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## Fermented mare milk product (Qymyz, Koumiss)

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1 **Fermented mare milk product (Qymyz, Koumiss)**

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24 **ABSTRACT**

25

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

34 **Keywords:** horse, mare milk, qymyz, koumiss, health

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58 **1. Introduction**

59

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

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

77           Many studies were carried out in 20<sup>th</sup> century in the USSR in Russian languages which  
78 are not accessible for the international community. This current review focuses mainly on data  
79 published by soviet authors which will help to understand the importance of mare milk and  
80 *qymyz* for human consumption.

81

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

83

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

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

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

103

### 104 **3. Mare Milk**

105

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

107

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 quite  
112 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).

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

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

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

132 of mare milk (1900  $\mu\text{m}/\text{kg}$ ) is higher than in cow's milk (560  $\mu\text{m}/\text{kg}$ ), but lower than in human  
133 milk (2930  $\mu\text{m}/\text{kg}$ ) (Salimei & Park, 2017).

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

154 Carbohydrates in mare milk mainly consist of lactose and trace amounts of  
155 monosaccharides and oligosaccharides. Lactose content in mare milk (6-7g·100 ml<sup>-1</sup>) is similar  
156 to that in human milk and significantly higher than in cow's milk (4-5g·100 ml<sup>-1</sup>). Among



157 monosaccharides, glucose was reported to change during the lactation period and range between  
158 50 -150 mg·L<sup>-1</sup> (Uniacke-Lowe & Fox, 2012). According to Seitov (2005) colostrum has a  
159 lower amount of carbohydrates than milk, which equals 2.9-3.4%. The amount increases  
160 rapidly and a day after foaling the amount equals 5%. The maximum amount of carbohydrates  
161 is present 1-3 months after foaling and ranges between 6.4-7%. Oligosaccharide concentration  
162 was reported to range between 79.8 - 217.8 mg·L<sup>-1</sup> and includes 58.3% neutral, 33.3% Neu5Ac,  
163 6.25% fucosylated and 2.1% NeuGc oligosaccharides (Karav et al., 2018).

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

173 Data on vitamin content of mare milk is variable. Some authors note lower  
174 concentrations of fat-soluble vitamins in mare milk due to a low amount of milk fat (Salimei &  
175 Fantuz, 2013). Others report similar content in mare and cow milk (Fuquay et al., 2011). Both  
176 works agree on higher content of vitamin C in mare milk (150 mg·L<sup>-1</sup>). The works of Berlin  
177 and Gricenko as reported by Seitov (2005) found concentrations of vitamins B<sub>1</sub> – 291-390; B<sub>2</sub>  
178 – 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  
179 milk.

180

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

182

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

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

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

193

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

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

205

## 206 4. Qymyz

207

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

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

220

### 221 4.1. Traditional *qymyz*

222

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

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

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

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

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

267

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

269

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

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

285 In present times, most *qymyz* hospitals use *qymyz* from the previous year as a starter.  
286 The scheme of *qymyz* production in hospitals can be seen from Figure 7 (Mursalimov & Satyev,  
287 1989; O. N. Popova, 1996). There is a possibility that due to the standardization of *qymyz*  
288 production in the USSR all hospitals had to use MRTU 46-573-69 in their production, but later  
289 on hospitals started using old *qymyz* as a starter.

290

#### 291 4.3. *Qymyz* quality

292

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

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

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

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

316

#### 317 *4.4. Qymyz process modifications*

318

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

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

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

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 kefir* var *kumis* with *S. lactis* and  
349 *Lb. bulgaricus* leads to better growth with *Lb. bulgaricus*, that has a higher production of lactic  
350 acid (Shigaeva & Ospanova, 1983). In contrast, yeast such as *Saccharomyces lactis* has an  
351 optimum pH for lactose hydrolysis at 6.5 (Guy & Bingham, 1978). According to Fox et al.  
352 (2015), usage of LAB with lactose-fermenting yeast is optimal in *qymyz* fermentation as lactic  
353 acid formation and alcoholic fermentations occur simultaneously and the products of  
354 fermentation are in definite proportions. However simultaneous fermentation leads to



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

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

376         The cost and availability of mare milk made authors seek ways to prepare *qymyz* from  
377 cow's milk. Hrisanova (1966) simply decreased pH of cow's milk to 5.2 and added *qymyz*  
378 starter cultures. The resulting cow milk *qymyz* had the same antagonistic effect against *M.*  
379 *tuberculosis* (H37Rv strain) as *qymyz* from mare milk. Shamgin et al. (1977) proposed using a

380 mixture of whole milk (33-36%), skimmed milk (0.6-1%) and whey (63-67%), which results  
381 in 1:1 ratio of casein to whey, similar to mare milk. Küçükçetin et al. (2003) used ultrafiltration,  
382 microfiltration and nanofiltration to modify cow's milk so that the final composition was equal  
383 to mare milk in terms of protein, lactose and other compositional factors. Prepared *qymyz* had  
384 no significant compositional or sensorial difference from mare milk *qymyz*.

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

393

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

395

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

405 horses in Europe remains to be recreational riding and horse racing (Liljenstolpe, 2009). In  
406 Central Asia, mare milk is generally used to produce *qymyz*, but it is also served fresh in special  
407 “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  
412 tuberculosis. Its anti-tuberculosis effect was reported in 1861 by a military doctor N. Zeland  
413 (Kudayarova et al., 2010). *Qymyz* is believed to inhibit growth of bad bacteria due to low pH,  
414 bactericidal substances produced by *qymyz* microbiota and high levels of lysozyme and  
415 lactoferrin in the original mare milk. Gricenko (1964) found that lactose- negative yeast isolated  
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*  
418 was due to *qymyz* microbiota as *qymyz* prepared from cow’s milk showed the same antibacterial  
419 effect against *M. tuberculosis* (H<sub>36</sub>Rv strain) as *qymyz* from mare milk.

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

424 Jirillo et al. (2010) found that mare milk strengthens the immune system. It was  
425 explained by a high content of albumin and globulin in the milk, which are building materials  
426 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

430 beneficial for lactose intolerant people. 88% of Mongolians are lactose intolerant, but they can  
431 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).

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

439

## 440 **7. Conclusion**

441

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

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.

<b>Country</b>	<b>Horse population</b>	<b>Year reported</b>	<b>References</b>
Kazakhstan	2.85 million	2019	<i>(Committee on Statistics, 2020)</i>
Kyrgyzstan	0.52 million	2019	<i>(Agriculture - Official Statistics - Statistics of Kyrgyzstan, 2020)</i>
Mongolia	4.21 million	2019	<i>(Mongolian Statistical Information Service-Livestock, 2020)</i>
Russia	1.37 million	2014	<i>(Knyazev &amp; Timchenko, 2016)</i>
Uzbekistan	0.24 million	2018	<i>(FAOSTAT, 2020)</i>

**Table 2.**

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

References	Mare milk	Cow milk	Human milk
<b>Protein</b>			
(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
<b>Fat</b>			
(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
<b>Carbohydrate</b>			
(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



**Table 3**

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

References	Mare			%			Human		%		Cow			%		
	(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	-

**Table 4.**

Milk composition and productivity of different horse breeds

Horse breed	Protein (%)	Dry matter (%)	Lactose (%)	Fat (%)	Milk production (kg/day)	Average liveweight (kg)	Author
A dai	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)

**Table 5**Major microorganisms in *qymyz* from different regions

<b><i>Qymyz</i> regions</b>	<b>Bacteria</b>	<b>Yeast</b>	<b>References</b>
Bashkortostan	<i>A. aceti</i> , <i>L. bulgaricum</i> , <i>S. thermophilus</i>	<i>Kluyveromyces lactis</i> <i>Saccharomyces</i> <i>cartilagenosus</i> Mycoderma yeasts	(Gricenko, 1964)
China	<i>L. helveticus</i> <i>L. casei</i> <i>L. plantarum</i>	<i>Kluyveromyces marxianus</i> <i>Kazakhstania unispora</i> <i>Saccharomyces cerevisiae</i>	(Mu et al., 2012; Sun et al., 2010)
Kazakhstan	<i>L. bulgaricus</i> , <i>L. casei</i> , <i>Str. lactis</i>	<i>Torulopsis kefir</i> <i>Torulopsis sphaerica</i> <i>Kazakhstania unispora</i>	(Montanari et al., 1996; Shigaeva & Ospanova, 1983)
Mongolia	<i>L. helveticus</i> , <i>L. kefirifaciens</i> , <i>L. lactis</i> , <i>L. mesenteroides</i>	<i>Kluyveromyces marxianus</i>	(Miyamoto et al., 2010)

**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).

Characteristics	Types of <i>qymyz</i>		
	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 1

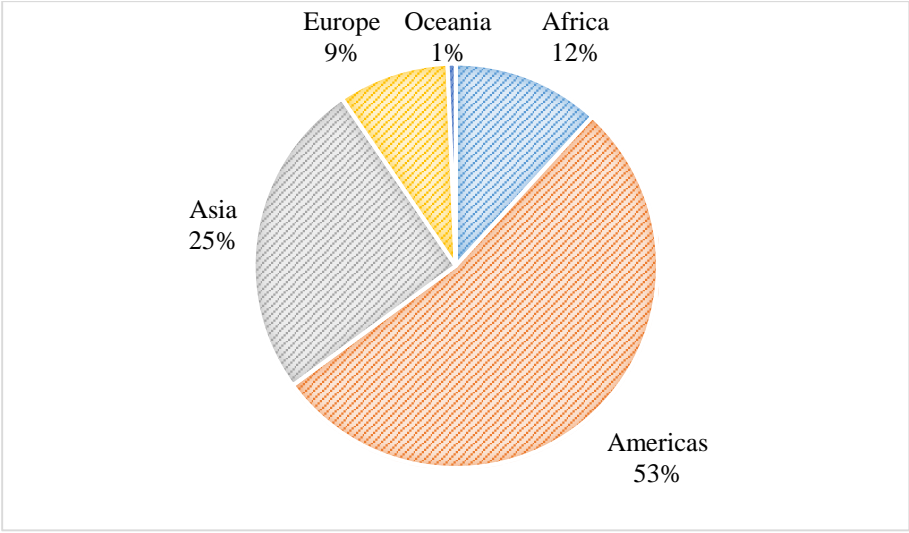


Figure 2

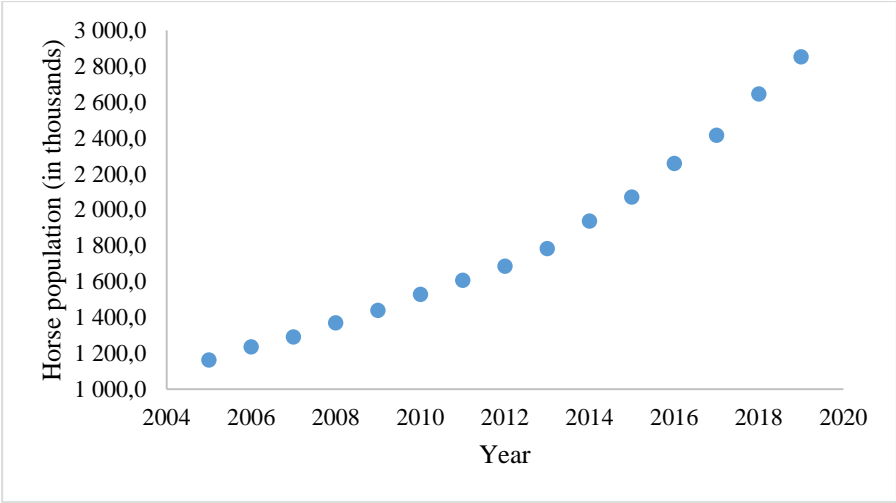
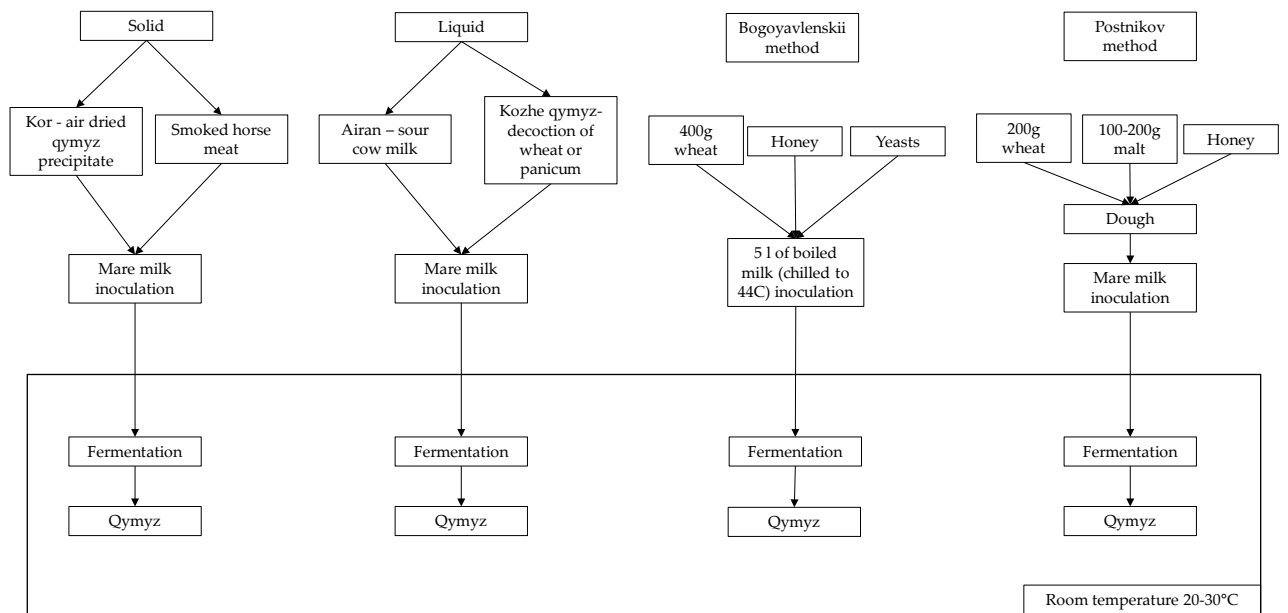


Figure 3



**Figure 4**





A



B

Figure 5

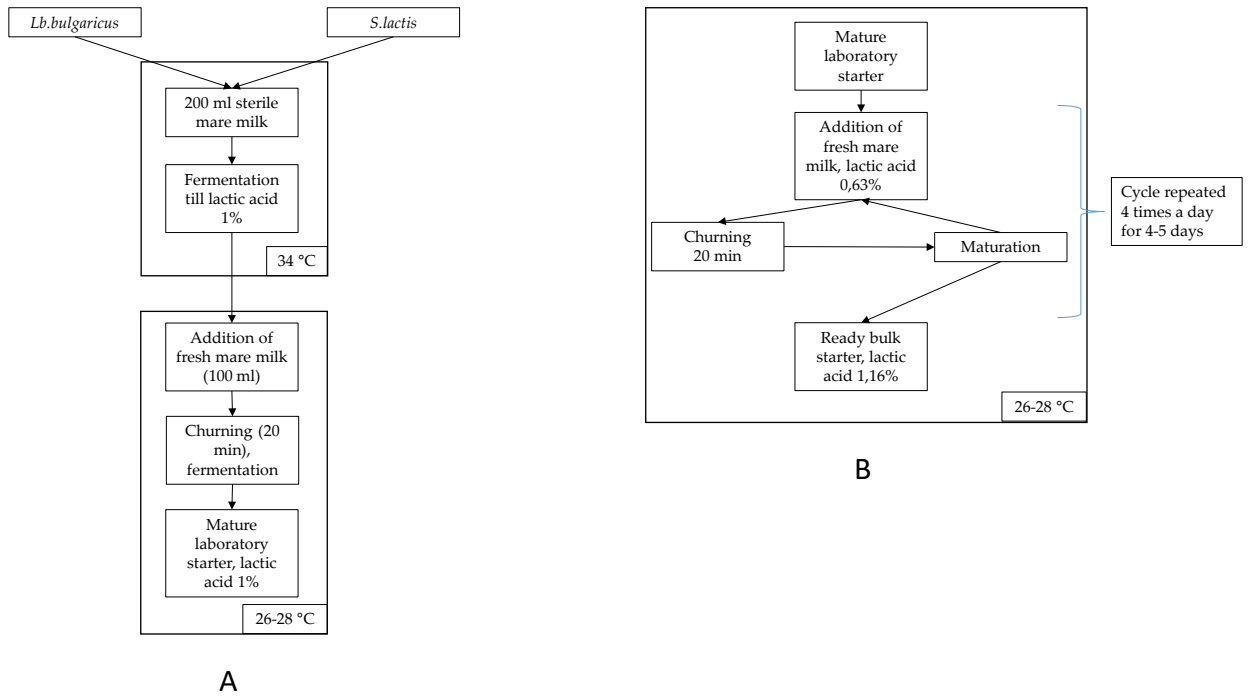


Figure 6

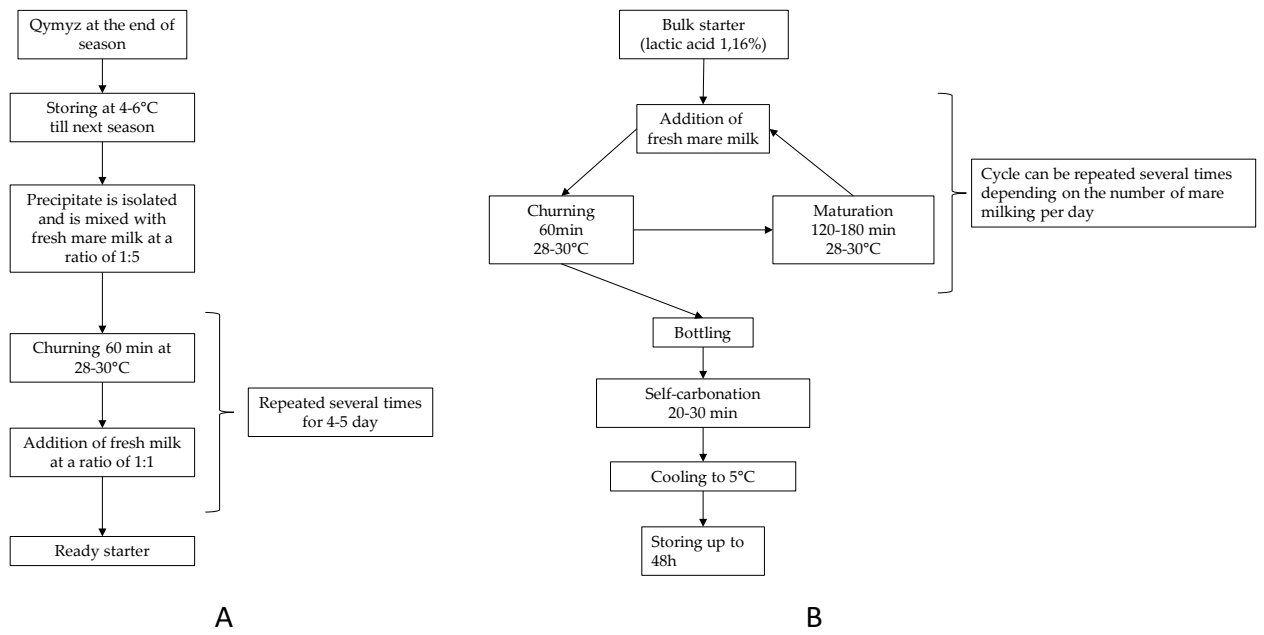


Figure 7

