

# Fermented mare milk product (Qymyz, Koumiss)

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### 24 ABSTRACT

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Mare milk has been consumed for thousands of years in Central Asia and neighboring regions. 26 Its composition is similar to human milk, but different from cow's milk. Fermentation with 27 lactic acid bacteria and yeasts leads to the production of a traditional drink called *qymyz*. *Qymyz*, 28 which is a symbol of good health, is a central part of the national cuisine and of the traditional 29 30 cycle of horse husbandry, as well as playing a role in the cultural identity of Central Asian people. Mare milk, along with qymyz, has been reported to have a wide range of therapeutic 31 effects. In this article, the authors have compiled information on mare milk composition, qymyz 32 production and the therapeutic effects of mare milk and its products. 33 **Keywords:** horse, mare milk, qymyz, koumiss, health 34 35

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#### 58 **1. Introduction**

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Despite the widespread use of cow's milk throughout the world, it is necessary to bear 60 in mind the possibility of providing the population with milk from other farm animals. Of the 61 702.1 billion tons of milk produced globally, 118.7 (16.9%) billion tons are acquired from 62 species other than dairy cattle. This proportion has been increasing regularly (B. Faye & 63 64 Konuspayeva, 2012). Mare milk has been consumed for thousands of years in Central Asia and neighboring regions. Herodotus in the 5th century BC described how Scythians used to 65 consume it (West, 1999). According to Outram et al. (2009) the first domestication of horses 66 67 and consumption of mare milk took place in present day Akmola province of Kazakhstan approximately in 3500 BCE. Nowadays horses are mainly used as dairy animals in former 68 USSR countries, such as Mongolia, and parts of China (Doreau & Boulot, 1989). 69

Due to its composition (proteins, lipids, vitamins, carbohydrates, macro and microelements, enzymes, hormones and a number of other important substances), raw horse milk has high nutritional value and digestibility. However, in Central Asian countries it is more widely consumed fermented under the name of *qymyz*. Fermented mare milk is phonetically pronounced as *qymyz*, but due to Russian domination in the region, the international literature term for fermented mare milk became better known as *koumiss* or *kumis*. It is an acidic and slightly alcoholic drink, the fermentation of which involves lactic bacteria and yeasts.

Many studies were carried out in  $20^{\text{th}}$  century in the USSR in Russian languages which are not accessible for the international community. This current review focuses mainly on data published by soviet authors which will help to understand the importance of mare milk and *qymyz* for human consumption.

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#### 82 2. Horse numbers, economic figures, market

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According to the Food and Agriculture Organization of the United nations (FAO), in 84 2017 there were about 60 million horses worldwide (Figure 2). 85

In most of the world except Central Asia, horses are not traditionally used as dairy 86 animals. (Salimei & Park, 2017). In Kazakhstan horses are used as dairy animals and on top of 87 that horse meat is consumed. About 30% of horses are slaughtered for meat annually in 88 Kazakhstan and as a result, Kazakhstan has become the second largest horse meat producer in 89 the world behind China (Bernard Faye, 2017). This has led to the steady growth in the number 90 of horses in Kazakhstan. In fact, the number of horses has more than doubled since 2007 from 91 92 1.16 million to 2.85 million (Figure 3).

In Russia, on the other hand, a decrease of 15% (from 1.61 million to 1.37 million) 93 between 2001-2014 was reported (Knyazev & Timchenko, 2016). However, in regions with 94 95 developed horse meat production such as Yakutia, Bashkortstan, Altai, etc. the number of horses has increased by 22% between 2008-2010 (Kalashnikov & Kalashnikov, 2011). In 96 Kyrgyzstan, an increase of 50% (from 347 thousand to 450 thousand) between 2006-2019 is 97 reported. In Mongolia during the period 2009-2019 the number of horses increased from 2,2 98 million to 4,2 million. Uzbekistan has also seen an increase in horse numbers from 168 thousand 99 100 to 242 thousand during the period 2008-2018. One of the other reasons for the steady increase in the number of horses is the low cost of horse breeding. Horses are bred in herds and feed on 101 pasture during winter and summer, which means they do not rely on fodder. 102

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#### 3. Mare Milk 104

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*3.1. Differences and similarities with other types of animal milk* 106

108 The comparison of the composition of mare milk with other types of milk cited by 109 various references show important differences (Table 2). The reported values are relatively 110 variable, which can be explained by different geographies, genetics and methods of analysis.

111 When comparing the content of sugar, protein and mineral salts, mare milk is quite112 similar to human milk but differs significantly from cow's milk

113 Milk protein from all animals is divided into two major groups: caseins ( $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ -,  $\kappa$ -114 and  $\gamma$  -caseins) and whey proteins, which include  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, serum 115 albumin, lactoferrin, immunoglobulins and lysozyme (Medhammar et al., 2012).

The protein in cow's milk is dominated by casein, which makes up about 72-77% of all nitrogen, while whey proteins account for only 13-18% of nitrogen, which is around 6 g/kg. This means cow's milk is defined as caseinic milk as it is rich in casein. Mare milk and human milk on the other hand are rich in whey protein containing 8.3 and 7 g/kg respectively, which is around 36 and 53% of all nitrogen respectively. Consecutively, mare milk and human milk are considered to be albumin-types of milk –high in albumin content (Malacarne et al., 2002; Uniacke-Lowe et al., 2010).

Mare milk casein mainly consists of β-casein (78.5%) while the  $\alpha_s$ -caseins and  $\kappa$ -casein are present at 20% and 1.8%, respectively. Whey proteins in mare milk are present in the following proportions: β-lactoglobulin, 30.7%, α-lactalbumin, 28.5%, serum albumin-4.4%, immunoglobulins- 19.6%, lactoferrin – 7%, lysozyme 10,5% (Salimei & Fantuz, 2013). The percentages differ in other studies, for example Duisembaev (1989) reported that β-casein and  $\kappa$ -casein concentrations are similar and amount to 39.9 and 46.0% respectively, while that of  $\alpha_s$ -casein is equal to 7.2%.

Non-protein nitrogen (NPN) include urea, free amino acids, amino sugars, bioactive
peptides, uric acid, creatine, creatinine, nucleic acids, and nucleotide. Free amino acid content

of mare milk (1900 μm/kg) is higher than in cow's milk (560 μm/kg), but lower than in human
milk (2930 μm/kg) (Salimei & Park, 2017).

Mare milk is poor in fat. In fact, according to Jennes (1988) mare milk, along with 134 donkey milk, contains the least amount of fat among milk types regularly consumed by humans. 135 As a result, horse milk has a lower calorific value (52 kcal/100g) than human (72 kcal/100g) or 136 cow's (66 kcal/100g) milk. The lipids in milk are organized in globules, that have different 137 138 sizes in different species. The lower the amount of fat in milk the smaller the diameter of the globules. Thus, mare milk has lipid globules with a small diameter. The diameter of mare milk 139 globules ranges from 2-3 µm, while human and cow milk globules have an average diameter of 140 141 3-4 µm. (Gantner et al., 2015). Mare milk globules are similar to human milk globules in having branched oligosaccharide structures on the surface, which may enhance digestion by binding to 142 lipases. Concerning lipid classes, mare milk is different from both human and cow's milk, in 143 144 that it has 80% triglycerides, 9 - 10% free fatty acids and 5 - 19% phospholipids. Human and cow milk fat consists almost entirely of triglycerides (97-98%) (Malacarne et al., 2002; Miraglia 145 146 & Martin-Rosset, 2006). In terms of free fatty acids mare milk, like human milk, has higher 147 amounts of unsaturated fatty acids and a lower amount of saturated fatty acids compared to cow's milk (Claeys et al., 2014; Salimei & Fantuz, 2013; Sinyavskii, Yakunin, et al., 2016). 148 From a dietetic point of view mare milk has an index of atherogenicity of 0.51 and 149 thrombogenicity of 0.99, which are significantly lower than in cow's milk (2.87 and 3.53 150 respectively) (Sinyavskii, Yakunin, et al., 2016). The lower the atherogenic index the less likely 151 lipids will adhere to cells of the immunological and circulatory system, and thus prevent 152 atherosclerosis. The thrombogenicity index shows tendency to form clots in the blood vessels. 153

154 Carbohydrates in mare milk mainly consist of lactose and trace amounts of 155 monosaccharides and oligosaccharides. Lactose content in mare milk ( $6-7g \cdot 100 \text{ ml}^{-1}$ ) is similar 156 to that in human milk and significantly higher than in cow's milk ( $4-5g \cdot 100 \text{ ml}^{-1}$ ). Among monosaccharides, glucose was reported to change during the lactation period and range between 50 -150 mg·L<sup>-1</sup> (Uniacke-Lowe & Fox, 2012). According to Seitov (2005) colostrum has a lower amount of carbohydrates than milk, which equals 2.9-3.4%. The amount increases rapidly and a day after foaling the amount equals 5%. The maximum amount of carbohydrates is present 1-3 months after foaling and ranges between 6.4-7%. Oligosaccharide concentration was reported to range between 79.8 - 217.8 mg·L<sup>-1</sup> and includes 58.3% neutral, 33.3% Neu5Ac, 6.25% fucosylated and 2.1% NeuGc oligosaccharides (Karav et al., 2018).

Minerals and trace elements play a major role in nutrition and aging of foals. Among 164 major minerals (Ca, P, K, Na, Mg) Ca (0.83 g·kg<sup>-1</sup>) and P (0.61 g·kg<sup>-1</sup>) have the highest 165 concentrations in mare milk, while Mg (0.06  $g \cdot kg^{-1}$ ) has the lowest concentration (Park et al., 166 2017; Seitov et al., 2005). All five minerals have lower concentrations in human milk and higher 167 concentrations in cow's milk (Salimei & Fantuz, 2013). Among trace elements (Zn, Fe, Cu) 168 169 Zn has the highest concentration in any milk. Mare milk when compared to human and cow's milk has a lower concentration of Zn (0.02 mg·kg<sup>-1</sup>). Fe and Cu have 0.01 and 0.005 mg/kg 170 171 concentration in mare milk respectively (Medhammar et al., 2012). Similar concentrations of minerals were found in Mongolian mare milk (Ishii et al., 2014) 172

Data on vitamin content of mare milk is variable. Some authors note lower concentrations of fat-soluble vitamins in mare milk due to a low amount of milk fat (Salimei & Fantuz, 2013). Others report similar content in mare and cow milk (Fuquay et al., 2011). Both works agree on higher content of vitamin C in mare milk (150 mg·L<sup>-1</sup>). The works of Berlin and Gricenko as reported by Seitov (2005) found concentrations of vitamins  $B_1 - 291-390$ ;  $B_2$ - 261-373; PP - 229; B<sub>5</sub>- 1547; B<sub>7</sub> (biotin) -10.2; B<sub>9</sub> (folate) - 263; B<sub>12</sub>- 2,5-3.3 µg·L<sup>-1</sup> in mare milk.

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#### 181 *3.2. Horse breed effect on mare milk composition*

The effect of breed on milk composition is hard to differentiate from the effect of a 183 certain feeding regimen or herd conditions. Additionally, individual variability of mares 184 complicates the assessment. The stage of lactation plays a major role in the composition of mare 185 milk. Authors note a higher content of fat, cholesterol, protein, total solids and citric acid, and 186 a lower content of lactose and vitamin C in colostrum compared to milk (Coenen et al., 2011; 187 Markiewicz-Kęszycka et al., 2015). Mare milking starts 20-45 days after foaling (Salimei & 188 Park, 2017). The table below shows differences in milk composition and production between 189 various breeds from post-Soviet countries. 190

191 Milk production in mares is even throughout the day, so most authors use the following formula proposed by Saigin to calculate daily milk production: 192

$$Us = (Uf * 24)/T$$

193

where Us- daily milk production (kg/day); Uf- amount of milk obtained during milking 194 (kg); *T*- duration of milking. 195

According to Doreau and Boulot (1989) the yield of milk increases until the age of 11-196 15 years, but the age effect on milk composition is low. Milk yield differences according to 197 198 breed is dependent on adult mare liveweight; 2.5 to 3.35 kg of milk per 100 kg of body weight (Salimei & Park, 2017). Duisembaev (1989) found that Kazakh zhabe type mares producing 199 milk with a higher fat content produce less overall milk per day. Subsequently, milk 200 201 productivity can be attributed to the characteristics of individual mares. According to reviews on the effect of nutrition of the mare, the energy supply of the diet above requirements has a 202 negative effect on protein content in milk and the amount of milk produced can be increased by 203 increasing the level of energy fed (Martuzzi & Doreau, 2006). 204

206 **4. Qymyz** 

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Qymyz is a traditional beverage of nomadic people in central Asia, which is obtained by 208 209 fermenting mare's milk with lactic acid bacteria and yeasts. Mare milk undergoes two-step fermentation - lactic fermentation and alcoholic fermentation. The final products of these 210 211 fermentations are lactic acid, ethyl alcohol, carbon dioxide and other by-products such as 212 volatile acids, fusel alcohols, and other compounds with a strong and distinctive aroma and taste (Fuquay et al., 2011; Kondybayev et al., 2018). In 1858 N.V. Postnikov, a Russian doctor, 213 discovered the effectiveness of the use of sour mare's milk in the treatment of patients with 214 215 pulmonary tuberculosis and decided to introduce the use of *qymyz* into medical practice by opening a 'qymyz' hospital near Samara, Russia. From this moment, hospitals became very 216 217 popular and widespread in the USSR (Kudayarova et al., 2010).

There are two types of *qymyz*: i) traditional *qymyz*, which is prepared using starters containing unspecified microorganisms and ii) *qymyz* prepared with a mixture of pure cultures.

221 4.1. Traditional qymyz

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223 In traditional processing, mare milk is inoculated using substances such as boiled millet, malt, soured cow's milk, soured soup, smoked horse meat, etc. Shigaeva and Ospanova (1983) 224 described several types of traditional qymyz starters (Figure 4) and classified them into 3 main 225 groups: a) spontaneous fermentation caused by microorganisms in fresh mare milk; b) 226 227 fermentation caused by microorganisms from fermented milk products or sour dough; c) mixed combination of both types of starters. After inoculation mare milk is churned for 20-30 minutes 228 229 and left to ferment at room temperature. During the day, fresh mare milk may be added several times depending on the number of milkings, which is around 6 milkings per day. The process 230

of churning and fermentation is repeated each time fresh mare milk is added. The purpose of adding milk and churning it is to increase *qymyz* volume and to increase acid and ethanol production, giving it a stronger taste.

Qymyz is churned in special containers called saba (made from horsehide) or kubi (made 234 from wood). The purpose of churning is to spread the starter in the milk and to provide aeration 235 236 for the yeasts. In times prior to the creation of the Soviet Union, leather containers for *gymyz*. were very convenient for nomads, as they were attached in a special way to the saddle, and 237 during a long trip, due to constant shaking from horse movement, *qymyz* acquired its true taste 238 and strength. The wooden container, Kubi, has a cylindrical shape, tapering upwards, with a 239 240 diameter of 20-40 cm and a height of about a meter, closed with a lid with a hole in the center. A plunger (a pole with a cross at the end or a disc with holes) is inserted into the hole and the 241 *qymyz* is churned. 242

243 Microbiota of traditional *qymyz* are not well defined. The study of Bashkirian *qymyz* found bacteria, A. aceti, L. bulgaricum and S. thermophilus and yeasts, Saccharomyces lactis 244 245 (previous name of Kluyveromyces lactis) and Saccharomyces cartilaginosus, along with yeast 246 belonging to the genus mycoderma (Gricenko, 1964). A study conducted on qymyz from Mongolia found bacteria such as L. helveticus, L. kefiranofaciens, L. lactis, L. mesenteroides 247 248 and yeasts, *Kluyveromyces marxianus*. Authors found that the most abundant bacteria in *gymyz*. from Mongolia was L helveticus and concluded that the final bacterial composition of qymyz is 249 related to the high acid stress tolerance of lactobacilli (Miyamoto et al., 2010). Similarly in the 250 Xin Jiang region of China the most abundant bacteria in *qymyz* was found to be *L. helveticus*, 251 while L. casei and L. plantarum were prevalent in Inner Mongolia and the Qing Hai regions 252 respectively (Sun et al., 2010). Yeast composition of *qymyz* in China showed the most dominant 253 yeast species to be K. marxianus, K. unispora and S. cerevisiae, but their frequencies differed 254 in different regions resulting in a distinct aroma and taste of *qymyz* belonging to these regions. 255

There was no study on correlation between yeast species and flavor of *qymyz* (Mu et al., 2012). 256 In Kazakhstani qymyz bacteria such as L. bulgaricus, L. casei, Str. lactis and yeasts Torulopsis 257 kefyr and Torulopsis sphaerica can be found (Shigaeva & Ospanova, 1983). According to 258 259 Montanari et al. (1996), the most abundant yeast species in Kazakhstani qymyz is Saccharomyces unisporus (Kazakhstania unispora), which does not ferment lactose but can 260 261 ferment galactose. Krekker (2001) confirmed that coculture of lactic acid bacteria (LAB) and 262 yeasts naturally leads to domination of non-lactose fermenting yeast over other types of yeasts. This means that Kazakhstani qymyz has a 2-step fermentation where lactose is first hydrolyzed 263 by LAB that metabolize glucose but secrete the remaining galactose to the environment where 264 265 it is consumed by yeasts. Among LAB that have this type of metabolism are L. bulgaricus, L. lactis, L. acidophilus and S. thermophiles (Gadaga, 2001; Hickey et al., 1986). 266

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#### 268 4.2. Qymyz making with pure cultures

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270 The first studies of qymyz production using pure cultures, carried out by Ginzburg and 271 Bachinski-Raichenko (Seitov et al., 2005), date back to 1910. However, the guidelines to be followed for producing qymyz were defined and published in 1969 through Inter-Republican 272 Technical Specifications (MRTU 46-573-69), which were approved by the Ministry of 273 Agriculture and the Ministry of Meat and Dairy Industry of the USSR (Figure 5). According to 274 this standard method there are 2 steps in the preparation of *qymyz*. First the laboratory starter is 275 prepared with a mixed culture of L. bulgaricus and Saccharomyces lactis, which ferment lactose 276 and have antibacterial properties (Figure 6). According to the standard, mare milk didn't have 277 to be pasteurized, but it had to have acidity  $\leq 0.06\%$  w/v lactic acid, a density of 1,029-1,033 278 g/cm<sup>3</sup> and fat content of at least 1% w/v. Second, the bulk starter is prepared on the basis of 279 the obtained laboratory starter over four to five days at 26-28 °C. *Qymyz* is obtained by using a 280

bulk starter and adding fresh mare milk 2-3 times a day until it loses its activity. An indicator
of good activity is the formation of bubbles (foam) when churned, detection of at least 8-10
yeast cells and abundance of LAB in the field of view of a microscope (Seitov et al., 2005;
Shigaeva & Ospanova, 1983).

In present times, most *qymyz* hospitals use *qymyz* from the previous year as a starter. The scheme of *qymyz* production in hospitals can be seen from Figure 7 (Mursalimov & Satyev, 1989; O. N. Popova, 1996). There is a possibility that due to the standardization of *qymyz* production in the USSR all hospitals had to use MRTU 46-573-69 in their production, but later on hospitals started using old *qymyz* as a starter.

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291 *4.3. Qymyz quality* 

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Three categories of *qymyz* obtained from pure culture were defined according to their sensory quality in National standard of the Republic of Kazakhstan - ST RK 1004-98 (Kazakh Research and Development Institute of Meat and Dairy Industry of the Ministry of Agriculture of the Republic of Kazakhstan., 1999): weak – one day fermentation, medium – two-day fermentation, and strong – three-day fermentation (Table 4).

A study conducted on people's preferences of *qymyz* resulted in the following sensory characteristics being ranked from 'liked most' to 'liked least': taste, freshness, acidity, color, smell. The results show that aroma of *qymyz* is the least significant for consumers. The same study showed that a higher proportion of consumers preferred strong *qymyz* (30%) when compared to weak (12%) and medium (5%) *qymyz*. 54% of responders couldn't perceive the different *qymyz* types (Real, 2019).

The content of *qymyz* protein or fat remained relatively the same as in the mare milk used for the preparation, but amino acid composition changed: the essential amino acids, lysine,

valine, isoleucine, phenylalanine and tyrosine decreased; methionine, cysteine and leucine 306 307 didn't change, but tryptophan increased; the nonessential amino acids, histidine, aspartic acid, glutamic acid and proline increased; alanine, serine and arginine decreased (Lebedev, 1963; 308 309 Urbisinov, 1992). According to Damdinsurengiin (2000) concentrations of all amino acids decreased, except for threonine and alanine. Gafiatullin (2006) reported an overall 11% 310 decrease in protein; subsequently casein and whey concentrations also decreased by 11.2% and 311 312 10.7%. Weak *gymyz* usually still contains a high amount of lactose and as a result has a slightly sweet taste. Overall *qymyz* has a sour taste and slightly spicy flavor, without any extraneous 313 flavors or odors. A yeasty flavor is acceptable in qymyz. The consistency of qymyz is liquid, 314 315 homogeneous, carbonated, slightly foaming, without any particles of fat.

- 316
- 317 4.4. Qymyz process modifications
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Most of the studies on controlled preparation of *gymyz* proposed methods similar to the 319 320 MRTU 46-573-69 standard but with a number of changes; i) pasteurization of mare milk, ii) 321 fermentation with different or additional species of microorganisms, iii) preparation of qymyz with cow's milk, iv) extending storage of mare milk and qymyz using different methods. Mare 322 milk pasteurization has been a topic of a number of studies. In general heating mare milk above 323 65 °C precipitates milk whey protein and albumin, oxidizes polyunsaturated fatty acids and 324 vitamin C, and changes the taste of the milk. Due to these reasons and the relative resistance of 325 horses to such diseases as brucellosis and tuberculosis, the use of unpasteurized mare milk for 326 qymyz production is assumed to be safe (Rahimov et al., 2018; Seitov et al., 2005). 327 Pasteurization of mare milk would be essential when it comes to producing *qymyz* made from 328 pure strains, because unpasteurized mare's milk can contain a variety of microorganisms 329 (Skorodumova, 1961). Shigaeva and Ospanova (1983) proposed the pasteurization of mare 330

milk at 65°C for 15 min and reported no difference in taste, color or consistency. Authors reported a slower fermentation rate in pasteurized milk as the utilization of lactose and the production of ethanol reached maximal rates after 7-14 days, while in unpasteurized milk 3-5 days was enough to get the same results. Kanareikina and Kanareikin (2017) compared timetemperature combinations for pasteurization ranging from 63-94°C and found that 30-min pasteurization at temperatures of 63, 65 and 67 °C resulted in the same color and consistency, while the taste and aroma was close to raw mare milk.

The use of different strains of microorganisms in starter conditions improves not just 338 the taste and aroma of qymyz, but also its shelf-life and probiotic properties. The main difficulty 339 340 during *qymyz* making is the control of the kinetics of acidification. Generally, in *qymyz* hospitals the maximum tolerated level of acidity of *qymyz* is reached in 72 hours. Beyond this, *qymyz* is 341 considered unsuitable for consumption, which has the consequence of limiting the shelf-life of 342 343 the qymyz. The use of selected microbial strains capable of producing less lactic acid and a slower fermentation rate appeared to be a means of extending the shelf-life of qymyz as shown 344 by (Shigaeva & Ospanova, 1983) with strains of S. lactis and Lb. bulgaricus which led to the 345 maximum formation of lactic acid of 1% and 2.25% respectively. 346

347 Yeasts in *qymyz* have been shown to grow better in more acidic environments (pH 4-5) 348 (Hrisanova, 1966). Indeed separate coculture of Torulopsis kefyr var kumis with S. lactis and Lb. bulgaricus leads to better growth with Lb. bulgaricus, that has a higher production of lactic 349 acid (Shigaeva & Ospanova, 1983). In contrast, yeast such as Saccharomyces lactis has an 350 optimum pH for lactose hydrolysis at 6.5 (Guy & Bingham, 1978). According to Fox et al. 351 (2015), usage of LAB with lactose-fermenting yeast is optimal in *qymyz* fermentation as lactic 352 acid formation and alcoholic fermentations occur simultaneously and the products of 353 fermentation are in definite proportions. However simultaneous fermentation leads to 354

competition between LAB and lactose-fermenting yeasts and results in a lower number ofyeasts and thus a lower amount of ethanol (Krekker, 2001).

Several authors have used acetate forming bacteria such as Acetobacter aceti (Gricenko, 357 1964; Shigaeva & Ospanova, 1983; Urbisinov, 1992). The features of A. aceti include uptake 358 of lactic acid and ethanol. Gricenko (1964) reported production of vitamin B<sub>12</sub> by A.aceti. 359 360 Shigaeva and Ospanova (1983) reported production of diacetyl, that gives dairy products a 361 buttery flavor, by A. aceti. In their work, addition of A. aceti to the usual starter consisting of LAB and yeasts leads to a decrease in ethanol concentration and an increase in lactic acid 362 concentration in the final composition of qymyz. Thus, they concluded that in the mixture of 363 364 LAB and ethanol producing yeasts, A. aceti prefers to consume ethanol rather than lactic acid and overall recommended the inclusion of A. aceti in starters as the aromatic quality of qymyz 365 366 increased. However, a decrease in ethanol could be due to the inhibition of yeast growth as 367 Gricenko (1964) reported a slower growth rate of lactose fermenting yeast and the total suppression of yeast that don't ferment lactose in the presence of A. aceti. Overall interaction 368 between microorganisms in qymyz is summarized in Figure 8. In general bacteria are 369 370 responsible for production of acids, while yeast is responsible for ethanol production. Acids lower the pH, which has a positive effect on yeast growth. LAB and lactose (+) yeasts compete 371 for lactose, which has a negative effect on growth of lactose (+) yeasts. Lactose (-) yeasts grow 372 on galactose produced by LAB. Acetic acid bacteria (AAB) inhibit growth of lactose (+) yeast 373 and suppress growth of lactose (-) yeasts, and thus inhibit production of ethanol in *qymyz*. 374 Aroma is mostly produced by yeasts and AAB. 375

The cost and availability of mare milk made authors seek ways to prepare *qymyz* from cow's milk. Hrisanova (1966) simply decreased pH of cow's milk to 5.2 and added *qymyz* starter cultures. The resulting cow milk *qymyz* had the same antagonistic effect against *M*. *tuberculosis* (H37Rv strain) as *qymyz* from mare milk. Shamgin et al. (1977) proposed using a mixture of whole milk (33-36%), skimmed milk (0.6-1%) and whey (63-67%), which results in 1:1 ratio of casein to whey, similar to mare milk. Kücükcetin et al. (2003) used ultrafiltration, microfiltration and nanofiltration to modify cow's milk so that the final composition was equal to mare milk in terms of protein, lactose and other compositional factors. Prepared *qymyz* had no significant compositional or sensorial difference from mare milk *qymyz*.

Extending mare milk storage is mainly done by spray drying. Before drying, mare milk 385 386 is thickened in a vacuum. Reconstituted milk had a lower amount of vitamins C and A, 5-6% less protein, lactose and fat (Mursalimov & Satyev, 1989). Qymyz lyophilization and 387 reconstitution after 12 months leads to a lower amount of lactic acid (0.06% decrease), and 388 389 ethanol almost evaporates completely. Bacterial and yeast populations remain viable (Musaeva, 1996). Damdinsurengiin (2000) was able to prolong shelf life of *qymyz* to 21 days at 20 °C by 390 adding stabilizers, agar-agar (E406) and apple pectin (E440) and pasteurizing bottled *qymyz* at 391 392 95 °C for 5-10 minutes.

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#### **5. Different products from mare milk**

395

In Europe mare milk is gaining interest in France, Germany and Italy as a source of 396 397 nutrition and as a substitute for children allergic to cow's milk (Malacarne et al., 2002). In Kazakhstan, in addition to qymyz, several other types of products such as yogurt and curd are 398 made for children (Sinyavskii, Torgautov, et al., 2016; Yakunin et al., 2017). Iannella (2015) 399 developed a method of cheese making from mare milk using camel chymosin at 0.4 g to 5 L. 400 401 Before addition of chymosin authors advocated adding starter cultures as they suppress undesirable bacteria, and produce lactic acid, which increase curd firmness and lower pH to 5.1 402 (optimal for camel chymosin). Mare milk has also been used in cosmetics such as shampoo, 403 shower gel, body cream, soap, hand cream (Stuparu et al., 2016). However, the main use of 404

horses in Europe remains to be recreational riding and horse racing (Liljenstolpe, 2009). In
Central Asia, mare milk is generally used to produce *qymyz*, but it is also served fresh in special
"saumal" (fresh mare milk) sanatoriums.

408

### 409 **6. Health benefits of mare milk and** *qymyz*

410

411 Qymyz is accepted as a therapeutic drink in the former USSR countries mainly against tuberculosis. Its anti-tuberculosis effect was reported in 1861 by a military doctor N. Zeland 412 (Kudayarova et al., 2010). Qymyz is believed to inhibit growth of bad bacteria due to low pH, 413 bactericidal substances produced by qymyz microbiota and high levels of lysozyme and 414 lactoferrin in the original mare milk. Gricenko (1964) found that lactose- negative yeast isolated 415 416 from *qymyz* had a higher bactericidal effect than lactose positive yeasts against non-pathogenic 417 B. subtilis and B. mesentericus. Hrisanova (1966) reported that the antibacterial effect of qymyz was due to *qymyz* microbiota as *qymyz* prepared from cow's milk showed the same antibacterial 418 419 effect against *M. tuberculosis* (H<sub>36</sub>Rv strain) as *qymyz* from mare milk.

Mare milk and *qymyz* are beneficial against atherosclerosis (Chiofalo et al., 2006). Zhang et al. (2012) have found that a strain isolated from *qymyz* reduces high cholesterol. Chen et al. (2010) report *qymyz* to be rich in angiotensin I-converting enzyme (ACE) inhibitors, which indicates the beneficial effect of *qymyz* on cardiovascular health.

Jirillo et al. (2010) found that mare milk strengthens the immune system. It was explained by a high content of albumin and globulin in the milk, which are building materials for antibodies (Stuparu et al., 2016).

427 Mare milk is beneficial for children with cow milk protein allergy (CPMA). As a result, 428 in European countries such as France, Germany, Belgium and Italy mare milk is sold as an 429 alternative to powdered milk formula for babies (Pieszka et al., 2016). Low lactose in *qymyz* is

beneficial for lactose intolerant people. 88% of Mongolians are lactose intolerant, but they can
still consume *qymyz* without any problem (Fox et al., 2015).

432 Mare milk was found to have anti-proliferative and anti-tumor effects against lung 433 cancer cells (Mao et al., 2009). Consequently authors have recommended use of mare milk in 434 treatment of cancer, as along with its anti-tumor effects, *qymyz* can be beneficial in fighting 435 chemotherapy side effects (Stuparu et al., 2016; Uniacke-Lowe et al., 2010).

Other than tuberculosis, diseases such as asthma, hyperlipidemia, anemia, nephritis,
diarrhea, and gastritis, as well as other digestive diseases, are treated with *qymyz* and mare milk
(Hou et al., 2019; Salimei & Park, 2017).

439

#### 440 **7. Conclusion**

441

Mare milk and *qymyz* have been the topic of a vast number of studies since the beginning 442 of the 20th century. Mare milk composition is closer to human milk than cow's. Its composition 443 varies due to horse breed, feed, location and lactation period. Qymyz is the main product made 444 445 from mare milk. The microbiota of *qymyz* is highly variable, but the main microorganisms in qymyz are LAB and yeasts. Mare milk, along with qymyz, has long been used in the treatment 446 447 of various diseases. Recent studies are reporting new beneficial properties of mare milk and *qymyz*. Due to these findings *qymyz* production has a lot of market potential. The quality of 448 *qymyz* depends on the use of specific microorganisms and specific fermentation conditions. The 449 interaction between these microorganisms is one of the more complex and important areas of 450 451 qymyz production, which requires further research. Optimizing succession (non-lactose fermentation yeast) or co-fermentation (lactose-fermenting yeast) of lactic and ethanol 452 fermentations in *qymyz* is still unclear, and thus deserves further study. 453

454

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456

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1	Figure legends
2	
3	Fig. 1. Countries where horses are used as dairy animals (Doreau & Boulot, 1989)
4	
5	Fig. 2. Horse population worldwide (FAOSTAT, 2019)
6	
7	Fig. 3. Horse population growth in Kazakhstan between 2005-2019 (stat.gov.kz, 2019)
8	
9	Fig. 4. Traditional qymyz starters (Shigaeva & Ospanova, 1983).
10	
11	Fig. 5. Qymyz preparation containers: (A) different forms of saba; (B) kubi.
12	
13	Fig. 6. Qymyz starter preparation scheme according to MRTU 46-573-69 (Seitov et al., 2005;
14	Shigaeva & Ospanova, 1983): (A) preparation of laboratory starter; (B) preparation of bulk
15	starter.
16	
17	Fig. 7. Starter preparation (A) and qymyz production (B) in 'qymyz' hospitals (Mursalimov &
18	Satyev, 1989; O. N. Popova, 1996).
19	
20	Fig. 8. Diagram of interaction between microorganisms in qymyz. Green lines - stimulation,
21	promotion; yellow- competition, inhibition; red - suppression. Dotted lines - overall
22	relationship between microorganisms.
23	

### Table 1

Horse population in Central Asian and bordering countries.

Country	Horse population	Horse population Year reported			
Kazakhstan	2.85 million	2019	(Committee on Statistics, 2020)		
Kyrgyzstan	0.52 million	2019	(Agriculture - Official Statistics - Statistics of Kyrgyzstan, 2020)		
Mongolia	4.21 million	2019	(MongolianStatisticalInformationService-Livestock,2020)		
Russia	1.37 million	2014	(Knyazev & Timchenko, 2016)		
Uzbekistan	0.24 million	2018	(FAOSTAT, 2020)		

### Table 2.

References	Mare milk	Cow milk	Human milk						
Protein									
(Park et al., 2017)	2	3,3	1,3						
(Claeys et al., 2014)	1,4-3,2	3-3,9	0,9-1,9						
(Akhatova, 2008)	1,6-2,3	2,5-3,3	1,8-3,5						
(Jenness, 1988)	2,5	3,2	0,9						
	Fat								
(Park et al., 2017)	1,2	3,8	4,2						
(Claeys et al., 2014)	0,3-4,2	3,3-5,4	2,1-4						
(Akhatova, 2008)	1,2-2,8	3,7	3,7						
(Jenness, 1988)	1,9	3,9	4,5						
	Carbohyd	rate							
(Park et al., 2017)	6,5	4,8	7						
(Claeys et al., 2014)	5,6-7,2	4,4-5,6	6,3-7						
(Akhatova, 2008)	6,2-7	4,7	6,3						
(Jenness, 1988)	6,2	4,6	7,1						

Mare, cow and human milk composition (% W/V)

# Table 3

		Mare			%		Hu	man		%		Cow			%	
References	(Uniac ke- Lowe et al., 2010)	(Malaca rne et al., 2002)	(Gafiatul lin, 2006)	(Uniac ke- Lowe et al., 2010)	(Malaca rne et al., 2002)	(Gafiatul lin, 2006)	(Uniac ke- Lowe et al., 2010)	(Malaca rne et al., 2002)	(Uniac ke- Lowe et al., 2010)	(Malaca rne et al., 2002)	(Uniac ke- Lowe et al., 2010)	(Malaca rne et al., 2002)	(Gafiatul lin, 2006)	(Uniac ke- Lowe et al., 2010)	(Malaca rne et al., 2002)	(Gafiatul lin, 2006)
Whole protein	24	21	20	100	100	100	12	14	100	100	34	33	33	100	100	100
Whey protein	8,3	8,3	11,7	34,2	38,8	54,7	6,2	7,6	53,0	53,5	6,3	5,7	6,6	18,4	17,5	20,4
Casein	13,6	10,7	8,0	56,0	50,0	37,4	2,4	3,7	20,5	26,1	26,0	25,1	25,9	76,0	77,2	79,4
Non- protein nitrogen * 6.38	2,4	2,4	-	9,9	11,2	-	3,1	2,9	26,5	20,4	1,9	1,7	-	5,6	5,2	-

Protein composition of mare, human and cow milk in g/kg. In brackets % of total nitrogen.

## Table 4.

Horse breed	Protein (%)	Dry matter (%)	Lactose (%)	Fat (%)	Milk production (kg/day)	Average liveweight (kg)	Author
Adai	2.12	10.2	6.48	1.62	10,2	376-430	(Imangaliev, 1967; Nurushev et al., 2008)
Bashkir	2,06-2,25	10,2-10,7	5,8-6,2	1,3-1,8	8,8	370-420	(Timerbulatova, 2015)
Kazakh zhabe type	2,03-2,09	-	6,24-6,33	1,5-1,58	9,5	475-529	(Duisembaev, 1989)
Kirgiz	2,1	-	6,9	1,8	12,9	-	(L. A. Popova & Gromova, 2014)
Kushum type	1,7	-	-	1,4	7,7-11,3	495-570	(Zeinullin, 1992)
Russian heavy draft	1,84-1,96	9,65-10,2	6,35	1,46-1,74	14,1	496	(Gladkova, 1990)
Soviet heavy draft	1,81-2,16	9,67-9,9	6,35	1,6	16,5	702	(Gladkova, 1990)
Yakut	2,27	10,5	8,46	0,93	8,8-13,3	-	(Pavlova, 2004)

## Milk composition and productivity of different horse breeds

# Table 5

Qymyz regions	Bacteria	Yeast	References		
Bashkortostan	A. aceti,	Kluyveromyces lactis	(Gricenko, 1964)		
	L. bulgaricum,	Saccharomyces			
	S. thermophilus	cartilaginosus			
		Micoderma yeasts			
China	L. helveticus	Kluyveromyces marxianus	(Mu et al., 2012; Sun et		
	L. casei	Kazakhstania unispora	al., 2010)		
	L. plantarum	Saccharomyces cerevisiae			
Kazakhstan	L. bulgaricus,	Torulopsis kefyr	(Montanari et al., 1996;		
	L. casei,	Torulopsis sphaerica	Shigaeva & Ospanova,		
	Str. lactis	Kazakhstania unispora	1983)		
Mongolia	L. helveticus,	Kluyveromyces marxianus	(Miyamoto et al., 2010)		
	L. kefiranofaciens,				
	L. lactis,				
	L. mesenteroides				

Major microorganisms in qymyz from different regions

### Table 6

*Qymyz* sensory types classification according to ST RK 1004-98 (Kazakh Research and Development Institute of Meat and Dairy Industry of the Ministry of Agriculture of the Republic of Kazakhstan., 1999).

	Types of <i>qymyz</i>					
Characteristics	Weak	Medium	Strong			
Lactic acid %	0.7	0.9	1.08			
Ethanol %	1	1.5	3			
Density g/cm <sup>3</sup>	1.025-1.021	1.020-1.018	1.017-1.015			





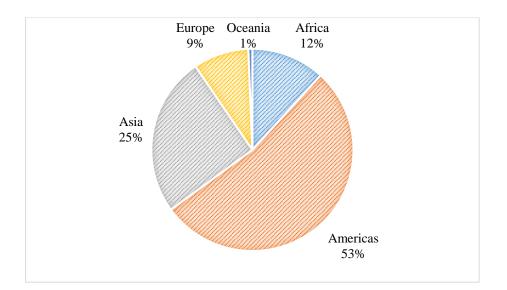


Figure 2

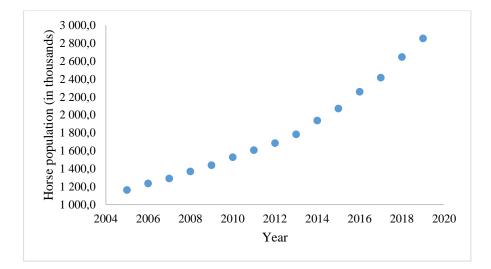


Figure 3

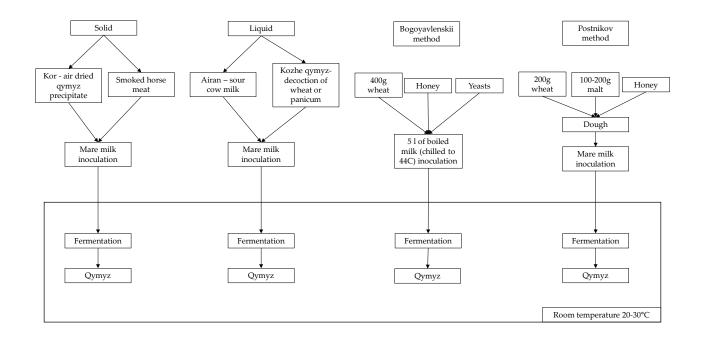
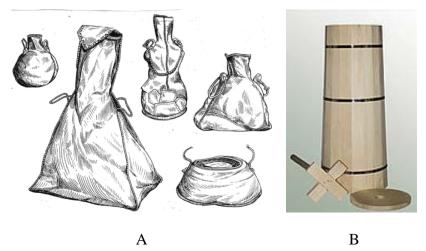
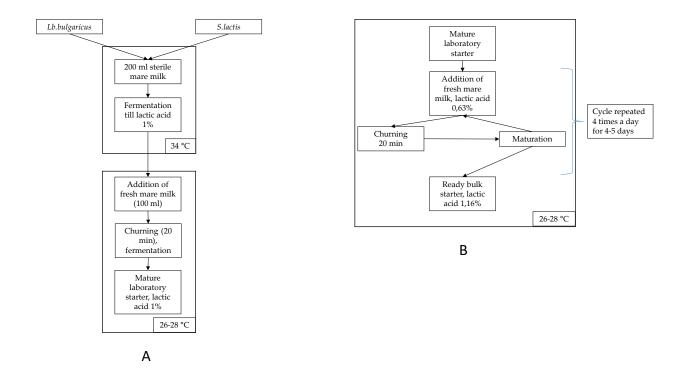


Figure 4



В

Figure 5





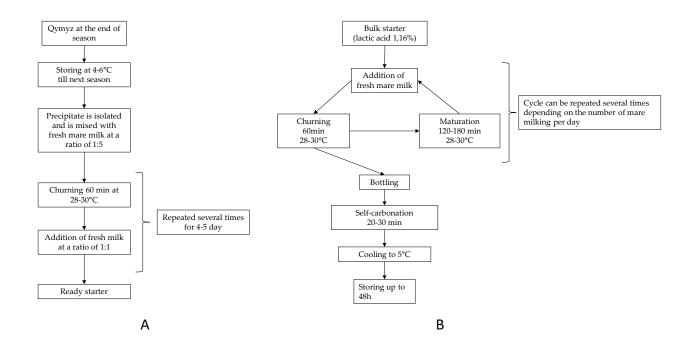


Figure 7

