiss. Kucukcetin *et al.*^[52] used membrane filtration technology to adjust cow milk composition to resemble mare milk by fermenting it with *Kazakhstani maxima*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus*. After nutritional analysis and sensory evaluation, the resulting product was found to be similar to koumiss. Due to the lower yield of mare milk, especially in winter, using modified cow milk can alleviate seasonal shortages of raw materials, ensuring market supply. Wang *et al.*^[53] studied the effects of the inoculation amount (lactic acid bacteria/yeast),

fermentation temperature, fermentation time, and sugar addition on koumiss fermentation. The optimal fermentation conditions were found to be an inoculation ratio of 3% lactic acid bacteria to 5% yeast, a fermentation temperature of 32 °C, a fermentation time of 80 h, and a sugar addition of 6%. These conditions produce fragrant, mildly sour koumiss. However, the industrial production of koumiss continues to rely on natural fermenting agents with unknown microbial species. The hygiene of raw mare milk and the environment cannot be guaranteed during the expansion of natural fermenting agents, posing a risk of contamination. Additionally, the proportion of microbial strains changes during repeated expansion, directly affecting the flavour and texture of the fermented product. Therefore, isolating and identifying dominant microbial strains in koumiss and developing direct-vat-set fermenters are key to the industrial and standardised production of koumiss products. Figure 7 shows a modern factory for fermented koumiss.



Figure 7 Pictures of industrial production workshop of koumiss.

7 Development of koumiss products

7.1 Koumiss beer

Koumiss beer is an innovative dairy-based alcoholic beverage produced mainly from fresh mare's milk with added sugar and fermented with lactic acid bacteria and yeast. This low-alcohol drink is sweet and sour in taste and possesses a unique texture. Enriched in nutrients and various probiotics, it is considered healthy. The characteristics of koumiss beer cater to the

consumption preferences of younger consumers and exhibit a promising market potential.

7.2 Koumiss powder and mare's milk powder

Koumiss powder, which is produced using freeze-drying or spray-drying techniques to remove most of the water content, offers ease of preservation, portability, and an extended shelf life. This overcomes the drawbacks of traditional koumiss products, such as the need for refrigeration and short shelf life, enabling a convenient and quick healthy food. Furthermore, the nutrient

composition of mare's milk closely resembles that of human breast milk, indicating its great potential for the development of infant formula powders.

7.3 Koumiss beauty products

In Chinese Mongolian medicine, koumiss is considered beneficial for beauty. Chemically extracted and formulated into creams, koumiss effectively treats skin conditions, such as melasma, freckles, and acne. As a naturally fermented beverage, it is safe for both internal and external use. Currently, beauty products containing koumiss, such as eye creams, foot creams, and facial masks, are being developed and sold in the Chinese market.

7.4 Koumiss tablets

Liquid koumisses have a short shelf life. By converting it into tablets through freeze-drying, not only are the nutritional components and bioactive substances of koumiss retained, but the taste of traditional koumiss is also enhanced, and its shelf life is extended. Furthermore, these tablets are easy to carry and suitable for long-distance transportation and sale in various environments.

7.5 Koumiss fruit beverage

Koumiss fruit beverages are prepared by adding suitable amounts of honey and fruit juice to koumiss, reducing its acidity and improving its taste to cater to a broader range of consumer preferences. Honey and fruit juice, which are rich in amino acids, vitamins, and minerals, produce even richer nutrition when combined with koumiss. These beverages have moderate sweetness and a low sugar content, thereby serving as a new healthy dairy drink that is less likely to contribute to obesity.

7.6 Koumiss bread

Most commercially available breads use industrial yeast for fermentation, resulting in a lack of a distinct flavour. Koumiss, however, is rich in yeast, can replace industrial yeast in bread-making. The use of koumiss in bread preserves its nutritional content and enriches texture and flavour, thereby improving its overall quality.

7.7 Probiotic products obtained from koumiss

Probiotic products are becoming increasingly popular due to their ability to balance intestinal microbiota and improve health and sub-health conditions. The selection of high-quality probiotic strains from fermented foods is important. Koumiss, rich in lactic acid bacteria and yeast, has led many research teams to isolate premium probiotic strains and develop them into health products.

8 Future prospects

As living standards improve, consumers are becoming increasingly focused on dietary health. Koumiss, a nutritionally rich and naturally fermented dairy product containing many probiotics, has the potential to be developed into a functional food due to its therapeutic effects on various diseases. However, there are some urgent issues with koumiss products that need to be addressed.

First, currently, koumiss produced using either traditional techniques or modern equipment (such as fermenters, homogenizers, and filling machines) still relies on traditional fermenting agents. The lack of clarity about microbial species in

these agents and the risk of contamination by other microbes during repeated propagation create challenges in controlling fermentation conditions, resulting in inconsistent product quality. Development of direct-vat-set fermenters is key to solving this issue.

Second, although koumiss has potential therapeutic effects associated with various diseases, the optimal dosage for disease prevention and treatment has not been thoroughly studied, and the mechanisms and pharmacological basis of the therapeutic effects are still unclear. Clinical research, animal experiments, and cell studies are needed to further evaluate the clinical efficacy, optimal therapeutic dosage, mechanisms of action, and pharmacological basis of koumiss.

Third, the taste and acidity of koumiss differs from sour cow's milk. To further expand the consumer market for koumiss, it is necessary to adjust its taste and acidity based on consumer feedback to better meet market preferences.

In conclusion, it is imperative that koumiss, as a health beverage, undergoes further market promotion. This will not only amplify its presence in the market but also extend its benefits to a larger consumer base.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work. Min-Hui Li is the Associate Editor of *Food & Medicine Homology*.

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