

THE PROCESS OF YAK MILK FERMENTATION BY POLYCOMPONENT STARTER CULTURE

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➤ Supporting Information

ABSTRACT: The paper presents a comparative characteristic of the fermentation processes of yak and cow milk samples with a high fat content of 1.5% and 6% with a multicomponent starter culture, which includes *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp *Lactis* and *Streptococcus thermophilus*. Acid formation in the process of milk fermentation under the influence of the starter microflora was assessed by the dynamics of changing in titratable (Ac) and active (pH) acidity over time. The course of the formation of the structure of the resulting clot was monitored on a rheometer, fixing the viscosity characteristics of the fermented milk clot in dynamics. It has been established that the increase in acidity occurs more intensively in yak milk in comparison with cow's milk with a corresponding acceleration of the formation of a fermented milk clot. In conclusion, the resulting clots were subjected to sensory analysis with the identification of the best sample, which was fermented yak milk with a fat mass fraction of 6%.

Keywords: Cow milk, Fermentation, Rheological properties, Starter culture, Yak milk.

INTRODUCTION

Milk is the most valuable food product and makes a significant contribution to the human diet (Pronko et al., 2020; Duguma, 2022). However, some countries of the world, such as Mongolia, Nepal, and China, have relied on milk from other animals like yak for the production of dairy products. Yak milk is unique dairy product in terms of organoleptic, physico-chemical, and technological properties, as well as nutritional value (Agyare and Liang, 2021; Singh et al., 2023). There are many factors that are attributed to these variations, including the type of animal, its health indicators, rearing conditions, feeding characteristics, climatic conditions, season, inter alia. In particular, studies of the composition of milk depending on the season revealed differences in lactose content, fractional composition of protein, fat and other components (Elemanova, 2022; Li et al., 2011). It should also be noted that the milk of different animal species has different biological potential. Studies have shown that yak milk is more nutritious than cow's milk (Elemanova, 2022, Saalieva and Usubaliev, 2020). Yak milk is distinguished by a higher content of fat - 5.5-7.2%, protein - 4.9-5.3%, milk sugar - 4.5-5.0% and minerals - 0.8-0.9% (Nikkhah, 2011), in contrast to the cow, where the corresponding figures were: 3.8%, 3.3%, 4.8% and 0.7% (Smirnova et al., 2020). The range of nutrient content varies depending on various factors: genotype, age and diet of the animal (Nikkhah, 2011; Saalieva and Usubaliev, 2020; Smirnova et al., 2020; Elemanova, 2022). In these study, milk from yak selected at Arpa pasture in the Naryn region of the Kyrgyz Republic contained protein, fat, and lactose in proportions of 4.8%; 4.95% and 5.1%, respectively (Aralbek kzy et al., 2022).

The chemical composition of milk affects the technological process of obtaining dairy products (Elemanova, 2022). Therefore, the study of the influence of the chemical composition of alternative milk on its technological properties is of considerable scientific and practical interest for the production of various products. The range of dairy products produced from yak milk according to national recipes inherited from time immemorial is diverse, but their production has so far been adapted only for home conditions in rural areas. On an industrial scale, the processing of yak milk in the Kyrgyz Republic is difficult, since there is no developed regulatory documentation and scientifically based technology for dairy products from yak milk, which is of particular relevance in light of the predicted increase in the number of yaks in different regions of Kyrgyzstan (Saalieva and Usubaliev, 2020).

Among the variety of dairy products, fermented milk or fermented milk (diet) drinks are of particular interest from a physiological functionality point of view. For the preparation of these are used - pure cultures of lactobacilli or their combinations with other microorganisms (yeast, propionic acid bacteria) (Savaiano and Hutkins, 2021; Kaur et al., 2022).

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Most lactobacilli are now classified as probiotics, which in recent years have been key components of functional foods, of which dairy accounts for more than 65 percent (Taye et al., 2021; Fesseha et al., 2021).

The choice of starter cultures is influenced by the type of product and, accordingly, the technology for preparing fermented milk products. The study of the process of fermentation of milk, in particular yak milk, using various types of starter cultures, is the scientific basis for the technology of developing new fermented milk products. In this connection, the purpose of these studies was to study the acid-forming ability of a consortium of microorganisms (*Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp *Lactis* and *Streptococcus thermophilus*) in yak milk, as well as the rheological properties of the fermented milk clot formed during fermentation.

MATERIALS AND METHODS

Milk samples

Yak milk was obtained directly from high mountain pastures (Arpa) in the Naryn region of the Kyrgyz Republic. For the study, the average milk batch was taken, which was collected from 5 yaks, which had similar calving periods. After milking, the milk was filtered, poured in sterile plastic bags and transported to the laboratory in a special thermal bag ($8\pm 1^{\circ}\text{C}$) for storing perishable products. The object of comparison was pasteurized cow's milk with a mass fraction of fat of 1.5% and 6%, obtained from a local supermarket (Bishkek, Kyrgyz Republic).

Starter cultures

A multistrain starter (Lyofast SAB 430A, Italy) containing pure cultures of lacto- and bifidobacteria (*Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp *Lactis* and *Streptococcus thermophilus*) was used as a starter culture. This starter culture is mainly used in the production of probiotic fermented milk products with a soft and delicate texture of medium viscosity. The optimal temperature for the growth of microorganisms is in the range of $37\text{--}45^{\circ}\text{C}$. The standard mode of operation of the starter culture starts at a temperature of 43°C , $\text{pH}=4.5\pm 0.15$, the fermentation time is on average 6-7 hours.

Physical measurements

Determination of titratable and active acidity was carried out by standard methods (AOAC, 2005). Studies of the rheological properties of fermented milk samples were carried out on a Rheometer MCR-302 device (Anton Paar, Graz, Austria) with a cylindrical geometry (CC27-SN26341) in the time base mode. The choice of this instrument for measuring non-Newtonian fluids, which include fermented milk drinks, is due to the fact that the rheometer allows to analyze such media without disturbing their structures (Smanalieva et al., 2021). The control of the gel formation process and the control of this process make it possible to obtain a clot with the necessary structural and mechanical properties. The temperature was chosen in accordance with the recommended one (Elemanova et al., 2022), optimal for the growth of starter cultures (43°C).

Sample preparation

Raw yak milk was separated on a "Neptune" laboratory separator with subsequent normalization according to the mass fraction of fat 1.5% and 6.0%. Normalized milk was pasteurized at a temperature of $90\text{--}95^{\circ}\text{C}$ with a holding time of 5-8 minutes and cooled to the fermentation temperature ($43\text{--}45^{\circ}\text{C}$). The starter culture was added into the prepared milk with thorough mixing at the rate of 1 UC/100 liters of milk. Then stirring was continued for 10 minutes and the acid-forming ability of lactobacilli in yak milk was studied. In a similar way, comparison samples were prepared for the study - cow's milk with a mass fraction of fat of 1.5% and 6.0%. The same samples (fermented cow's and yak's milk) were used to study the process of structure formation.

Sensory analysis

Sensory tests were done to evaluate fermented yak and cow milk samples. Descriptive tests were conducted using descriptors adopted from Drake (2007). Total of ten trained panellists evaluated sensory properties: colour, texture, odour, taste, mouthfeel, and overall acceptance of the fermented yak and cow milk samples. All panellists were trained under the "Sensory Analysis" study program.

RESULTS AND DISCUSSION

In this study, the biochemical properties and starter microflora activity of yak milk and the formation of the associated structure of the fermented milk clot were studied in order to establish technological parameters for the production fermented milk products from yak.

Patterns of acid formation during the fermentation of yak milk by lactobacilli

The main biochemical process that occurs during the production of most fermented milk products is lactose fermentation, the end product of which is lactic acid. Lactic acid determines the sensory properties of the product, namely

texture, taste, and smell. The activity of acid formation of lactobacilli depends on a number of factors, including the composition of the nutrient medium in which they are located. Comparative studies were carried out on the acid-forming activity of lactobacilli in starter cultures added to cow's and yak's milk of different fat content. After adding the starter, the milk samples were placed in a thermostat with a temperature favorable for the starter cultures (43 °C). The parameters, determined every 60 minutes, were titratable (Ac) and active (pH) acidity (Figures 1 and 2).

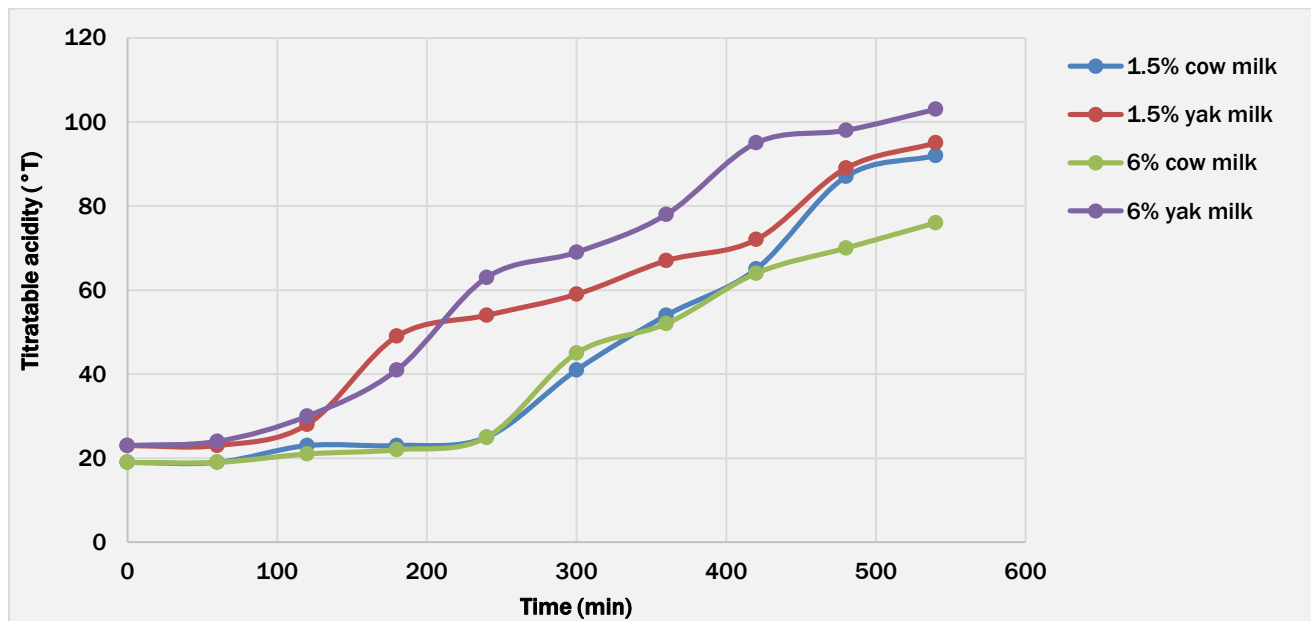


Figure 1 - Dynamics of changes in titratable acidity in yak and cow milk samples, fermented with a thermophilic starter culture (*Lactobacillus acidophilus*, *Bifidobacterium animalis subsp Lactis* and *Streptococcus thermophilus*)

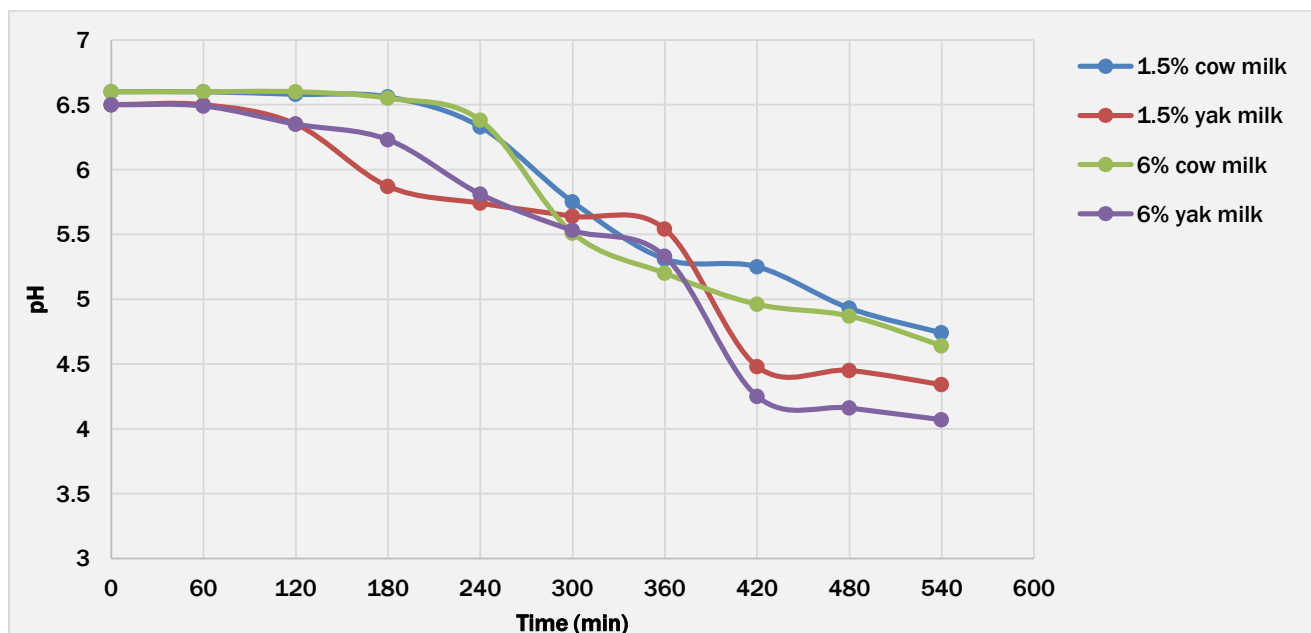


Figure 2- Dynamics of changes in active acidity (pH) in yak and cow milk samples, fermented with a thermophilic starter culture (*Lactobacillus acidophilus*, *Bifidobacterium animalis subsp Lactis* and *Streptococcus thermophilus*)

Figure 1 demonstrates the differences in the rate of passage of biochemical processes, namely lactic acid fermentation, in yak and cow milk. These differences are especially noticeable starting from the second hour of observations, when there is a rather sharp increase in titratable acidity in yak milk samples. At the 180 minute of fermentation, the difference between the acidity of yak and cow milk reaches almost 30 °T for 1.5% milk and about 20 °T for 6% milk. A similar sharp increase in titratable acidity for cow milk begins only after 4 hours from the moment the starter is added and until the end of fermentation, the rate of acid formation slowly decreases for 6% milk, reaching an acidity of 75 °T. For 1.5% cow milk, after almost the same fermentation process as 6% milk, at 420 minutes there is a second noticeable jump in the level of acidity, which is still difficult to explain reliably. Perhaps this is due to the species composition of the starter microflora, one of the components which is bifidobacteria. It is known that these microorganisms develop slowly in milk, because they are obligate anaerobes, and also do not show caseinolytic activity.

These anaerobes can absorb casein only after partial hydrolysis (Funk and Irkitova, 2016). It can be assumed that at the first stages of fermentation, bifidobacteria do not participate in the fermentation of lactose for the reason indicated above. As the lactic acid fermentation develops, lactobacilli, capable of breaking down casein, lead to the formation of poly- and oligopeptides from it, stimulating the growth of bifidobacteria, which are now able to ferment lactose. In this regard, we can observe a second noticeable acceleration of acid formation, starting from the 420 minute of the process.

In this context, yak milk demonstrates similar dynamics in the development of lactic acid fermentation. After a noticeable increase in the level of acidity, there is a slight slowdown, starting from the 3 hour for 1.5% milk and from the 4 hour for 6% milk, and a second jump in acidity from 75 °Th to 90 °Th for 1.5% - milk from 7 to 8 hours of the fermentation process, and for 6% milk - from 80 °T (6 hours) to 95 °T (7 hours). At the same time, the minimum desired acidity of the final product, equal to 75 °T, is achieved after 540 minutes for 6% cow's milk and after 340 minutes for 6% yak milk. This indicates an intensification of the technological process of processing the latter into fermented products. This is also evidenced by the moment of the beginning of the formation of a fermented milk clot, which occurs when the acidity reaches about 35 °T. Such acidity is reached at the 140 minute for yak milk and at the 270 minute for cow milk.

A similar picture can be observed in Figure 2, which shows the dynamics of changes in active acidity in the fermented samples of the studied milk and the object of comparison.

The obtained data indicate that the studied yak milk is a favorable nutrient medium for the development of starter microflora with a reduction in the duration of the technological process for the production of fermented milk products by more than 3 hours.

Patterns of acid coagulation of yak milk proteins in the process of its fermentation by a polycomponent starter culture

The lactic acid formed during the fermentation of lactose under the action of lactobacilli causes coagulation of casein, which leads to gel formation of milk, i.e. the colloidal system from a freely dispersed state (structureless) changes into a coherently dispersed state (structured - gel) (Bylund, 1995). And this process is the most important in the development of fermented milk products, and the consistency, thixotropy, and structural integrity of the finished product depend on this process. The formation of the structure of a fermented milk clot during the fermentation of milk samples with a multicomponent starter culture was observed by changing the viscosity on a Rheometer MCR-302 device. The results of the analysis are presented in Figure 3.

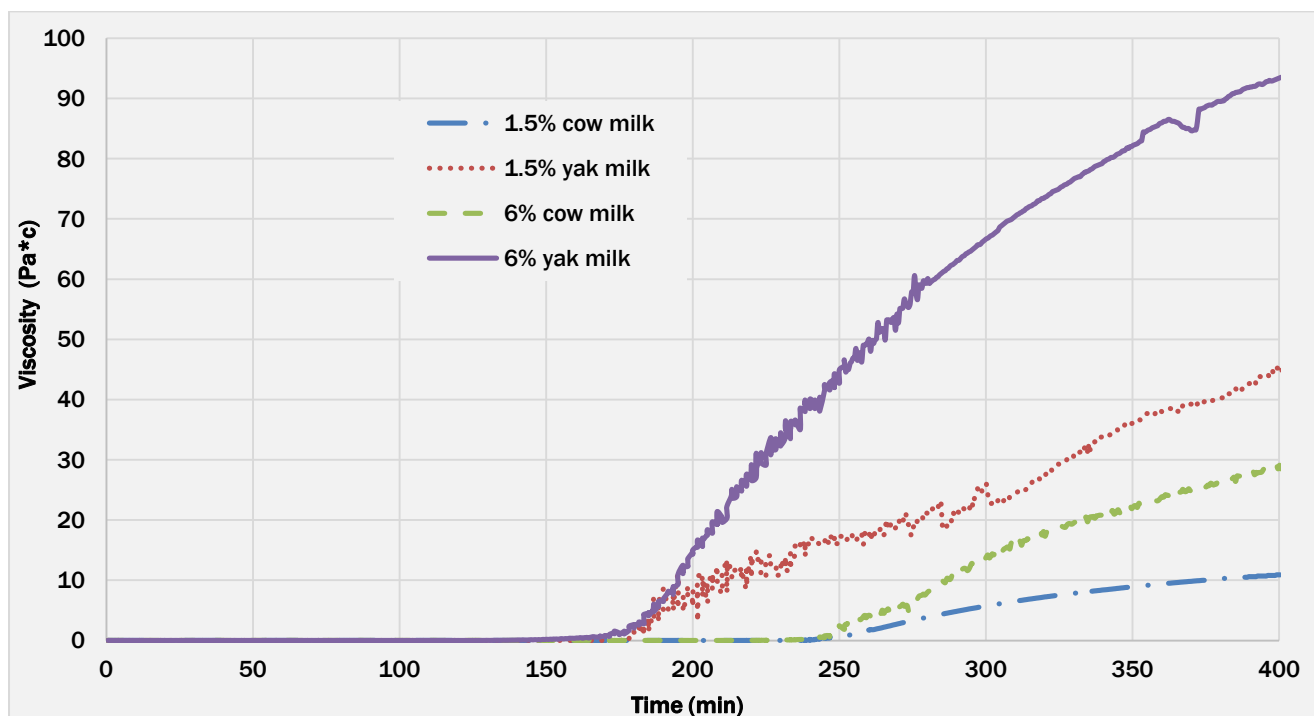


Figure 3 - Viscosity change in the process of structure formation during fermentation of samples of yak and cow milk with polycomponent starter.

Figure 3 shows the duration of individual stages of acid coagulation of yak and cow milk proteins differs markedly. In particular, the induction period of the structure formation process in yak milk lasts 180 and 200 min for 6% and 1.5% samples, respectively, while for cow milk this period lasts 250 min. This more than an hour difference is primarily due to the composition of milk, because all other experimental conditions are equal for the two studied types of milk. The amount of casein and the size of casein micelles determine the rate of acid coagulation of proteins and the strength of the resulting clots (Bylund, 1995). The influence of the composition of milk, namely the content of protein and lactose, on

the process of gel formation is also confirmed by the data of other researchers (Elemanova et al., 2022; Yang et al., 2018).

Yak milk is characterized by a high protein content (4.9-5.3%) (Nikkhah, 2011), which leads to a reduction in the induction period of structure formation under the influence of lactic acid. Yak milk also contributes to a significant increase in the viscosity of the formed clot at the 400 minute in comparison with cow milk fermentation - about 3 times for 6% milk and 4 times for 1.5% milk. The obtained data indicate the intensification of the technological process for the production of fermented milkdrinks from yak milk with the formation of clots with improved rheological characteristics.

Sensory analysis of fermented yak and cow milk

The results of the analysis carried out by a group of tasters consisting of 10 people are presented in Figure 4. All samples had a pleasant sour-milk taste and smell. Samples of fermented yak milk had a slight specific taste. The consistency of the clots differed significantly according to t-tests (paired-sample tests) with a 95% confidence interval with SPSS software (SPSS Inc., Chicago, IL, USA). When fermented, yak milk gives a well-formed clot with a creamy, viscous consistency, while cow milk has a more viscous clot, characteristic of acidophilic drinks. The best performance was noted for a sample obtained from 6% yak milk.

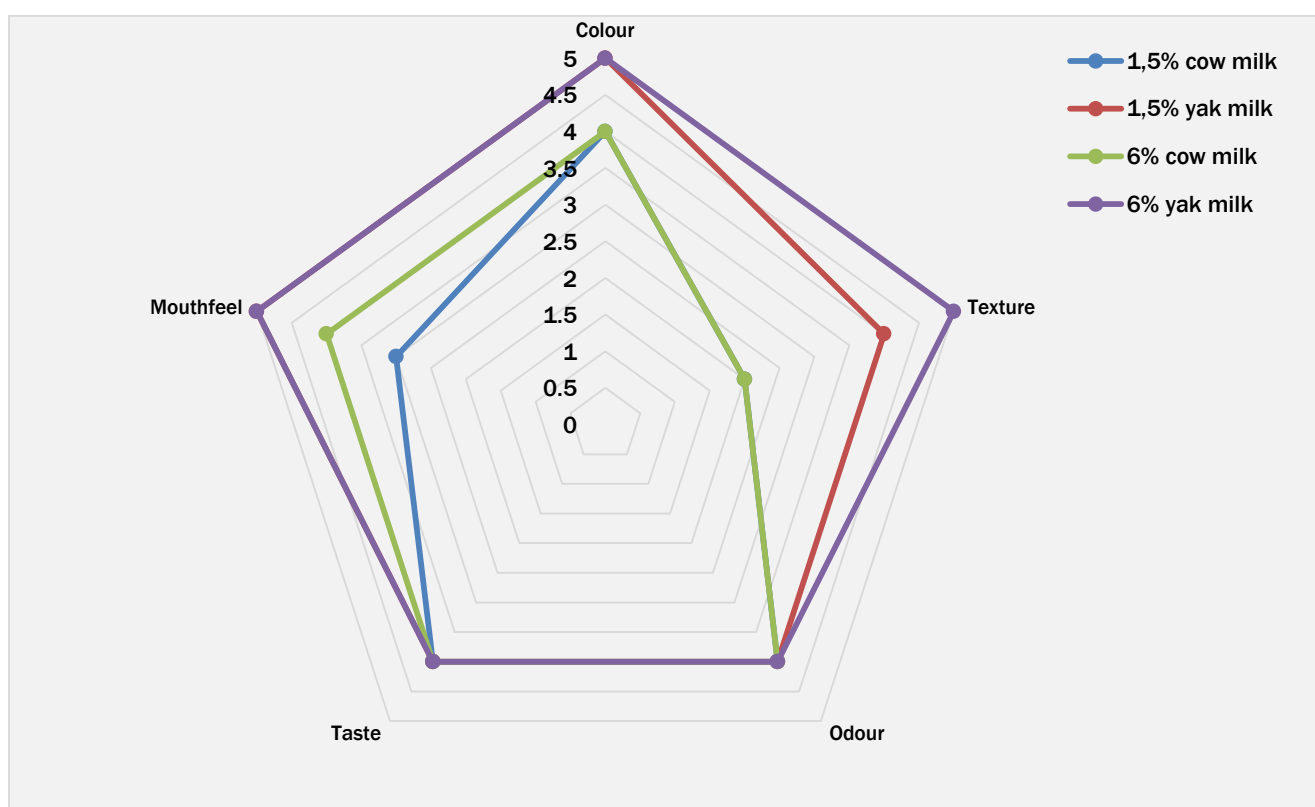


Figure 4 - Sensory analysis of fermented milk samples

CONCLUSION

The current study found that the acid-forming activity of lactobacilli manifests itself to a greater extent in yak milk, which is evident from the rate of increase in titratable acidity and the time it takes to reach the desired acidity of the final product (75 °T), which was ≈ 340 min for 6% yak milk and ≈ 540 min for 6% cow milk. That is, the duration of the technological process for producing a fermented milk drink from yak milk using the studied complex of microorganisms is reduced by 3 hours. This is due to the composition of the raw materials, characterized by a high content of dry substances, including protein. With regard to rheological properties (viscosity) of the fermented milk clot formed during the fermentation, this study revealed noticeable differences in the value of the induction period for two types of milk: 180 minutes for yak milk and 250 minutes for cow milk. By the end of the fermentation process, the viscosity of the fermented milk clot obtained from yak milk with a 6% mass fraction of fat and 1.5% is approximately 3 (93 Pa·s) and 4 (45 Pa·s) times, respectively, higher than the case is for cow milk. Organoleptic (sensory) analysis of the fermented samples confirmed 6% fermented yak milk as the best. According to the results of the research obtained in this work, yak milk is recommended for the production of yogurts and fermented milk drinks.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contributions

A. Usubalieva = organization of the experiment, literature search, discussion of the results of the analysis, writing the article, reviewing and editing; M. Musulmanova = guidance and consultations, direction of research and discussion of the results of the analysis, correction of the article; N. Saalieva = delivery of yak milk, preparation of samples, control over the fermentation process of yak and cow milk, processing of the data obtained; Z. Ozbekova = work on the rheometer, conducting, discussing, plotting and processing the rheological part of the experiment, reviewing and editing; A. Aralbek kyzy = conducting an experiment - determining titratable acidity, pH, monitoring the fermentation process of yak and cow's milk; A. Deidiev = sensory analysis of fermented yak and cow milk and tasting.

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Consent to publish

All authors agree to the publication of this manuscript.

Competing Interests

The authors have not declared any competing interest.

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