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The effects of modifications at milk and incubation conditions on the production of cow's kumiss

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Abstarct: This study was carried out with the aim of improving the knowledge on the applicability of cow's milk to prepare a mare's kumiss like drink. Commercial mare's kumiss was purchased and analysed in parallel to the cow's milk kumiss and used as inoculant for making cow's milk kumiss. Cow's milk was used for making Control samples. Nevertheless, Control samples of cow's milk kumiss were more viscous and prosaic for a kumiss drink. The commercial mare's kumiss was not also liked by the panel members. The most admired sample determined to be modified cow's milk kumiss. Modification made as addition of 50% water to full-fat cow's milk, then fortification of this milk with 3.8% (w/v) lactose and 0.9% (w/v) whey protein appeared to be more valuable for a pleasant-tasting kumis-like dairy product development. As inoculation culture, mare's kumis or kumiss like sample developed in the study were found to be used at a rate of 5%. Also, a consortium of dominant bacterial and yeast culture selected from mare's kumiss appeared to be applicable for making a kumiss like cow's milk drink. Static incubation of milk at 28 °C for 24 h, and 4.4 pH and 0.7% lactic acid levels after incubation; also, a pH of 3.5 to 4, a maximum of 1.5% lactic acid at the 14th day of cold storage appeared to be good values for such a drink. Nevertheless, more detailed studies are needed to develop cow's milk kumiss or kumiss-like drinks.
Keywords: Cow's milk, kumiss, kumiss culture, modified milk, process.

Süt ve inkübasyon koşullarında yapılan değişikliklerin inek kımızı üretimine etkileri

Özet: Bu çalışma, inek sütünün kımız benzeri kısrak içeceği yapımında kullanılabilirliği konusundaki bilgileri artırmak amacıyla yapılmıştır. Ticari kısrak kımızları satın alınarak inek sütü kımızları ile paralel olarak analiz edilmiş ve inek sütü kımızlarının yapımında kültür olarak kullanılmıştır. Kontrol örnekleri yapmak için inek sütü kullanıldı. Bununla birlikte, inek sütü kımız kontrol numuneleri, bir kımız içeceği için daha viskoz ve yavandı. Ticari kısrak kımızı da panel üyeleri tarafından beğenilmedi. En çok beğenilen örneğin modifiye inek sütü kımız olduğu belirlendi. Tam yağlı inek sütüne %50 su ilavesi şeklinde yapılan modifikasyonun ardından bu sütün %3,8 (w/v) laktoz ve %0,9 (w/v) peynir altı suyu proteini ile zenginleştirilmesinin hoş içimli bir kımız için daha uygun olduğu görüldü. Mayalama kültürü olarak kısrak kımızı veya çalışmada geliştirilen kımız benzeri örneğinin %5 oranında kullanılabileceği tespit edildi. Ayrıca, kısrak kımızlarından seçilen baskın bakteri ve maya kültüründen oluşan bir konsorsiyumun kımız benzeri inek sütü içeceği yapmak için uygun olduğu da ortaya çıktı. Sütün 28 °C'de 24 saat statik inkübasyonu ve inkübasyondan sonra pH 4,4 ve %0,7 laktik asit seviyeleri; ayrıca, soğuk muhafazanın 14. gününde pH 3,5 ila 4, maksimum %1,5 laktik asit böyle bir içecek için iyi değerler olarak tespit edildi. Ancak inek

sütü kımız veya kımız benzeri içeceklerin geliştirilmesi için daha detaylı çalışmalara ihtiyaç vardır.
Anahtar kelimeler: İnek sütü, kımız, kımız kültürü, modifiye süt, proses.

Introduction

Food safety and accordingly public health may be at risk due to microbiological and chemical contaminants (Sevilmiş, 2016). Tourists are particularly affected by this situation (Erdem & Gündogdu, 2018a). The majority of diarrheal diseases are related to food hygiene (Ucar et al., 2016). It is known that probiotics and fermented milk products such as kefir and kumiss support intestinal health and general health (Behera et al., 2017). Drinkable dairy products are produced all over the world and these products such as kumiss are consumed in different ways in every society. These products have created tastes in the course of history and have become special for producer society (Marsh et al., 2014).

It has been reported that mare's milk contains properties similar to human milk in terms of nutritional value and health benefits, and no side effects have been observed, although it has been consumed for thousands of years (Musaev et al., 2021). Kumis is an acidic and slightly alcohol-containing dairy product that has been traditionally produced by Central Asian communities using mare's milk since ancient times (Tegin & Gönülalan, 2014a). Kumis has started to become widespread outside of these communities as well (de Melo Pereira et al., 2022). As the positive effects of functional dairy products such as kumiss and kefir on health are revealed, their prevalence is accelerating (Shaimardanova, 2012; Afzaal et al., 2021). Another feature of kumis is that it offers the opportunity to meet the water needs in rural areas where access to potable water is difficult. Kumis is a product that also meets the vitamin C needs of people such as herdsmen who do not have the opportunity to consume vegetables and fruits in the Asian steppes for a long time (Dugan, 2009; McGuire, 2017). Today, kumiss is used as a product that supports health tourism (Erdem & Gundogdu, 2018b). There are researchers who describe kumiss as a unique beverage (Yangılar et al., 2016). It has been reported that kumiss has significant health gains as a result of studies performed on experimental animals (Eliş Yildiz et al., 2015; Gülmez & Atakişi, 2020). Studies on both the nutritional and therapeutic properties of kumis have been compiled and presented in great detail (Dhewa et al., 2015).

Horse milk has its own characteristics (Hinz et al., 2012). Since the traditional form of kumis is made from mare's milk, it has high lactose and low protein content (Nurtazin et al., 2015). However, where kumiss has the potential to become widespread, sufficient mare's milk cannot be supplied in commercial kumiss production, milk of the other farm animals, especially

cow's milk, which is more economically produced in every month of the year, is recommended for kumiss production (Küçükçetin et al., 2003). It is not easy to obtain the characteristics of traditional kumiss by using cow's milk. For this reason, when milk other than mare's milk is used, it has been suggested that the milk should be modified as to mare's milk. There are differences among researchers in terms of material, method and starter cultures used for cow's milk kumiss production (Malacarne et al., 2002; Liu et al., 2019; Li et al., 2020; Rakhmanova et al., 2021).

Kumiss culture consists of a consortium of bacteria and yeasts. The dominant culture consists of *lactobacilli, lactococci, leuconostocs* and yeasts (*Saccharomyces* sp., *Candidia* sp. and *Torula* sp.) (Sun et al., 2010). Along with these microorganisms, *Streptococcus (Sc.)* and *Enterococcus (Ec.) faecium* were also isolated in mare's milk (Ying et al., 2004). It has been reported that while *Enterobacter* and *Rhodotorula* constitute the dominant flora in mare's milk, *Lactobacillus* and *Dekkera* are the dominant flora during the formation of kumiss, and the flora and the metabolites that give its character to kumiss, differ (Xia et al., 2021). *Lactobacillus, Lactococcus, Acetobacter, Streptococcus, Serratia* and *Leuconostoc* bacteria; and the fungi *Kazachstania, Kluyveromyces, Trichosporonaceae, Pichia,* and *Candida* were predominantly isolated in traditionally produced mare's milk kumiss (Wang et al., 2008). It has previously been reported that 112 volatile substances detected in the same study were mainly produced by 4 groups of bacteria (*Lactobacillus, Acetobacter, Lactococcus* and *Pseudomonas*) and 2 groups of fungi (*Kazachstania* and *Candida*). Yao et al. (2017) have identified *Lc. otakiensis, Sc. macedonicus,* and *Ruminococcus torques*. The use of raw mare's milk in production causes the diversity of the flora. So, the use of pasteurized milk in commercial production has been suggested (Rakhmanova et al., 2021). Researches continue to achieve the technological development that will contribute to the spread of commercial kumiss production (Kozhahmetova & Kasenova, 2013; Maksyutov et al., 2013). The traditional form of kumis must be produced from horse milk. However, due to the inadequacy of horse milk, it shows that other most aboundantly produced milks can be used in the production of kumiss or a kumiss like drink. As can be understood from the summary information given above, it cannot be claimed that such a commercial product is produced and consumed worldwide after the studies on the production of kumiss from cow's milk (Kırdar & Tegin, 2022). After that, more studies will be done probably to develop a cow's milk kumis or a kumiss like drink that is appropriate to produced and consumed at more countries. In this study, it was aimed to find out the effect of modification of UHT cow's milk, incubation conditions (static and/or shakingly) and

inoculant on the acidity, pH and flavour development after incubations and at a 14 d of cold storage time period.

Materials and Methods

Preparation of milk samples: UHT cow's milk purchased from a local market was transferred aseptically in autoclaved (121 °C for 15 min) erlenmayer flasks before use. Unmodified UHT milk was used for making Control samples. Modified samples were diluted by adding autoclaved (121 °C for 15 min) drinking water to the UHT milk at a 1: 1 rate (v/v). Then, the prepared milk saples were modified by adding each of sugars (sorbitol, sucrose, glucose, maltodextrin, inulin and lactose) at a rate of 3.8% (v/w) and/or whey protein (WP, Maybi, Smart code: 058.320.50) at a rate of 0.9% (v/w).

Inoculation: A commercial mare's kumiss (sold as bag of 12 of 200 mL in glass bottle) bought from manufacturer was used as inoculant by adding at a rate of 5% to the prepared milk samples before incubations.

Incubation: Static incubation or one from 5 different shaking procedures (continuous, 30 min shaking at every 3 h, 6 h static + 30 min shaking in every 3 h, 12 h static + 30 min shaking at every 3 h, 12 h static + 12 h continuous) were applied during incubation. All the samples were incubated at 28 ºC for 24 h. Then, the samples were kept at 4 °C for 14 d for further analysis.

Instrumental analysis of milk: Analysis of milk and modified milk samples used in the experiements were made by using a milk auto-analyzer (Lactoscan LS, Nova Zagora, Bulgaria).

Acidity: Titratable acidity (lactic acid, %) of milk and kumiss samples were measured by using 0,1N NaOH and phenolphtalein indicator (Sadler & Murphy, 1984).

pH: The pH milk and kumiss samples were measured by using a digital pH meter (Milwaukee, AZ8686).

Coliforms and coagulase positive staphylococci: The reference methos were applied as mentioned by Pouch & Ito (2001) for the analysis of milk and kumiss samples. Briefly, 10 g of each sample was used for making ten-fold serial dilutions in diluted in 90 ml of steril physiological saline (PS). For coliforms, Violet Red Bile Lactose Agar (VRBLA, Oxoid CM0107) was used. Petri dishes were incubated for 24 hours at 30 °C, the growing pink-red colonies with a pink precipitation ring around were counted. For coagulase positive staphylococci, Baird Parker Agar (BPA, Oxoid, CM1127) plates with Egg Yolk Tellurite Emulsion (Oxoid, SR0054) were used and the plates were incubated at 37 °C for 48 hours.

Black shiny coagulase positive colonies with a diameter of 1.5 – 2.5 mm with a transparent zone around were counted.

Selection and characterization of strains of predominant microflora in kumiss: Possible starter culture members were selected from Modified Cow's Milk Kumiss (MCMK). The MCMK was obtained at the end of static incubation after adding 50% water, 3.8% lactose and 0.9% WP to cow's milk. Serial dilutions of the sample up to 10-7 were prepared. A 100 µL from each of the last three serial dilution tubes were streaked on 10 parallel petri dishes (Pouch & Ito, 2001). de-Man Rogosa Sharpe Agar (MRS, Merck, Germany) was used for the isolation of lactobacilli and M17 Agar was used for the isolation of lactococci. Petri dishes were incubated at 37 ºC for 3 d. (Malacarne et al., 2002; Küçükçetin et al., 2003; Rakhmanova et al., 2021). For the isolation of yeasts, the petri dishes of Potato Dextrose Agar (PDA) pH adjusted to 3.5 with 10% lactic acid were inoculated and incubated for 4 d at 24 ºC (Hinz et al., 2012). Colonies were randomly picked from the plates, and each colony was purified on its own agar plate (MRS, M17 or PDA). A total of 300 colonies, 100 colonies from each, were selected on MRS, M17 and PDA agar. Gram positive, catalase negative, citrate negative bacteria that can utilise lactose, fructose, sucrose, glucose, maltodextrin and inulin were selected from these colonies. Among the yeasts, those that were catalase positive, citrate negative and able to use lactose, fructose, sucrose, glucose, maltodextrin and inulin were selected. Carbohydrate fermentation tests were made according to Bansal et al. (2013) and Reiner (2012) using Phenol Red Carbohydrate Broth. Ten colonies belonging to each petri dish were selected. So, each group of 10 isolates were made. Then, each group strains were multiplied by incubating them together in their own broth (MRS, M17 or PDA) for 24 hours at 30 ºC. One mL from each of these broth cultures was inoculated to 100 mL of milk by making different combinations of MRS, M17 and PDA colonies. The most liked sample was used as inoculum for the next kumis production. The process was repeated three consecutive times. Colony selection and isolation procedures reported above were repeated from the most favored sample. Finally, selected 15 isolates, 5 from each of the MRS, M17 and PDA colonies, were propagated and used as kumiss milk inoculant as stated above. Identifications of the 15 colonies were made using a mass spectrometer “Matrix-Assisted Laser Desorption/ Ionization - Time of Flight (MALDI-TOF)” system (Liu et al., 2019).

General liking scores: General liking scores at kumiss samples were performed by six panel members as mentioned by Yao et al. (2017).

Statistical analysis: The mean values of the samples and the standard deviation values between the samples were calculated by using the Microsoft Excel program. Data were

analyzed using the general linear model procedure in SAS soft-ware (Sun et al., 2010). Differences between the samples were determined using the least significant difference (LSD) test. A probability level of $P< 0.05$ was considered statistically significant.

Results

The results of the analyzes performed on the cow's milk and modified cow's milk samples before inoculations are given in the Table. There was no information in the user manual of the device that such an analysis could be made. But, we trained to test them by using milk analyzer. Sugars and WP added to UHT milk made it difficult for the milk analyzer to read. So, only pH and acidity values were considered in this study. It was observed that the pH value decreased by 0.3 units when water and sugar added to the milk, and increased by 0.3 units when WP together with water and sugar added (Table).

Table. Analysis of milk and modified milk samples used in the experiments.

Additives Water: milk 1:1 (v / v) Lactose 3.8%, (w / v) Whey protein (WP), 0.9% (w/v)	**Density**	**Conductivity (ms/cm)**	**Freezing point**	**Water, %**	**Nonfat dry mater, %**	**Fat, %**	**Protein, %**	**Lactose, %**	**Minerals, %**	**pH**	**Lactic acid, %**
UHT cow's milk (as Controls)	26	5.6	0.5	82	7.6	3.2	2.8	4.2	0.6	5.7	0.31
Milk + water	15	3.9	0.3	49	4.4	1.7	1.6	2.4	0.4	5.4	0.23
Milk + water + lactose	21	3.7	0.4	31	5.9	1.9	2.2	3.2	0.5	5.2	0.23
Milk + water + WP	15	3.6	0.3	49	4.5	1.8	1.6	2.4	0.4	6.0	0.24
Milk + water + lactose+ WP	20	3.1	0.3	33	5.8	1.5	2.1	3.2	0.5	6.1	0.23

The study was conducted at 7 consecutive steps. No coliforms or coagulase positive staphylococci were detected in the samples. The acidity values were given all the steps of the study while pH was only given at the 4, 6 and 7th steps (Figure 4b, 6b and 7b), and general liking scores at the 6 and 7th steps (Figure 6c and 7c).

On the 7^{th} d of cold storage, the acidity (L.a.%, mean ± SD) was 0.52±0.07 in the samples that did not add water to the milk (Figure 1), while it was 0.95±0.08 in the samples that water and sugar added (Figure 2), and 1.45±0.1 in the samples that water, sugar and WP added (Figure 3). These average values of each group are not given separately. There was no effect of shaking in terms of acidity development between the groups in the first 3 steps of the study (Figure 1-3) ($P> 0.05$). There was no significant difference between the samples made by addition of different sugars and whey protein. Although not obvious, it was observed that the

addition of glucose had a greater effect on the development of acidity (Figure 1-3). It was observed that the addition of water, sugar and WP to the samples supported the fermentation. The taste panel members found the kumiss samples that made by using non-diluted milk samples to be very thick, difficult to drink and tasteless even after the 7 d of cold storage (data not shown).

On the 7th d of cold storage, the mean pH value was 5.68±0.13 in the sample that did not add water to the milk. Also, 4.79±0.04 in the samples that added water and sugar, and 4.68±0.03 in the samples that added water, sugar and WP. There was no effect of shaking in terms of pH development between the groups (P> 0.05). No significant pH change was observed between the samples that different sugars addition. The addition of water, sugar and WP together to the samples supported pH reduction (P< 0.05).

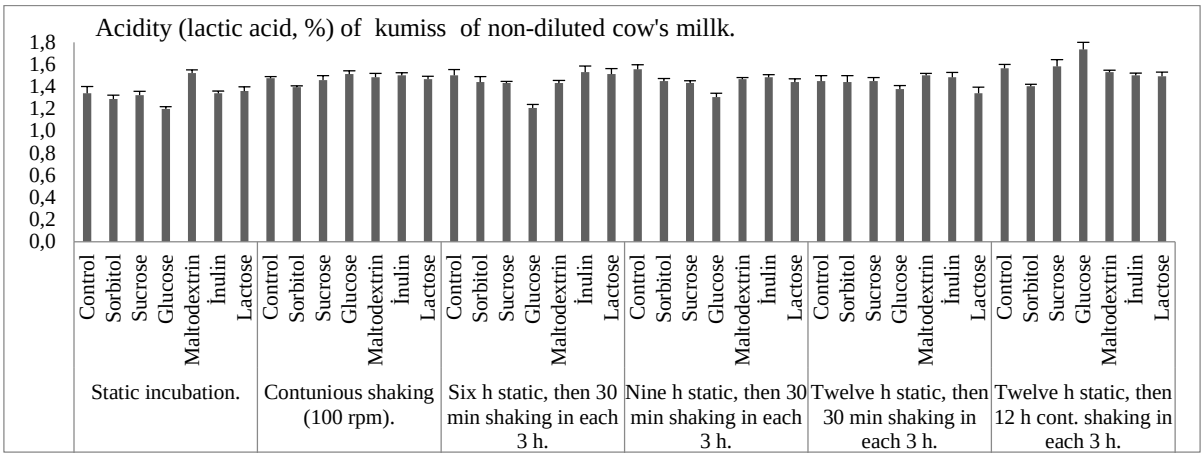

Figure 1. Acidity (lactic acid, %) of kumiss samples that made from non-diluted but sugar added milk samples and incubated in 6 different ways, then maintained at 4 ºC for 7 d.

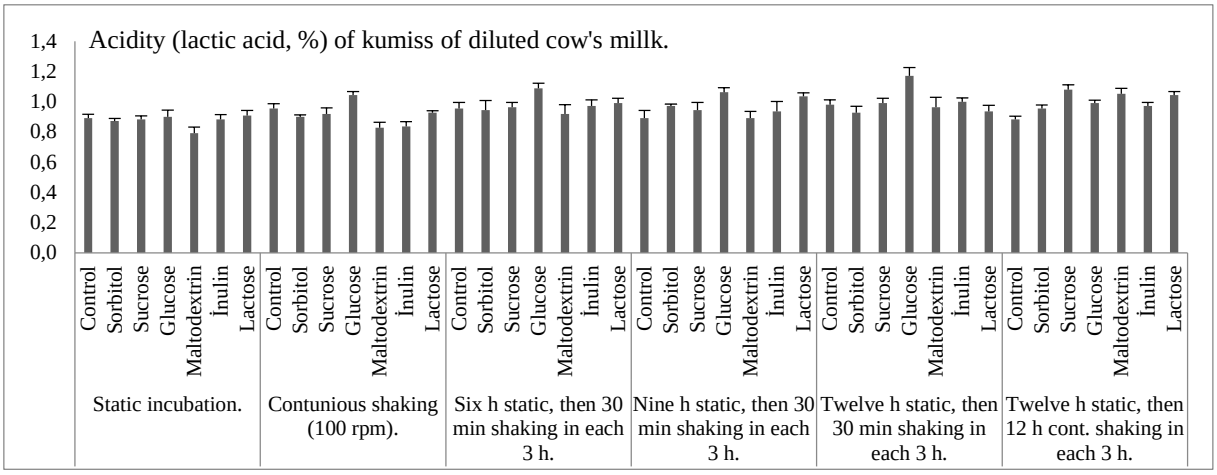

Figure 2. Effect of modification of cow's milk (50 mL) by adding water (50 mL) and each of 6 sugars (3.8 g) on the acid development after incubation.

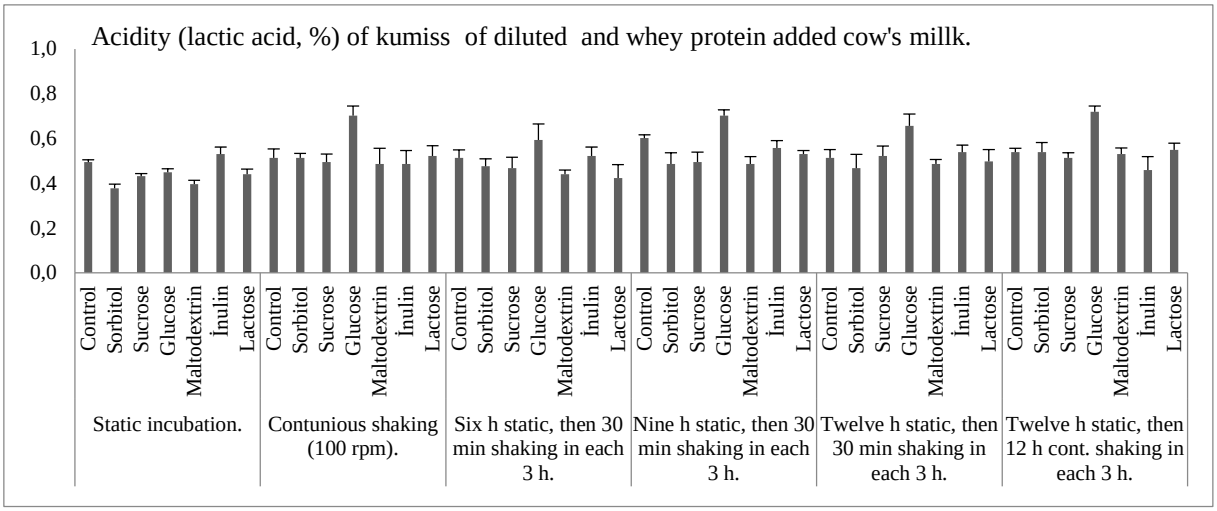

Figure 3. Effect of modification of cow's milk (50 mL) by adding water (50 mL), each of 6 sugars (3.8 g) and whey proteins (0.9 g) on acid development of the samples after incubation.

In the 4th stage of the process development, 16 different samples inoculated with 2 different inoculants and incubated with static or shaking process were compared with the Control samples. Acidity, pH and sensory analyzes were performed at the 1st h, 7th d and 14th d of cold storage (Figure 4a, b). Acidity values in cold storage were higher in samples inoculated with fresh cow's kumiss (Figure 4a). In addition, it was observed that the addition of lactose and WP had a positive effect on acidity in both Control and modified samples. Acidity development continued during the 14 d of cold storage. However, the increase in acidity in the modified samples was less than in the Control samples. There was no effect of shaking in terms of acidity development between the groups. Sugar type or shaking regime were not effective on the acid development. The effect of lactose and WP on the acidity of 7th and 14th d was clearly demonstrated ($P< 0.05$). It was observed that the addition of water, sugar and WP to the samples supported the fermentation. The mare's kumiss culture adapted to cow's milk was used as inoculant in repeated incubations, making it more effective in acidity development. Control samples were quite viscous and tasteless even after shake incubations.

In the 1st h, 7th d and 14th d of cold storage, pH in both Control and MCMK samples were found to be lower in samples fermented with new inoculum than kumis inoculum (Figure 4b). It was observed that adding lactose and WP to the samples had a positive effect on pH ($P< 0.05$). The pH became more stable with the addition of lactose and WP in all samples in 14th d of cold storage. The pH decrease in the experimental samples was less than in the Control samples. There was no effect of shaking in terms of pH decrease between groups. It was observed that the addition of water, lactose and WP to the samples supported the pH decrease ($P< 0.05$).

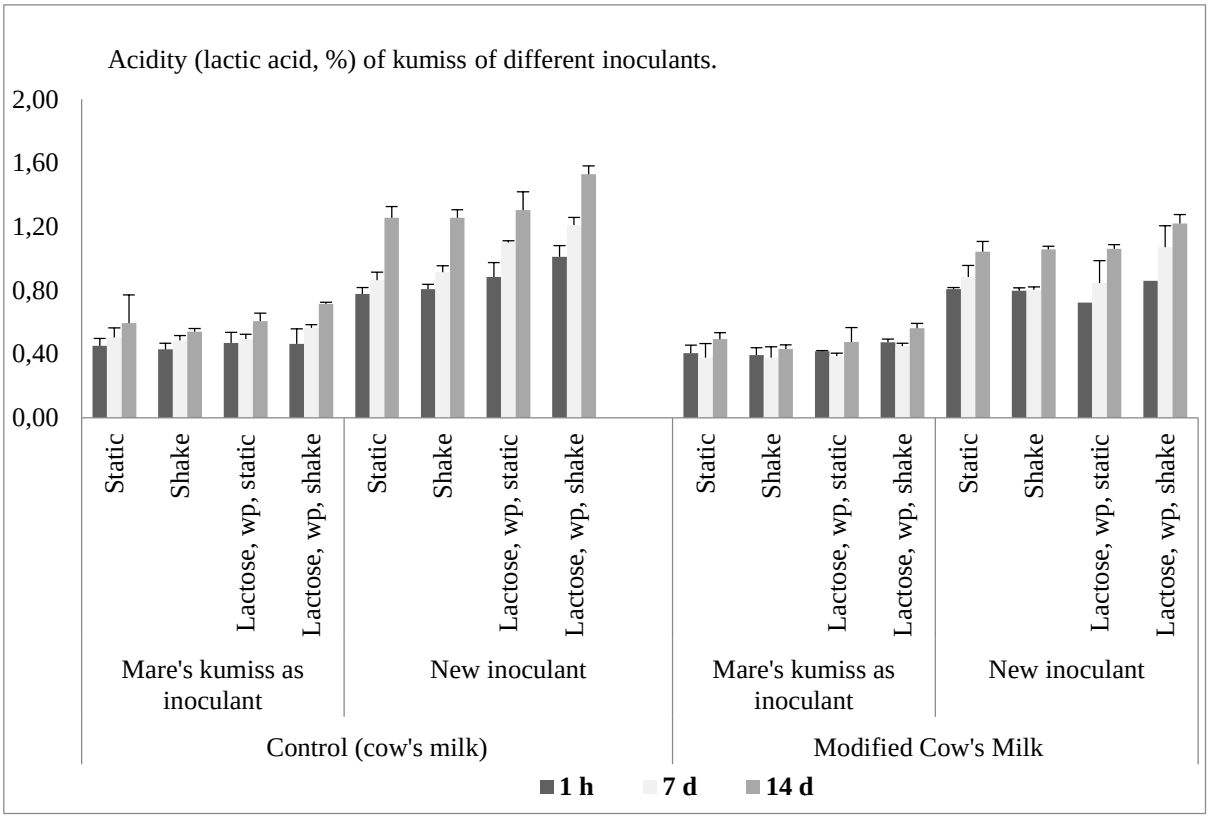

Figure 4a. Effects of addition of water, lactose and whey proteins (WP) to cow's milk inoculated with mare's kumiss or newly developed inoculum: Acidity (lactic acid, %) after static and shake incubation.

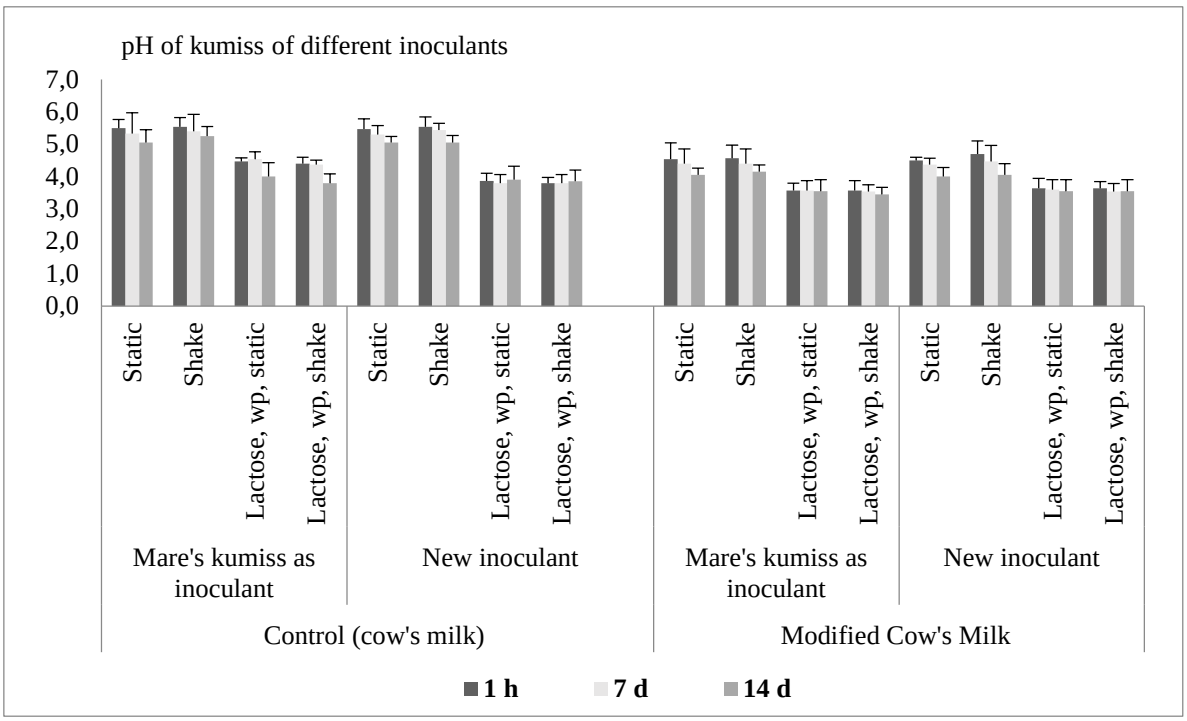

Figure 4b. Effects of addition of water, lactose and whey proteins (WP) to cow's milk inoculated vith mare's kumiss or newly developed inoculum: pH after static and shake incubation.

As can be seen in Figure 5, the acidity values of the samples made with modified cow's milk increased gradually in the 14th d cold storage. The highest acidity value (1.34±0.13) was observed in samples of cow's milk fermented with new inoculant and kept in cold storage for 14 d. Acidity were found in modified cow's milk fermented with fresh yeast as 1.1±0.8 in cow's milk fermented with mare's kumiss as 0.61±0.07 and in modified milk fermented with mare's kumiss as 0.49±0.05. Observation of 1.1±0.12% acidity on the 14th d of cold storage in the samples of modified cow's milk inoculated with new inoculum indicated that the product will have a longer shelf life. As a result of taste panel, it was seen that the samples made with cow's milk were thick and tasteless. When dilution was made by equal amount of water to the kumiss, the samples were become more tasteless (data not shown).

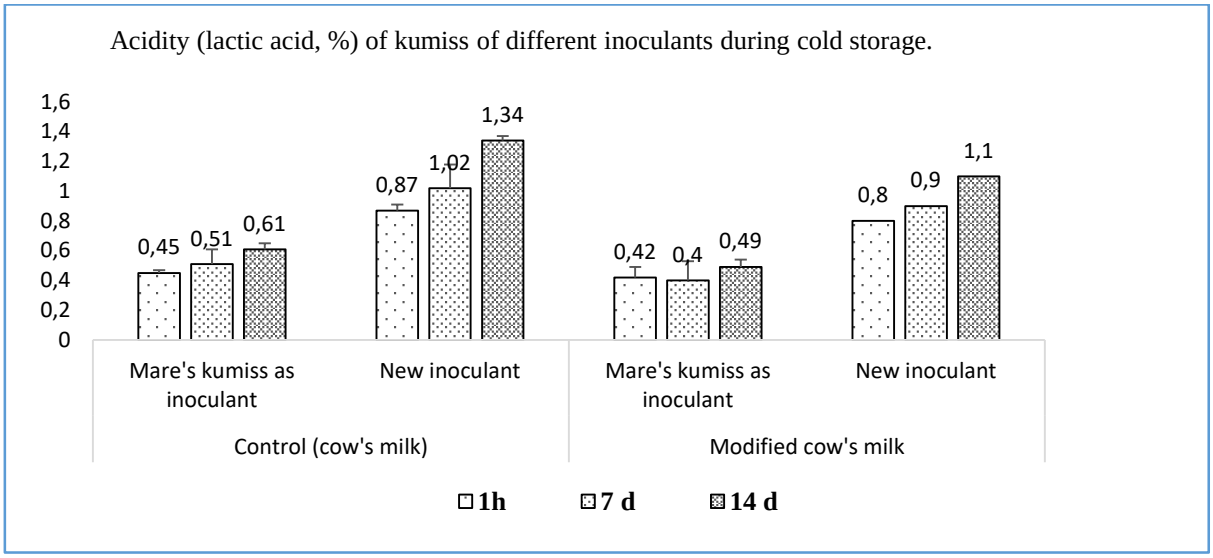

Figure 5. Effect of different inoculants on acid development in 4 different kumiss samples at 1st h, and 7th and 14th d of cold storage, 4 °C.

In the 6th step of the process development, the most admired sample (the MCMK sample) in the previous step (Step 5) was used as inoculant at a rate of 5%. Static incubation was applied to the samples and analyzes were made at the 1st h and 7th and 14th d of the cold storage (Figure 6a, b, c).

It was observed that adding lactose, WP or both lactose and WP to the water added milk wappeared to be important for the development of acidity during cold storage, which was not obvious at the end of the incubation. The best acidity development was observed on the 14th d of cold storage in the samples added water, lactose and WP (1.1±0.1) (Figure 6a).

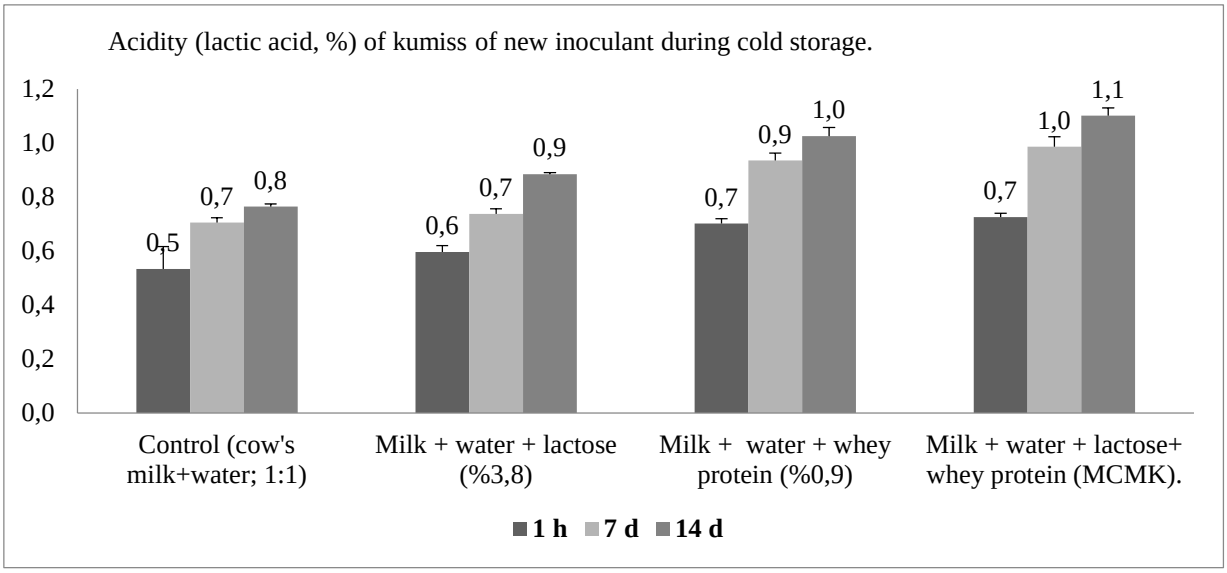

Figure 6a. The acidity (lactic acid, %) changes of kumiss samples during cold storage, 4 ºC.

No difference was observed in terms of pH change in the kumiss samples produced after adding lactose and/or WP to the milk to which water was added. However, on the 7th d of cold storage, the highest pH decrease was observed in the samples added together with lactose and WP. This change did not persist and no significant difference was observed between the groups in the 7th and 14th cold storage d (Figure 6b).

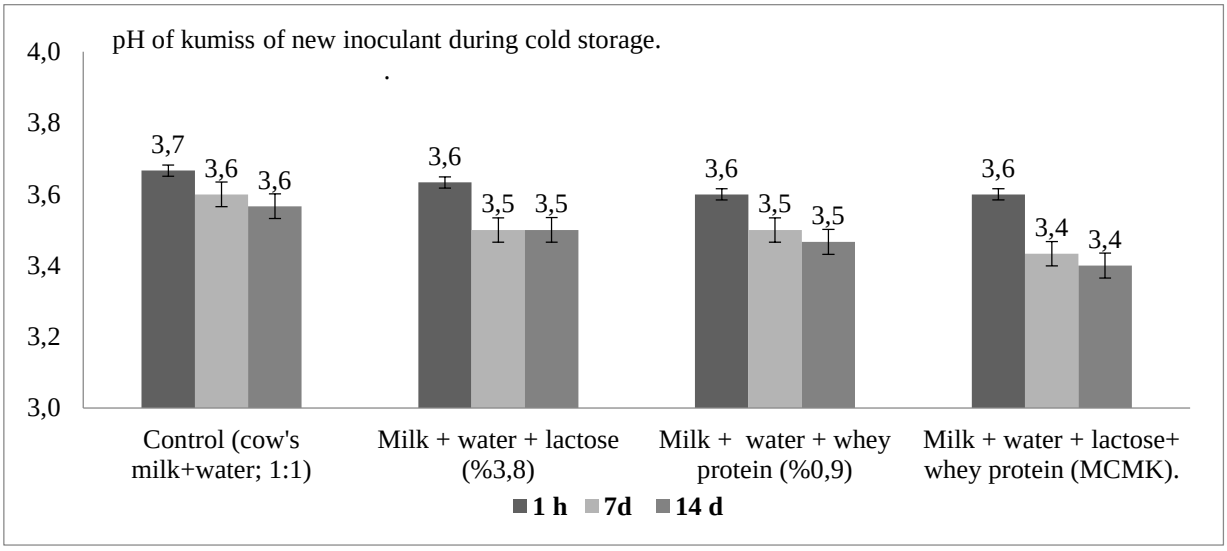

Figure 6b. The pH changes of kumiss samples during cold storage, 4 °C.

The general liking scores of the samples are given in Figure 6c. Control samples were liked the least and the MCMK samples the most. The panelists sought this taste because they were familiar with the taste of buttermilk. Control samples were reported to be thick, sticky,

and tasteless. It was reported that some of the water added samples were bitter and tasteless. Adding lactose to milk together with water created a sourer taste, and adding WP created a more aromatic and tart taste.

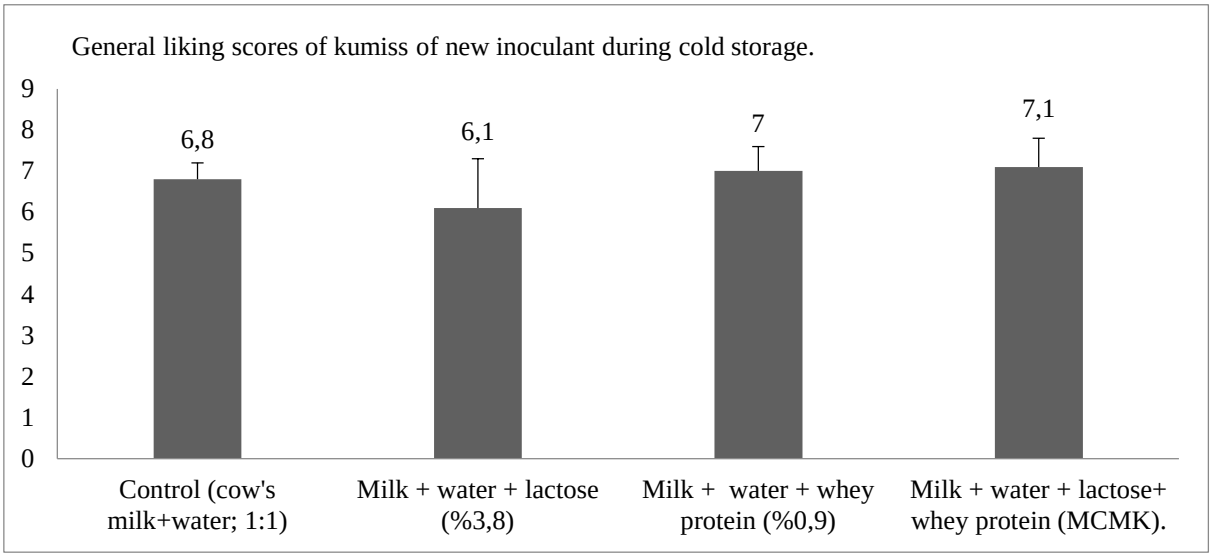

Figure 6c. General liking scores of the different kumiss samples during cold storage, 4 °C.

The mare's kumiss that purchased from a commercial sell point was not liked by the panel members. Nevertheless, MCMK has found to be more aromatic and attractive for drink. All the panelists reported that there was no similarity between this new product (MCMK) and mare's kumiss, but that they would prefer to consume this product over mare's kumiss (data not shown). After this step, 4 different inoculants were prepared by selecting 5 each from MRS, M17 and PDA colonies. After the obtained MCMK samples were kept at 4 °C for 1 h, acidity, pH and general liking tests were performed (Figure 7). The results of the analysis were compared with the results obtained from the commercial mare's kumiss. Horse kumiss had a general liking score of 3.4±2.1 and panelists did not like the acrid and bitter taste in it. The M17 + PDA sample had the lowest appreciation score, with the highest acidity and lowest pH values. MRS + M17 + PDA samples containing 15 isolates were the best samples from other samples in terms of acidity development, pH decrease and general liking score.

As a result of Maldi-Toff analysis, 9 out of 15 isolates could not be identified. Two of the isolates were *Lb. bulgaricus*, 2 of them *Str. thermophilus*, one was *Saccharomyces* sp. and the other was *Torula* sp.

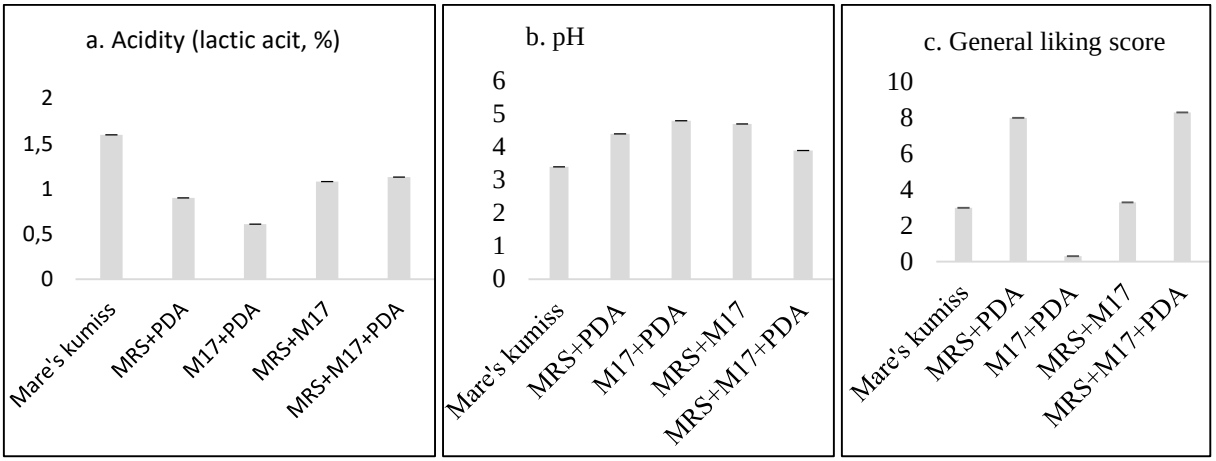

Figure 7. Acidity (lactic acid, %) (a), pH (b) and general liking scores (c) of kumiss samples inoculated with consortia of different strains of bacteria and yeasts.
*Mare's kumiss; a commercial product purchased for using as benchmark sample. Predominant five isolates taken from each MRS; de-Man Rogosa Sharpe agar, M17; M17 agar, PDA; Potato dextrose agar plates inoculated from kumiss samples.

Discussion

Kumis is a product developed by the Central Asian and Caucasian communities in the historical process and produced and traded in homes and industry until today (Tegin & Gönülalan, 2014b). It is known that mare's milk is different from cow, sheep, goat and buffalo milk (Hinz et al., 2012). It is similar to albuminous milks such as human and goat milk in terms of serum proteins and high lactose content (Hinz et al., 2012). It is known that more than a century ago, products obtained as a result of fermentation of cow's milk with kumiss instead of mare's milk are used in the treatment of sick and weak individuals (George et al., 1872; Dhewa et al., 2015; Musaev et al., 2021). It has been reported that kumiss has the potential to be consumed widely in other communities (Yangılar et al., 2016; de Melo Pereira et al., 2022). However, the biggest reason limiting this potential is the inability to produce sufficient mare's milk (Shaimardanova, 2012; Afzaal et al., 2021). Efforts are constantly being made to produce kumiss by using other milks, especially cow's milk, which is the most abundant milk in the world. Legielski (1874) has mentioned that soft kumiss, hard kumiss and diabetic kumiss are made from cow's milk. In this study, although mare's kumiss was not liked by the panelists, MCMK was liked.

In some studies, kumiss was produced without adding water to cow's milk (Malacarne et al., 2002; Rakhmanova et al., 2021). Modification from the cow's milk has been made in different ways. The water ratio that add to milk is not similar in studies (Özer, 1997; Teichert et al., 2020). In this study, after adding water to milk at a ratio of 1: 1, 3.8% lactose and 0.9% WP were added to increase lactose to 6% and WP to 1.2%, as in mare's milk. We could not

find any other study that modified cow's milk in this way. Therefore, comparing the findings of this study with the findings of other studies may not be confirmatory. It has been also stated in the previously made studies that many different parameters may affect one or more quality parameters of kumis (Malacarne et al., 2002; Teichert et al., 2020).

There is no production and consumption of mare's milk in Türkiye. On the other hand, mare's kumiss is produced only by a Kyrgyz family business and sold directly to the consumer. Kumiss obtained from this family business was used as inoculant in the study and as reference sample in taste tests. The acidity of the kumiss we supplied was 1.57±0.1 and the pH was 3.9±0.1. The samples came in 24 of 250 mL glass bottles in a parcel. It was observed that there was no difference between the samples in the analyzes performed on 3 randomly selected samples. None of the panelists liked these kumiss and refused to drink it. However, one of our students, who is a citizen of Turkmenistan, stated that this kumis, which we do not like, is very tasty, very pleasant and has the same characteristics as the mare's milk kumiss that he is accustomed to drinking in his country. This result showed us that food consumption habits should be taken into consideration more. Even if enough of our MCMK is produced in Türkiye, it will not be easy for its consumption to become widespread. For this reason, it may not be appropriate for mare's kumiss consumers. The MCMK developed in this study, which our panelists liked to drink and defines as a product that does not resemble yoghurt, kumiss or ayran, may not have pleasent taste for some other consumers.

In this study, UHT market milk from the same batch was used. In this way, we have avoided the mistakes that may arise from heating, cooling, homogenization and standardization. Coliform group bacteria and *staphylococci* were not found in milk, commercial kumiss and experimental samples. In this study, MCMK was obtained by adding 5% of kumiss to milk. Then MCMK itself was used as 5% inoculant. Later, the inoculating culture was developed. More than 5% inoculant has been used in traditional kumiss production (Pastukhova & Gerbeda, 1982; Ishii et al., 2014; Wu et al., 2021). Traditional mare's milk is made in the form of increasing the amount by adding raw mare's milk to the kumis made before (Wu et al., 2021). Possibly, thanks to the constantly high acidity value in the product and the continued fermentation of the kumiss dominant culture, the reproduction of harmful bacteria in raw milk is suppressed and the hygienic quality of the product increases as the product is fermented. It is probably possible to use raw milk in this way. However, there are also researchers who report that the use of raw mare's milk in kumiss may be harmful in terms of hygiene and public health (Kınık et al., 2000; Dankow et al., 2006; Pastukhova & Gerbeda, 1982). Probably such a production method is not suitable for commercial kumiss production. It is clear that this

production model will cause difficulties in hygienic and standard production. For this reason, it has been suggested that milk should be pasteurized, as in yoghurt, ayran and kefir, for the standard production (Özer, 1997; Küçükçetin et al., 2003; Zhang et al., 2020).

While there is less protein and fat in the composition of mare's milk than cow's milk, there is more serum protein and lactose (de Melo Pereira et al., 2022). In order to make these values of cow's milk similar to mare's milk, full-fat cow's milk was modified. Values are given in Table. The MCMK is easy to produce, cheap and have the qualities to set an example for technological applications. Very different incubation methods have been used in the production of mare's kumis and other experimental kumiss studies. Shaking incubations were mostly made (Koroleva, 1988; Malacarne et al, 2002). Studies using static incubation have also been made (Küçükçetin et al., 2003). In this study, it was determined that a bitter and prosaic taste was formed in the shaken kumiss samples made after adding water to the cow's milk. These flavors did not occur in the shaken samples of cow's milk without modification. It was seen that excessive shaking would not be appropriate in the samples with added water. Static incubated samples yielded results similar to those produced with static for 12 hours and shaking every 3 hours for 12 hours (Figure 4b). In the light of these results, it was concluded that it would be appropriate to use static incubation because it is more suitable for the industry and economy and probably has a longer shelf life of kumiss.

In the Turkish Food Codex Legislation on Fermented Dairy Products (Legislation No: 2009/ 25), kumiss is stated as "*Lactobacillus delbrueckii* spp. *bulgaricus* and *Kluyveromyces marxianus* cultures are used as fermented milk product". In this Legislation, although it was not stated that mare's milk should be used in production, no information was given about the milk to be used. The reason for this may be that kumiss is not yet traded in Türkiye. The Legislation may not be fully detailed. The protein content of kumis is not reported in the Legislation. The ethanol content is at least 0.5%. The amount of lactic acid is at least 0.7%, and number of specific microorganisms (cfu/g) is at least 107, the number of added microorganisms (cfu/g) is at least 106 and the number of yeast is at least 104 (cfu/g). Salt content is not reported. The findings obtained from this study were found to be in compliance with the Legislation. The acidity of MCMK was at least 0.7% lactic acid (Figure 1 – 3, 4a, 6a, 7a). The amount of alcohol in the MCMK did not exceed 0.5%. It was observed that the bacteria and yeast contents were in compliance with the criteria reported in the Legislation even on the 14^{th} d of cold storage. In order for the kumis produced in this study to fully comply with the Legislation, it is necessary to increase the amount of alcohol. Alcohol content naturally increases especially in the first week of cold storage (Ishii et al., 2014; Dönmez et al., 2014; Tang et al., 2020). However, on

the 14th d of cold storage in this study, the alcohol rate remained below 0.5%, which was the desired value in the Legislation. Since the alcohol content is not higher than 0.5%, it is not possible to produce and trade this kumis in Türkiye. Because the product does not provide the values required in the Legislation. In this study, it was aimed to produce non-alcoholic kumiss and our findings are suitable for the purpose of our study. It has been reported that *Saccharomyces lactis* is the main yeast producing alcohol in kumiss, there are differences in the amount of alcohol according to the fermentation time, and the amount of alcohol is 1% even in sweet kumis (Li et al., 2020). In this study, samples were taken from low alcohol beer production models (Yaygın, 1994). Salt was not added to the samples for easier evaluation of the resulting flavors.

It has been mentioned that the trade of kumiss will be increased thanks to the development of production technology (Berlin, 1962; Özer, 1997). Küçükçetin (2003) modified cow's milk using the membrane filtration technique and it was reported that the produced kumiss was more popular than mare's milk kumiss. In another study, the shelf-life of the kumiss has been increased (Ender et al., 2006).

In previous studies, similar to our study, kumiss milk has been prepared by adding water, sugar, whey and other supplements to cow's milk (Özer, 1997; Küçükçetin et al., 2003). However, there is no complete similarity and harmony between the studies. Our study is probably most similar to the study by Küçükçetin (2003). The researcher used mare's kumiss as the main inoculant at a rate of 20% to modified cow's milk (90% water, 6.4% WP and 3.6% milk powder). We obtained kumiss from the same farm as the researcher and used as inoculant at first step of the study. In our study, the process was simplified by adding water to cow's milk in equal proportions, adding lactose and WP to make the content look like mare's milk. The researcher reported that mare's kumiss received the highest score in sensory tests. However, in this study, mare's kumiss was not liked by the panelists and MCMK was the most liked (Figure 7c). Küçükçetin (2003) made his inoculant at a different way from us. Inoculant production medium and the kumiss milk were incubated at 22 °C with shaking. We did it statically at 28 ^{o}C. Differences in alcohol levels may be due to different applications. It may not be accurate to make a one-to-one comparison with Küçükçetin's and our results.

Since different materials and methods were used in the researches, it is difficult to compare the results. Although the starter cultures of kumiss are traded, standard strains have not yet been found. In this study, mare's kumiss was used to develop inoculant and sequential MCMK were made for inoculant adaptation. We tried to select culture strains from MCMK dominant flora. At this stage, more research should be done to develop best starter culture

appropriate for material, method and cultural demands. Rakhmanova et al. (2021) selected a strain from mare's kumiss (colonies that grew by incubating the MRS agar plates used in strain selection at 37 °C and PDA agar plates at 28 °C) for cow's milk kumiss and fermented with a combination of the two strains. Researchers have suggested incubation at 36 ^{o}C for 16 hours by adding 4% of culture obtained by the combination of a bacterium and yeast to milk. The researchers have determined that yeasts were dominant in the mare's milk and bacteria in cow's milk. It has also been reported by the researchers that acidity and pH development are higher in mare's milk kumiss. Although the study has been designed differently from our study, the aims are similar.

Conclusion

The use of 5% freshly prepared kumiss or a consortium of bacteria and yeasts selected from kumiss appeared to be recommendable as inoculant. Modification of cow's milk bay addition of 50% (v/v) water, 3.8% (w/v) lactose and 0.9% whey proteins in it can be suitable for making kumiss like fermented dairy drink. Static incubation of milk at 28 °C for 24 h, and 4.4 pH and 0.7% lactic acid levels in kumiss appeared to be appropriate for making such a drink. A pH of 3.5 to 4, a maximum of 1.5% lactic acid level in the drink appeared to be good values for such a drink at the 14th d of cold storage. It is concluded in this study that more detailed studies on the subject are needed to develop cow's milk kumiss or kumiss-like drinks.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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