Original Paper

Properties of camel milk liquor (''shubat'') in the Republic of Kazakhstan

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Abstract

In the Republic of Kazakhstan, both one-humped (*Camelus dromedaries*) and two-humped (*C. bactrianus*) camels are occurring in pure or mixing herds. A beverage, named ''shubat'', is prepared by fermenting camel milk without prior thermal processing. The production procedure is the same as those for the other camel milk beverages named ''qaris'' and ''garris'' in African countries, Mongolian ''hoormog'', and also ''kumiss'' that is prepared from raw mare's milk. In this paper we analyzed 5 samples of Kazakhstan made ''shubat'' prepared from the milk of one-humped camel. The pH of the samples was 3.8–4.1, and the ethanol level varied from 0.6 to 2.8%. There was no significant difference in the chemical composition. The highest levels of amino acid, Ala and Pro contents were 7.8 and 12.3 mg/100 ml, respectively. Regarding trace components, the Zn content was highest in all samples. Sixty-five lactic acid bacterial strains isolated from the samples were identified as *Lactobacillus plantarum*, *L. paracasei subsp. paracasei*, *L. casei*, *L. helveticus*, and *Lactococcus lactis*. The identification of yeast is ongoing, and most isolates exhibit a lactose-fermenting property. Since interest in health products has recently been increasing in Kazakhstan, the benefits of ''shubat'' may be re-evaluated in the near future.

Key words: camel, fermented milk liquor, shubat, Republic of Kazakhstan

Introduction

Camels are important domestic animals that can eat prickly plants non-edible for sheep and goats, and can tolerate marked dehydration[1,2,3]. In addition to carrying loads, their milk, meat, and fur are efficiently used, supporting the lives of people living in arid areas. Reportedly, the number of humps on the back changed with the domestication process, and they are classified into *Camelus dromedarius* and *C. bactrianus*.

Ninety percent of camels maintained in the world are *C. dromedarius*. They have been widely maintained in Africa and through central Asia, including the Republic of Kazakhstan[4] (Fig. 1). The bactrian, *Camelus bactrianus* has a lesser distribution and occurs in Mongolia[5,6], China, Kazakhstan, and Tyva Republic of Russia. Kazakhstan, located in central Asia, is a specific region where both *C. dromedarius* and *C. bactrianus* have been maintained in mixed herds[7,8,9].

Camel milk has been reported to be rich in fat

Fig. 1 Map of central Asia.

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and have a specific flavor[10,11]. Currently, nearly 800,000 L is milked annually from camels in the world, and in some countries such Chad and Djibouti in Africa, camel milk is consumed more than cow milk. In Ethiopia, it is consumed as fermented milk because fermentation occurs at a normal temperature during prolonged transport due to the poor infrastructure.

There are milk liquors with a low alcohol percentage prepared by fermenting camel milk with lactic acid bacteria and yeasts such as ''qaris''[12] and ''garris''[13,14] in Africa and ''hoormog'' in Mongolia[15,16]. Kazakhstan has achieved marked economic growth by utilizing its abundant natural resources after democratization, but there are also people with nomadic style of life, that produce traditional beverages such as camel milk liquor that called ''shubat''. However, there only few reports were published on the technology and properties of the beverages prepared from dromedary and bactrian camel's milk[17,18,19]. As obesity rates have recently increased in Kazakhstan, traditional food has attracted renewed interest.

Material and Methods

1. Survey region and period

We surveyed the maintenance of camels, their milking, and preparation and usage of ''shubat'' in Almaty City and its suburbs, located in the southeast region of Kazakhstan in August 2010 and May 2011, and collected samples of camel milk in March 2013. The samples were collected from camel farms around Almaty that produce and sell ''shubat'' in Almaty Province and from ''shubat'' producing factories in Aral sea coast area.

2. Sample analysis and viable cell number

Two samples of nomads' homemade ''shubat'' (Nos. 1 and 2) and 3 samples (Nos. 3, 4, and 5) of products manufactured at plants (5 samples in total) were collected in Almaty city market in 2011. In Kazakhstan, the samples were stored at 5℃. After bringing them back to Japan, the pH and ethanol content of the samples were measured. The general composition[20] and mineral components and free amino acids were analyzed following the standard procedures.

For viable cell numbers, the samples were diluted with sterile physiological saline, pour plates were prepared with MRS agar medium (Oxoid) for lactic acid bacteria, and the colonies formed on the plates were counted. Antibiotic Kabicidin (Nihon Seiyaku, Ltd.) was added to the medium to inhibit yeast growth. The yeast cell number was similarly measured by preparing pour plates with Potato dextrose agar medium (Eiken), and the colonies formed were counted. Four plates were prepared at each dilution, and the mean viable cell number was counted.

3. Isolation and identification of lactic acid bacteria

The samples were serially 10-fold diluted with sterile saline, and inoculated into four different media, namely MRS agar medium (Oxoid), plate count agar with BCP medium (Nissui), Acetate agar medium[21] and GAM agar medium (Nissui). The colonies formed on the culture medium were subjected to the purification by further inocluation into MRS agar medium. Isolated strains were separated based on their morphology[21]. Presence or absence of growth has been checked at 15℃, and at 45℃ for the rods, and at 10℃, and at 45℃ for the cocci. Gram stain, catalase test, litmus milk test, nitrate reducing ability test, gas production from glucose, and D-type, L-type optical activity of lactic acid were tested by Utimura and Okada method[21]. Also, the homo- and hetero-fermentation was subjected to samples. For the sugar fermentation test the bacterial suspension was inoculated into LB agar medium (Nissui) and cultured for 72 hours at 32℃. Lactic acid bacteria were identified according to the Bergey's Manual of Systematic Bacteriology[22].

Results and Discussion

1. Type of camel and milking

Reportedly, in Kazakhstan, dromedary camels outnumbered bactrian camels. The camel milk is very popular in this country due to its medicinal and dietary properties.

Farms maintaining as many as 1,000 heads of *C. dromedarius* have recently increased in grasslands around cities, aiming to selling milk in city market.

Camels give birth once per 2 years, and the duration of pregnancy is 13 months, that is allowing milking for 2 consecutive years. Reportedly, the annual milking yield for *C. dromedarius* is 2,000 L and that for *C. bactrianus* is 700 L. The dromedary produces about 5.5 L of milk per day. The mean daily volume per camel is about 3–5 L in August, in camel farms observed. The highest yields are milked in September. Based on a report on the volume of camel milk[8], it may be necessary to consider the timing of surveys and milking volume.

It is said that dromedary camels are easy to handle, because of its smaller physique and milder temperament than that of bactrian ones. Also, higher milk yields were the reasons to own the dromedary in Kazakhstan.

''Shubat'' in Kazakhstan is mainly prepared from dromedary camel milk, but in Aral sea coast area the bactrian milk is used. The Aral sea coast area is known as a popular ''shubat'' -producing center.

In small-scale camel farms, a young camel is taken to its mother and fed a small amount of milk, followed by milking twice a day in the morning and evening during summer and fall. Milking is women's work in Mongolia, but in Arab people of Africa and Western Asia, it is performed by men[23].

Milking is performed by 2 persons, and the method is similar to that performed by Mongolian nomads[16]. In large-scale farms, milking is performed throughout the year using an automatic milker.

Regarding camel milk properties, farm workers reported that the total volume milked from dromedary is larger than that from bactrian, but the milk is thinner in the former. According to a report from Russian researchers, the milk composition is different, and the milk solid, lipid, protein, and albumin contents are higher in bactrian milk[7]. However, it is unclear whether camels raised in the same region were surveyed. It may also be necessary to consider the grass type ingested by camels and milking season with regard to the quality of milk.

2. ''Shubat'' preparation method

Camel breeders that own few animals, collect daily milk to an exclusive fermentation container (plastic or wooden container). The fermentation container contains residual ''shubat'' at an about 20% volume, the milk added is raw, not subjected to thermal processing. The milk is stirred using a wooden paddle. Stirring appears to be less frequent than that in the process of preparing the same volume of the Mongolian ''kumiss'', made from mare's milk. This fermentation method is characterized by the use of raw milk not subjected to heating and is identical to the methods used to produce other fermented milk liquors including ''qaris''[12] and ''garris''[13,14] in Africa, Mongolian ''hoormog''[15,16], and ''kumiss'' made from mare's milk in Kazakhstan and Mongolia. It is assumed that immunological components in milk are retained and not inactivated because no heating is added, and various metabolic products produced through fermentation by various microorganisms contribute to the health-promoting effect of ''shubat''. We are planning to investigate the immunity-promoting characteristics of ''shubat''.

The ''shubat'' manufacturing method at plants is the same as that in small-scale production. The products are sold by weight/volume in containers brought by purchasers. Some products are transported to city markets within 3 days.

3. Properties of ''shubat'' and isolates

The color of the 5 samples was bluish milky white with micro foaming. As shown in Table 1, the pH was 3.8–4.1, and the ethanol content was 0.6–2.8%. The ethanol component and carbon dioxide presence is flavor ''shubat''. The most markedly fermented sample (No. 3) was prepared at a plant, its ethanol level was 2.8% with lactic acid bacterial cell number varied from 4.9×10^7/ml to 7.2×10^8/ml and that of yeast cells was 1.5×10^6/ml–3.9×10^7/ml. So far, tenfold difference between lactic acid bacteria and yeasts was found.

Table 1 Properties of shubat.

Sample	pH	Ethanol (%)	Lactic acid bacteria	Yeast
Shubat No. 1	4.1	0.6	4.9×10^7/ml	1.5×10^6/ml
Shubat No. 2	3.9	1.5	1.2×10^8/ml	3.4×10^7/ml
Shubat No. 3	3.8	2.8	3.8×10^8/ml	2.7×10^7/ml
Shubat No. 4	3.8	1.6	3.6×10^8/ml	3.9×10^7/ml
Shubat No. 5	4.0	0.6	7.2×10^8/ml	8.3×10^6/ml

This is similar to those in other milk liquors, such as ''hoormog'' and ''kumiss'', in our previous reports[16,24], and the counts were similar to those in ''shubat'' reported by Rahman *et al*.[19]. The yeast cell counts were 2–order lower of that of lactic acid bacterial cell counts only in one sample (No. 5).

The results of chemical composition analysis are shown in Table 2. The lowest solid content was noted in No. 3, in which fermentation had progressed and the ethanol content was high. Compared to Mongolian ''hoormog'' prepared from bactrian milk[15,16], the protein and ash contents were low in all samples. We will investigate whether these variations are due to differences in grass ingested by camels or depends on species features of dromedary and bactrian, as it was reported by Russian researchers[7].

Table 3 shows the results of mineral composition analysis. The Zn content was high in all samples, and this was consistent with that in ''shubat'' originated from the Aral Sea coast area reported by Saitmuratova *et al*.[17,18]. Table 4 shows the analysis of the free amino acids. The total amino acid content was lower than that of Mongolian ''hoormog'' in all samples. Sample No. 4 had the most favorable smell among the 5 samples and the total amino acid content was the highest, the Ala and Pro contents were 7.8 and 12.3 mg/100 ml, respectively. These values are higher than those of Mongolian ''hoormog''[16].

The 65 lactic acid bacterial strains isolated were identified as *Lactobacillus plantarum, L. paracasei* subsp. *paracasei*, *L. casei*, and *L. helveticus and Lactococcus lactis*. Of the samples taken from nomads, 30 strains were isolated, including 28 strains belong to *Lactobacillus* spp. and 2 strains belong to *Lactococcus* sp. The factory originated samples revealed 35 strains of lactic acid bacteria. In total, *L. plantarum* is represented by 27 strains, *L. paracasei* subsp. *paracasei* by 18 strains, *L. casei* by 11 strains, *L. helveticus* by 7 strains and *Lactococcus lactis* by 2 strains, respectively (Table 5. shown). The latter species was found only in samples from nomadic families. These samples contained diverse microflora. Abundance of lactic acid bacterial strains may suggest that the fermentation is not carried out by the one strain alone; it is highly possible that more than one strain is involved in flavor formation and fermentation process. Of the strains isolated, *L. plantarum* and *L. paracasei* subsp. *paracasei* were most dominant. These two bacterial species are also were reported

Table 2 General composition of camel milk, shubats and hoormog.

Sample	Water	Solid	Protein	Fat	Ash	Insolubled nitorogen
	%	%	%	%	%	%
Camel milk*	85.3	14.7	5.2	3.4	0.7	5.4
Shubat No. 1**	88.6	11.4	3.1	3.9	0.8	3.6
Shubat No. 2**	89.5	10.5	2.8	3.6	0.7	3.4
Shubat No. 3**	93.2	6.8	2.5	3.2	0.8	0.3
Shubat No. 4**	92.4	7.6	2.1	2.9	0.7	1.9
Shubat No. 5**	89.2	10.8	2.6	3.8	0.8	3.6
Hoormog***	85.2	14.6	4.3	5.3	0.9	4.3

*); Camel milk (*Camelus doromedarius*) was brought from Kazakhstan in 2013.
**); Samples: No. 1～No. 5 were collected in 2011 in Kazakhstan.
***); The Hoormog (This milk was *Camelus bactrianus*) sample was brought from Mongolia in 2010.

Table 3 Mineral composition of camel milk, shubats and hoormog.

Sample	Na	K	Ca	Mg	P	Fe	Zn	Cu
	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
Camel milk 1*	36	130	160	15	100	0.1	2.1	0
Shubat No. 1**	46	140	100	8	80	0.1	0.5	0
Shubat No. 2**	51	170	110	8	86	0.1	0.4	0
Shubat No. 3**	53	170	120	8	100	0.1	0.5	0
Shubat No. 4**	48	150	110	8	97	0.1	0.5	0
Shubat No. 5**	51	160	110	9	87	0.1	0.4	0
Hoormog***	54	160	150	5	120	0.1	0.7	0

*); Camel milk (*Camelus dromedarius*) was brought from Kazakhstan in 2013.
**); Samples: No. 1～No. 5 were collected in 2011 in Kazakhstan.
***); The Hoormog (This milk was *Camelus bactrianus*) sample was brought from Mongolia in 2010.

Table 4 Contents of free amino acid in camel milk, shubats and hoormog.

Sample	Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Leu	Tyr	Phe	Lys	Trp	Arg	Pro	Ile	His
	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml	mg/ 100 ml
Camel milk*	1.0	0	0	15.0	0	1.0	0	5.0	0	0	0	1.0	0	0	0	0	1.0	0
Shubat No. 1**	0.7	0.3	0.5	2.8	1.0	5.4	0	2.2	0.8	2.3	0	1.3	0.5	0	0	8.9	0.9	0
Shubat No. 2**	0.8	0.3	0.7	1.9	0.9	5.1	0	2.5	1.1	3.5	0	1.4	0.4	0	0	6.9	1.1	0.1
Shubat No. 3**	0.5	0.5	0.6	1.4	0.8	4.8	0	0.7	0.5	1.1	0	0.5	0.7	0	0.2	5.1	0.4	0.1
Shubat No. 4**	0.4	0.2	0.7	1.8	1.0	7.8	0	3.2	1.2	3.8	0	2.1	0.6	0	0	12.3	1.2	0.1
Shubat No. 5**	0.4	0.2	0.3	1.1	0.5	1.9	0	0.1	0	0.4	0	0	0.4	0	0	6.7	0.1	0
Hoormog***	2.8	1.4	2.9	5.5	0.5	3.9	0.2	3.5	2.2	6.9	3.1	2.1	4.2	0	0.4	11.3	2.1	0

*); Camel milk (*Camelus dromedarius*) was brought from Kazakhstan in 2013.
**); Samples: No. 1～No. 5 were collected in 2011 in Kazakhstan.
***); The Hoormog (This milk was *Camelus bactrianus*) sample was brought from Mongolia in 2010.

Table 5 Morphological and physiological characteristics of lactic acid bacteria isolated from shubat.

Characteristics	Nomad's home made shubat				Factory made shubat			
	L. plantarum	*L. paracasei.* subsp. *paracasei*	*L. casei*	*Lc. lactis*	*L. plantarum*	*L. paracasei.* subsp. *paracasei*	*L. casei*	*L. helveticus*
	(20 strains)	(8 strains)	(6 strains)	(2 strains)	(7 strains)	(10 strains)	(5 strains)	(7 strains)
Morphology	rods	rods	rods	cocci	rods	rods	rods	rods
Gram staining	+	+	+	+	+	+	+	+
Configuration of lactic acid bacteria	DL	L	L	L	DL	L	L	L
Growth at 10℃				+				
Growth at 15℃	+	+	+		+	+	+	−
Growth at 45℃	−	±	−	−	−	±	−	+
Gas from glucose	−	−	−	−	−	−	−	−
Acid from								
L-Arabinose	−	−	−	+	−	−	−	+
D-Xylose	+	−	−	+	+	−	−	+
Rhamnose	+	+	+	+	+	+	+	+
Sorbose	+	+	+	+	+	+	+	+
D-Ribose	+	−	+	+	+	−	+	+
Glucose	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	±
Fructose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	−
Maltose	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	−
Lactose	+	+	+	+	+	+	+	+
Treharose	+	+	+	+	+	+	+	±
Melibiose	+	−	−	−	+	−	−	+
Raffinose	+	+	−	−	+	+	−	−
Melezitose	−	+	+	−	−	+	+	±
Mannitol	+	+	+	+	+	+	+	−
Esculin	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	−
Amygdalin	+	+	+	+	+	+	+	−

from the fermented milk in Mongolia[24], which is similar in arid conditions with Kazakhstan. So far, above bacteria are might be the dominant bacteria in the fermentation of ''shubat''. The *L. helveticus* is reported by Watabe *et al.*[25] from the fermented milk in Inner Mongolia in China. The latter bacter-

ia were only separated from the Aral Sea coast area products (manufactured shubat sample) and might be used as the fermentation starter. Finding of *Lactococcus lactis* is possibly due to the freshness of the sample and short time to sample processing. Regarding ''garris'' in Sudan, Sulieman *et al*[14] reported that *Lactobacillus* spp. and *Lactococcus* spp. accounted for 74 and 12% of isolates in 20 samples, respectively, and *L. plantarum* was the most frequent isolate.

Our findings suggest that several lactic acid bacterial species are involved in the fermentation of ''shubat''. The bacterial strains *L. casei* and *L. helveticus* are frequently isolated from milk products and liquors produced by nomads[25,26,27]. *L. plantarum* is closely involved in the production of traditional fermented food such as Japanese Funazushi[28]. The involvement of several lactic acid bacteria in the fermentation of milk liquors produced by nomads has been previously reported[29]. The isolation of *L. plantarum* from Mongolian ''kumiss'' has recently been reported[30], suggesting that *L. plantarum* is a bacterial species playing an important role in milk liquor production in grasslands.

We isolated two strains of *Lc. lactis*, which is rarely found in fermented milk products. The *cocci* cannot be readily isolated unless the sample conditions are favorable, indicating that the samples were analyzed under favorable conditions.

The identification of yeasts is ongoing, it is characteristic that all 43 pure-cultured strains exhibited lactose-fermenting ability, and many strains produced ethanol in culture with glucose as a carbon source.

4. Drinking and effect of ''shubat''

''Kumiss'' and ''shubat'' are served at banquets in summer for middle-aged and older Kazakhstan people even nowadays, but only a small volume of ''shubat'' is consumed. Many young city residents said that they have never drank ''shubat'', showing that recognition of ''shubat'' varies among generations, in which the settlement policy strongly promoted by the government for nomads who have passed on the tradition of ''shubat'', and lifestyle changes in the 20th century, such as economic liberalization after democratization in 1991, may be closely involved. According to manufacturers, about 500 and 200 ml per day of ''shubat'' is consumed by adult males and females, respectively. These are smaller volumes than those of ''hoormog'' based on Mongolian manufacturers[16]. Diverse food products are available in Kazakhstan compared to Mongolia, and this may also affect consumption. To the question: 'what is the tastiest ''shubat'' ?', the answers were: 'one in which fermentation has not excessively progressed', 'one prepared in summer', and 'one prepared in the Aral Sea coast area'. In a survey on the effect of drinking ''shubat'', all subjects answered that it is 'good for health'. In a survey on the effect of drinking ''hoormog'' in the Gobi Province of Mongolia, in addition to 'good for health', clear effects were stated, such as 'good for visceral disease', 'good for people with a weak stomach and intestines', 'effective to improve the constitution with a diarrheal tendency', 'resolves leg edema', and has a 'diuretic effect'[16], suggesting that interest in ''shubat'' was not so high until recently in Kazakhstan. As interest in health has recently increased, the value of traditional beverage ''shubat'' containing abundant trace components, such as Zn, may be re-evaluated in the near future.

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カザフスタン共和国のラクダ乳酒(Shubat)の性質

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カザフスタンではヒトコブラクダとフタコブラクダが飼われ，その乳を加熱せずに発酵させ shubat をつくってきた。製法はアフリカ諸国の qaris, garris と同じだった。5 つの試料は全てヒトコブラクダの乳が用いられ，乳酸菌数は 4.9×10^7/ml から7.2×10^8/ml，酵母菌数は1.5×10^6/ml から3.9×10^7/ml，pH は3.8～4.1，エタノール含量は0.6～2.8％だった。一般成分分析値に差はなく微量成分は亜鉛が多かった。遊離アミノ酸量は試料 No. 4 でアラニンが7.8 mg/100 ml，プロリンが12.3 mg/100 ml と多かった。分離した65菌株の乳酸菌を，*Lactobacillus plantarum*，*L. paracasei* subsp. *paracasei*, *L. casei*, *L. helveticus*, *Lactococcus lactis* と同定した。Shubat の飲用が「健康に良い」と再評価が起きていた。