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Study on Production and Properties of Kumiss of Herders in Mongolian Dry Steppe

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Abstract: Kumiss, prepared in dry inland Asia, is a carbonated beverage containing about 2% alcohol, prepared by fermenting raw horse milk with lactic acid bacteria and yeast. The quality of Kumiss is markedly influenced by factors affecting the quality of horse milk: grass eaten by horses, habitat and microclimate conditions of the herders' home (temperature). The type of microorganisms and dominant flora in Kumiss vary among families. Many lactic acid bacteria involved in fermentation belong to Lactobacillus and yeast is mainly belonging to genus Kluveromyces. At present, plastic containers mostly replaced bovine leather vessels traditionally used by herders for fermentation. Men drink about 5 L of Kumiss daily on average, and the vitamin C level in Kumiss is 8-11 mg/100 ml. This beverage is an important source of vitamin C in the Mongolian herders'diet, which contains almost no vegetables. Thus, in summer, daily intake of vitamins and calories in Mongolian herders are supplemented by drinking a large volume of Kumiss.

Key Words: Composition analysis, Dry Inland Asia, Horse milk, Kumiss, Microbial flora

1. Introduction

Mongolian herders' life is markedly influenced by the weather. The diets of herders are mostly comprised of milk, dairy products and meat, and intakes of vegetables and fruit are very limited. Dairy products are prepared from milk of various domestic animals, but horse milk is not appropriate for processing to cheese (Kosikowski, 1982). For this reason, nomadic peoples have used unpasteurized horse milk for fermentation, to prepare an alcoholic beverage named kumiss. The Kumiss-producing region overlaps with the distribution of steppe zone. Up to date, there is not any standard for Kumiss in Mongolia. So, the quality of Kumiss is varying from family to family, and between natural zones. The purpose of this study is to investigate the characteristics and production methods of Kumiss in dry steppe zone conditions and in different herders' families.

2. Materials and Methods

2.1. Location

Herders' families in Tuv, Uburhangai, Arhangai, and Bulgan Provinces were selected for the survey. The selection was based on the grounds of Kumiss quality ranking among Mongolian people. The Kumiss in the survey area is ranking in the top five. Samples of horse milk were taken in the following places: one in Uburhangai and one in Bulgan Province. Samples of Kumiss were taken from one family in Tuv, one family in Uburhangai, 4 families in Arhangai, and 4 families in Bulgan Province.

2.2. Traditional meaning of milk quality by herder

Herders say that the following conditions lead to good milk quality: first, it is original 'Mongolian horse milk'; second 'horses eat only meadow grass'; third 'horses are maintained in a favorable environment', and fourth the 'hojir (natural salts) is needed'. Accordingly, environmental conditions would markedly influence milk quality and have been involved in the quality and characteristics of Kumiss.

2.3. Sample analysis

The number of viable microorganisms, pH, and alcohol content were measured using standard methods. The chemical and mineral composition and amino acids were analyzed following the standard procedure described in detail by Ishii (1999). The vitamin C level was measured using HPLC.

2.4. Isolation and identification of lactic acid bacteria and yeasts

Lactic acid bacteria were isolated from 22 samples (Surveyed; 2002-2012) as follows, referring to the method reported by Kozaki and Okada (1992). Colonies formed on MRS agar medium and GAM agar medium, plate count agar medium with BCP and ACE agar medium were picked and subjected to repeated streak cultures on MRS agar medium. Lactic acid bacteria strains were isolated and subjected to

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Table 1. Chemical composition analysis of horse milk and kumiss (%).

Sample and Province	Water	Solid	Protein	Fat	Ash	Insolubled nitrogen
Horse milk						
Uburuhangai Provunce '12	89.3	10.7	2.1	2.0	0.4	6.2
Bulgan Provunce'12	89.6	10.4	1.9	1.9	0.3	6.3
Kumiss						
Bulgan Province'06 NO1	94.7	5.3	2.1	1.8	0.3	1.1
Bulgan Province '06 NO2	96.1	3.9	1.7	1.2	0.3	0.7
Arhangai Province'07 NO1	96.6	3.4	1.8	0.7	0.3	0.6
Arhangai Province '07 NO2	94.9	5.1	1.5	1.1	0.3	2.2
Tuv Province '08	94.6	5.4	2.1	1.9	0.3	1.1
Uburhangai Province'12 NO1	95.0	5.0	2.0	1.6	0.3	1.1
Aruhangai Province '12 NO2	94.0	6.0	2.3	1.9	0.3	1.5
Aruhanngai Province'12 NO3	95.2	4.8	2.0	1.9	0.3	0.6
Bulgan Province '12 NO4	94.9	5.1	2.1	2.1	0.3	0.6
Bulgan Province'12 NO5	94.8	5.2	2.3	1.7	0.3	0.9

Table 2. Mineral composition analysis of Horse milk and kumiss (mg/100 ml).

Sample and Province	Na	Ca	P	Fe	K	Mg	Zn	Cu	Mn
Horse milk									
Uburhangai Province '12	12	62	38	0.1	44	5	0.03	0	0
Bulgan Province '12	13	67	37	0.1	46	5	0.03	0	0
kumiss									
Bulgan Province '06 NO1	13	62	34	0.1	49	5	0.03	0	0
Bulgan Province '06 NO2	14	55	31	0.2	52	5	0.03	0	0.01
Arhangai Province '07 NO1	14	73	44	0.1	56	7	0.03	0	0
Arhangai Province '07 NO2	13	70	37	0.2	51	5	0.03	0	0.02
Tuv Province'08	14	88	49	0.1	67	0	0	0	0.02
Uburhangai Province '12 NO1	12	60	43	0.1	47	6	0.02	0	0.02
Arhangai Province '12 NO2	11	66	54	0.1	59	5	0.02	0	0.02
Arhangai Province '12 NO3	12	59	46	0.2	55	5	0.02	0	0.02
Boulgan Province '12 NO4	13	59	39	0.1	42	6	0.02	0	0.02
Bulgan Province '12 NO5	13	58	49	0.1	64	7	0.03	0	0.01

morphological observation, gram straining, and 16s RNA-based identification. Yeasts were isolated from the same samples as follows, referring to the method reported by Iizuka and Goto (1980). Colonies formed on YM agar medium, PDA and GAM ager medium were picked and subjected to repeated streak cultures on YM agar medium. Yeasts strains were isolated and subjected to morphological observation.

3. Results and Discussion

3.1. Horse milking and milk quality

Horses' milking was performed from the end of July to early October. Milking was performed about 8 times a day during the summer season, and the volume was 4-5 L/day/animal. **Table 1** shows the chemical composition analysis of horse milk, and **Table 2** shows the results of mineral composition analysis. There were no differences among samples, but the characteristics of Mongolian horse milk cannot been evaluated by the values only.

3.2. Production method and fermentation container

In the evening, a one-day volume of raw milk was directly added to the fermentation container in which about 20% of Kumiss was left, and stirred with a rabble to prepare Kumiss. The number of stirs was 3,000 to 10,000 in consideration of the amount of milk, temperature, and fermentation state. This number was also closely involved in the quality and fermentation state of Kumiss. The temperature in the fermentation container was about 25°C. The fermentation containers have changed from traditional vessel named 'huhuul' (made from cow hide) to plastic containers.

3.3. Kumiss microbial isolates

The characteristic feature of Kumiss is the fermentation of raw milk, involving the processing of only horse milk. Processing may play an important role in retaining the active components of horse milk, such as antibodies, and have a marked influence on the effect of Kumiss. The number of microorganisms in Kumiss was 107-109 Lactic acid bacteria/ml and 106-107 yeast/ml, showing that Lactic acid bacteria accounted for a high ratio. The alcohol content was low: 1.2-2.4%, the pH was 3.7-4.0 and the acidity was 2.3-2.6%, which are advantageous for the storage of food (Wood and Hoge, 1985).

Table 1 shows the results of general component analysis. The solid content decreased in Kumiss from that in milk, but it did not vary among producing regions. Table 2 shows the contents of trace components. These are present in trace amounts, but ingestion of a large volume increases their intake. These components may play important roles in the adjustment of various physical functions. Amino acid analysis showed great variation in quantity (**Table 3**). Glutamic acid was among the dominant amino acids in Kumiss and horse milk, too. Amino acid composition of Kumiss varied not only between different locations, but also between seasons. Kumiss quality is supposedly related with the higher content of glutamic acid.

The main lactic acid bacterium involved in the fermentation of Kumiss was previously considered to be *L. bulgaricus*, but this information was from a textbook written by a German researcher in the 20th century (Rubinnsky, 1910). In this study, a total of 14 species of Lactic acid bacteria and 11 species of yeasts belonging to 4 and 5 genera were isolated, respectively (**Table 4**). The same species were also found during previous studies done from 2002 to 2012 in Mongolia (Ishii, 2013).

Based on these findings and reports from other researchers (Ishii, 1999, 2004, 2013), multiple species of Lactic acid bacteria and yeast are involved in the fermentation of Kumiss, and the species and their proportion varied among families. The types and constitution of microorganisms were characteristic to each family, suggesting the presence of a close

Table 3. Amino acid analysis of horse milk and kumiss (mg/100 ml).

Sample and area	Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Leu	Tyr	His	Phe	Lys	Trp	Arg	Pro	Ile
Horse milk																		
Uburhangai' Province '12	3.0	0	0	13	0	2.0	0	2.0	0	2.0	0	0	0	2.0	0	0	0	4.0
Bulgan Province '12	3.0	0	0	13	0	0	0	2.0	0	2.0	0	0	0	4.0	0	0	0	0
kumiss																		
Bulgan Province '06 NO1	21.0	33.4	55.1	13.2	15.1	71.6	3.1	45.6	25.4	96.7	7.8	0	43.3	65.5	7.2	0	45.3	50.4
Bulgan Province '06 NO2	4.3	5.3	9.9	21.5	3.0	18.4	0	7.1	4.8	24.5	4.5	0	11.7	10.3	1.5	4.9	10.5	7.2
Arhangai Province '07 NO1	6.4	12.3	7.4	26.7	4.0	17.0	3.0	7.6	7.0	29.3	7.0	0	20.0	8.8	1.5	2.2	15.4	0
Arhangai Province '07 NO2	1.8	1.7	2.0	6.5	1.7	7.4	0	0.8	1.0	5.5	1.2	0	2.6	2.7	0	3.6	13.2	0.6
Tuv Province '08	1.5	1.4	2.2	9.5	2.2	10.2	0	1.0	0.8	5.4	1.2	0	2.8	3.4	0	2.5	13.0	0.7
Uburhangai Province '12 NO1	2.0	2.0	12.0	18.0	5.0	16.0	0	3.0	1.0	9.0	4.0	3	4.0	3.0	0	6.0	29.0	3.0
Arhangai Province'12 NO2	4.0	4.0	3.0	28.0	4.0	16.0	0	4.0	2.0	11	0	0	5.0	3.0	0	0	10.0	0
Arhangai province12 NO3	1.0	1.0	2.0	5.0	3.0	9.0	0	2.0	1.0	5.0	0	0	3.0	2.0	0	0	22.0	0
Bulgan Province '12 NO4	2.0	1.0	2.0	13.0	2.0	7.0	0	2.0	2.0	9.0	4.0	0	3.0	3.0	0	4.0	12.0	0
Bulgan Province '12 NO5	4.0	4.0	6.0	16.0	4.0	15.0	0	5.0	2.0	15.0	5.0	3.0	5.0	8.0	0	0	11.0	5.0

Table 4. Microbial flora isolated from Mongolian kumiss.

Lactic acid bacteria		yeast	
Lactobacillus	*acidphilus*	*Kluyveromyces*	*marxianus var.marxianus*
Lactobacillus	*delbrueckii* subsp.*bulgaricus*	*Kluyveromyces*	*marxianus* var.*lactis*
Lactobacillus	*delbrueckii* subsp.*lactis*	*Kluyveromyces*	*mesenteroides*
Lactobacillus	*paracasei* subsp. *paracasei*	*Saccharomyces*	*cerevisiae*
Lactobacillus	*paracasei* subsp.*tolerans*	*Saccharomyces*	*florentinus*
Lactobacillus	*plantarum*	*Saccharomyces*	*fragilis*
Lactobacillus	*rhamnosus*	*Debaryomyces polymorphus*	
Lactobacillus	*lactis* subsp.*cremoris*	*Debaryomyces hansenii*	
Lactobacillus	*brevis*	*Candida kefyr*	
Lactobacillus	*helveticus*	*Candida tropicalis*	
Lactococcus	*lactis* subsp.*lactis*	*Torula delbrueckii*	
Streptococcus	*salivarius* subsp.*thermophilus*		
Pediococcus	*acidilactis*		
Leuconostoc	*oenos*		

symbiotic relationship. The optimal flora for fermentation has been selected by experience in each family. The isolated and identified strains showed a wide growth temperature range and grew even at lower than 25°C.

3.4. Ingestion and healing effect of Kumiss

In 2006 during the Bulgan Province survey, 35 of 40 men and 36 of 42 women said that they drank Kumiss every day (Ishii, 2013). Some people even said they drank as many as 20 L a day. The mean daily intakes of the 35 men and 36 women were 9.7 and 3 L, respectively. They drank a large volume of Mongolian milk tea before they started drinking Kumiss. Those who drank a large volume of Kumiss ate almost no food. Kumiss provided about 400 kcal/L, and it was rapidly excreted into urine.

The vitamin C level in Kumiss was 8-11 mg/100 ml. The daily vitamin C requirement of adults is supplied by 500 ml of Kumiss. Kumiss was a valuable source of vitamin C supply for herders who did not ingest vegetables or fruit. The blood vitamin C level was investigated in those who drank Kumiss in a large-scale study in 2002 (Ishii, 2004), and the intake and blood vitamin C level were positively correlated. The intake of dairy products was high in the traditional herder diets in summer, but ingestion of a large volume of Kumiss supplemented the traditional diets.

The effects of drinking Kumiss were described as: 'not getting a cold', 'good for the lungs', 'good for the stomach and intestine' and 'good for health' in many answers. They say that persons with 'fracture' and 'hypertension' must not drink it. Kumiss has played an important role in maintaining the health of nomads in steppes without a physician, and it is also a valuable source of water in dry regions (Takano and Yamamoto, 2002).

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