

COMPARATIVE STUDY OF FERMENTATION PROCESSES OF YAK AND COW MILK

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ABSTRACT

Yak milk is a valuable raw material for the production of functional food. Investigation of the fermentation process with commercial starter cultures is conducted to find suitable starters for producing fermented yak milk products on an industrial scale using modern rheological, microbiological, and sensorial methods. According to the acidification curve and rheological measurement, the gel-forming time of yak milk with mesophilic, thermophilic and mixed (meso-thermophilic) starter cultures was not significantly different from cow milk; but, yak milk had a significantly higher gel strength in all cases. The viable bacteria count of fermented samples with thermophilic starters was 8.23 log CFU·mL⁻¹ for cow milk and 9.12 log CFU·mL⁻¹ for yak milk. During cold storage (72 h), the CFU in yak milk remained higher (98.48% – 101.22%) during the storage period than that of cow milk (90.33% – 95.06%). The sensorial property scores of yak milk fermented with mixed starters were the highest, followed by those of milk fermented with mesophilic and thermophilic starter cultures. The obtained parameters show that mesophilic and mixed cultures are advantageous in terms of sensorial and probiotic properties, and thermophilic culture - in terms of gel strength. All this information is useful for the development of new dairy products.

Keywords: yak milk, cow milk, fermentation, rheology, gel strength, lactic acid production

INTRODUCTION

Yaks (*Bos grunniens*) are adapted to live at high altitudes (from 2,000 to 5,000 m above sea level) and under severe climatic conditions. They are an important cattle species in the highlands of the Nepalese Himalayas, Indian Kashmir, Tibet, Mongolia, Bhutan, and also in the Tian-Shan mountains of Kyrgyzstan (Wiener *et al.*, 2016). According to the latest statistical data, there are approximately 31,000 heads of yak in Kyrgyzstan (Smanalieva *et al.*, 2019).

Yak milk is a protein- and fat-rich, nutritious, traditional food for their herders and families who live in the high mountains (Chen *et al.*, 2021; Wiener *et al.*, 2016). According to the literature data, yak milk contains higher levels of dry matter 16.9 – 17.7% (w/w), protein 4.4 – 5.3% (w/w), fat 5.5 – 7.2% (w/w), lactose 4.5 – 5.0% (w/w) and minerals 0.8–0.9% (w/w) compared to cow (*Bos taurus*) and goat (*Capra aegagrus hircus*) milk (Li *et al.*, 2010; Mamet *et al.*, 2023; Nikkhah, 2011). These values are relatively similar to buffalo milk (Walstra *et al.*, 2006) and to khainak milk (a hybrid of yak and cow) (Elemanova *et al.*, 2022). Yak milk differs from cow milk by its higher concentration of total proteins (46.2 – 58.4 g/L), total casein (40.2 g/L) and the proportion of individual caseins (Chen *et al.*, 2021; Li *et al.*, 2010). According to Mamet *et al.* (2023), Pamir yak milk was rich in minerals such as Ca, Fe, Zn and Mg and vitamins: thiamine (B₁), niacin (B₃), pyridoxine (B₆) and cobalamin (B₁₂).

Yak milk is consumed as full-fat milk and is used also to manufacture butter, ghee, cheese and yoghurt. Tibetan nomads give diluted yak milk to babies to complement breast milk (Wiener *et al.*, 2016). In Tibet fermented food known as *Kurut* is produced using raw yak milk. Fermentation of yak milk takes at least 7–8 days at an ambient temperature of around 10 ± 25°C. A special characteristic of *Kurut* is the presence of alcohol and lactic acid (Sun *et al.*, 2010). In Bhutan, hard cheese from yak milk (locally known as *Chugo*) and fermented cheese called *Yidpa* are used as the main sources of protein. In Nepal cheese is produced from yak milk, and contains 46.8% butterfat on a dry matter basis (Ma *et al.*, 2017; Wiener *et al.*, 2016). According to Or-Rashid *et al.* (2008), yak cheese has a 24.8% (w/w) higher level of total long-chain saturated fatty acids (C17:0 – C26:0) and a 3.2 times higher content of total n-3 polyunsaturated fatty acids than cheddar cheese (Or-Rashid *et al.*, 2008). Besides, recent review work revealed that yak milk contains functional substances such as isracidin, casecin, lactoferricin, and other antimicrobial peptides (Kulyar *et al.*, 2021).

The concept of a 'functional food' is often associated with fermented food, especially fermented milk products with probiotic lactic acid bacteria (LAB), which are involved as starter cultures. Starter cultures used in the dairy industry

are divided into mesophilic, which work best at 25–30°C, and thermophilic, which work best at 40–45°C (Giraffa *et al.*, 2010). The combination of *Lactobacillus* and *Streptococcus* species is widely used in yoghurt production. The thermophilic starter culture enables a rapid fermentation process. Starters composed of *Lactococcus lactis* subsp. *lactis biovar diacetylactis* and *Leuconostoc cremoris* can produce diacetyl and are used to produce cultured buttermilk. The mixed cultures of thermophilic and mesophilic bacteria, for example, *Lactococcus lactis* subsp. *lactis biovar diacetylactis* and *Streptococcus thermophilus* are widely used for the rapid production of quarks. The fermentation regimes of cow milk with this starter culture are well known, but there is limited information about the fermentation process of yak milk with these starter cultures.

In this regard, the present study was undertaken to investigate the fermentation process of yak milk with different LABs, as well as their influence on the rheological and sensorial properties of fermented milk. For functional food which includes fermented dairy products, the most important quality characteristic is the presence of live probiotic microflora in the end product, in particular LABs. Therefore, the viability of starter cultures in fermented yak and cow milk during storage at 4 °C was investigated.

MATERIAL AND METHODS

Yak milk

Milk batches were collected from 20 yaks in July from Ak-Sai valley (Naryn region, Kyrgyzstan). The Ak-Sai valley is located at an altitude of 3000 – 3800 m above sea level. Milk immediately after milking was collected at ambient temperature in 500 mL glass bottles with screw caps (Duran Group, Germany) and kept in a thermal bag with ice during transportation. The milk samples were stored at –25°C in a freezer before analysis.

Cow milk

Cow milk fermentation was investigated as a reference. Pasteurised skimmed cow milk with a fat content of 1.5% was obtained from a local supermarket (OJSC 'Bishkeksut', Bishkek, Kyrgyzstan).

Starter cultures

The starter cultures were obtained from the SACCO System Company (Cadorago, Italy): Lyofast Y438B (thermophilic cultures: *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*) for yoghurt production; Lyofast MWO030 (mesophilic cultures: *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*) for quark production and Lyofast MS 064 CP (mixed cultures: mesophilic *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, and thermophilic *Streptococcus thermophilus*) for quark production by the fast method.

Samples preparation

For investigation, the average milk batch was taken, which was collected from 20 yaks. Yak milk was heated to a temperature of 35–40°C and was separated using a milk separator (Elecrem, Paris, France) and then standardised to the fat content of 1.5%, as described in [Elemanova et al. \(2022\)](#). Pasteurisation of standardised milk was conducted at 85–87°C for 15 minutes. Milk samples were fermented using thermophilic, mesophilic, and mixed bacteria at temperatures 43, 32, and 35°C, respectively. The dosage of starters was calculated according to the recommendation of the SACCO System Company (Cadorago, Italy): the thermophilic starters – 40 mg·L⁻¹, the mesophilic starters – 6.24 mg·L⁻¹, for the mixed cultures – 1.16 mg·L⁻¹.

Yak milk after adding the starter cultures was mixed on a magnetic stirrer for 15 min. The prepared samples were poured into 10 glass bottles and kept at the temperature required for each culture. The titratable acidity and pH of the samples were measured every 60 min. For rheological measurements, milk samples with starter cultures after stirring were placed immediately in a rheometer cylinder and measured until gel formation.

For sensorial analysis, yak milk samples were fermented with three cultures in the same way as for the determination of titratable acidity and pH. Sensorial indicators of the prepared samples were determined starting from the day of preparation and after 24, 48, and 72 hours. All analyzed samples for sensorial analysis were stored in a refrigerator at 4°C.

Preparing fermented cow milk samples and measuring their physical, rheological, and sensorial parameters were carried out in the same way as for yak milk.

Chemical content of yak milk

The crude protein content was measured by the Kjeldahl method (the conversion factor 6.38) as described by AOAC method 991.20 using an Extraction Unit EV6 All/16 (Gerhardt, Germany). The Babcock method for fat determination was carried out according to AOAC method 989.04. Determination of lactose content in yak milk was performed by the Munson–Walker gravimetric method according to AOAC method 930.28 (AOAC, 2019).

Physical measurements

The pH of the samples was determined during fermentation using a portable pH meter (Model 220, Denver Instrument, Denver, USA). A potentiometric titration with 0.1 M NaOH was used for the measurement of the titratable acidity according to AOAC method 947.05 (AOAC, 2019).

Rheological measurements

A small amplitude oscillatory rheology (SAOR) was used for the characterization of the structural changes in standardised yak milk during fermentation. The measurements were carried out using an MCR 302 rheometer (Anton Paar, Austria) fitted with cylinder geometry (CC27-SN26341) in a time-sweep regime according to [Smanaliev et al. \(2021\)](#). The temperature was chosen according to the fermenting bacteria used: for thermophilic bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*): 43°C; for mesophilic bacteria (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*): 32°C; for mixed bacteria (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, *Streptococcus thermophilus*): 35°C. Rheological parameters such as storage modulus G' , loss modulus G'' and loss tangent ($\tan \delta = G''/G'$) were determined in triplicates and results were averaged.

Enumeration of viable microorganisms

For the enumeration of viable starter cultures, 1 mL of fermented milk was diluted with 9 mL of sterile 0.9% NaCl (10⁻¹ dilution). Further, tenfold serial dilutions ranging from 10⁻¹ to 10⁻⁸ were prepared. The 10⁻⁷ and 10⁻⁸ dilutions were used for the inoculation of LAB to the culture medium and 1 mL of these dilutions was spread on a Petri dish consisting of MRS agar (CM1153, Oxoid, UK). The Petri dishes with inoculated mesophilic and mixed LAB starters according to the spread plate method ([Sun et al., 2010](#)) were incubated in microaerobic conditions at 30°C; and at 37°C for samples with the thermophilic starter.

Sensorial analysis

The sensorial properties of 3 batches of yak milk fermented with different starters were evaluated by 12 panellists trained in a descriptive sensorial panel at the Kyrgyz-Turkish Manas University. The descriptors used for the sensorial test such as overall acceptability, colour, texture, odour, creamy odour, taste and mouth feel, were adopted from [Drake \(2007\)](#). In the acceptance test, participants evaluated the fermented yak milk using a 5-point hedonic scale.

Statistical analysis

The comparison of mean values of three batches with each starter was analysed by the SPSS 22 software (SPSS Inc., Chicago, IL) using the One-Sample T-test (paired sample test) with a 95% confidence interval. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The protein, lactose, and fat contents of the investigated yak milk were 5.1±0.6%, 4.8±0.3%, and 6.3±0.4%, respectively. The protein content of yak milk (5.1 %) was higher than that of cow milk (2.9%), but the lactose contents of both milk samples were nearly identical, 4.7% and 4.8%. The protein, lactose and fat content of Kyrgyz yak milk, corresponds to the data of Mongolia ([Jianlin et al., 2000](#)). Since yak milk has a higher fat content than cow milk and to reduce influencing factors in the fermentation process, the fat content of yak milk was standardised to 1.5 %.

Acidification trend during milk fermentation

Generally, it is known that during milk fermentation, the pH decreases ([Salaün et al., 2005](#)). Three important points of pH were compared and discussed 1) Start time of acidification, pH=6.2; 2) Time of clot building, pH≤5.2, where calcium and inorganic phosphate are transferred to the aqueous phase; 3) Time of the whole fermentation process: the time when the pH decreases to 4.6 since that is the isoelectric point of casein ([Corrieu and Béal, 2015](#)). The fermentation ended at a pH of 4.5 or if the pH did not decrease significantly in a 1 h interval. Acidification of yak milk with thermophilic starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) was faster than with other starter cultures. During fermentation, a decrease in the pH to 6.2–6.0 was recorded at 180–190 min after the addition of the thermophilic starter cultures (Fig. 1, 2).

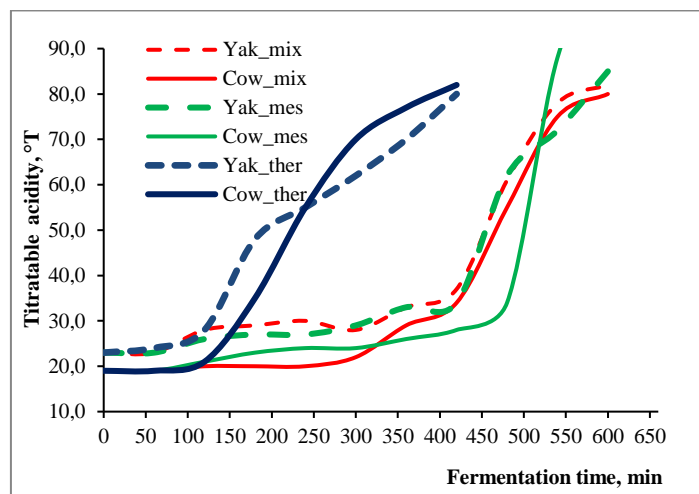


Figure 1 Acidification curve: titratable acidity vs fermentation time of cow and yak milk during fermentation with the three starter cultures: thermophilic (_ther), mesophilic (_mes) and mixed starters (_mix). Results are the average of three replicates with standard errors ≤ 0.5 %

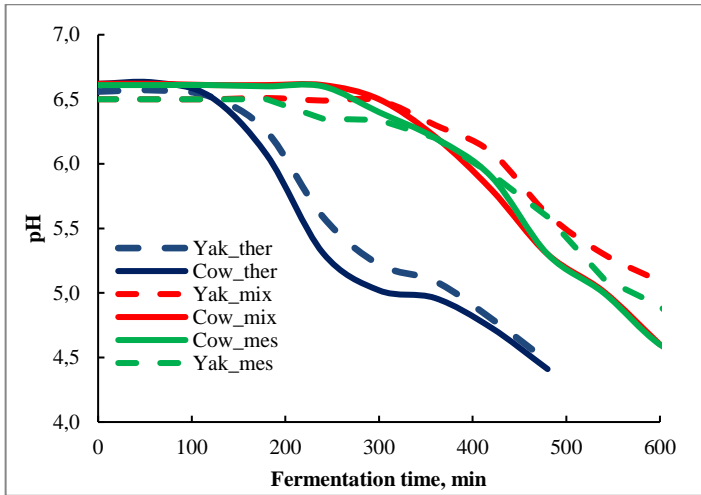


Figure 2 Acidification curve: decrease in the pH of cow and yak milk during fermentation with the three starter cultures: thermophilic (_ther), mesophilic (_mes) and mixed starters (_mix). Results are the average of three replicates with standard errors $\leq 0.5\%$

At 240 min the pH reached 5.6 in yak milk and 5.3 in cow milk for the thermophilic starters. At 480 min (6 h) when the thermophilic fermentation stopped, the titratable acidity (TA) was 80°Th (pH 4.47) for yak milk and 74°Th (pH 4.41) in reference cow milk. Ren et al. (2015) investigated the acidification properties of *S. thermophilus* and revealed that the fermentation time for yak milk was from 6 to 11 hours. However, the titratable acidity values found at the end of fermentation were from 57.16 to 59.03°Th (Ren et al., 2015).

In terms of the mesophilic starter culture of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, fermentation of yak and cow milk is the slowest and ends at 600 min. At the end of fermentation (600 min) with mesophilic starters, TA was 85°Th (pH 5.09) in yak milk and 88°Th (pH 4.60) in cow milk. During acidification with mixed starter cultures of cow and yak milk, at 360 min the lactic acid produced led to a decrease to pH 6.21 and 6.30, acidity to 29 and 33°Th , respectively (Fig.1, 2). After 530 min of fermentation, the pH in cow and yak milk decreased to 5.1 and 5.3, respectively, and the acidity increased to 75 and 78°Th , respectively.

As expected, the fermentation processes of yak and cow milk with the three starter cultures were different. The acidification of milk depends on the dry substances and especially the protein concentration of milk, the composition of bacterial starter cultures, heat treatment modes and the method of milk coagulation and other factors (Lucy, 2016). For comparison, in yak milk, the content of protein is higher than that in cow milk, while the lactose content is the same.

Structural changes in yak and cow milk during fermentation

The rheological data obtained makes it possible to judge the rate of chemical and biochemical processes occurring in food products. They can also be used to measure and control fermentation processes to take appropriate corrective actions to ensure that food products are of high quality and have the desired rheological properties.

Acidification of milk leads to coagulation of casein molecules and gel formation. The strength of milk gels can be measured with rheological parameters such as elastic or storage modulus (G') (Brückner-Gühmann et al., 2019; Smanalieva et al. 2021). Time-sweep measurements were used to characterise the fermentation process of yak and cow milk samples. The results of time-sweep measurements of cow and yak milk fermented with thermophilic starter cultures (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*) are shown in Fig. 3 and Table 1. The G' represents the elasticity, whereas G'' indicates the viscous or liquid character of the materials. Gel formation during milk fermentation results in a steep increase in the storage moduli G' in the time-sweep diagram. Milk fermentation by thermophilic starters was conducted at 43°C . The gel formation in cow and yak milk ($G' = G''$) began after 172 min and 167 min, respectively. At this point, the pH was 5.95 in yak milk and 6.00 in cow milk samples. The G' of both samples from this point grew exponentially and indicated gel formation. The gel strength (G') at the end of fermentation was higher for yak milk (210 Pa), compared with cow milk (131 Pa).

The fermentation process with mixed bacteria was completed for both milk samples at the same time, 480 min (Fig. 4). Gel formation ($G' = G''$) of yak milk begins after 388 min of fermentation (pH=6.19), whereas in cow milk structure formation starts at 324 min (pH=6.20). However, the gel strength (G') at the end of fermentation was significantly higher in yak milk (69 Pa) than in cow milk (40 Pa).

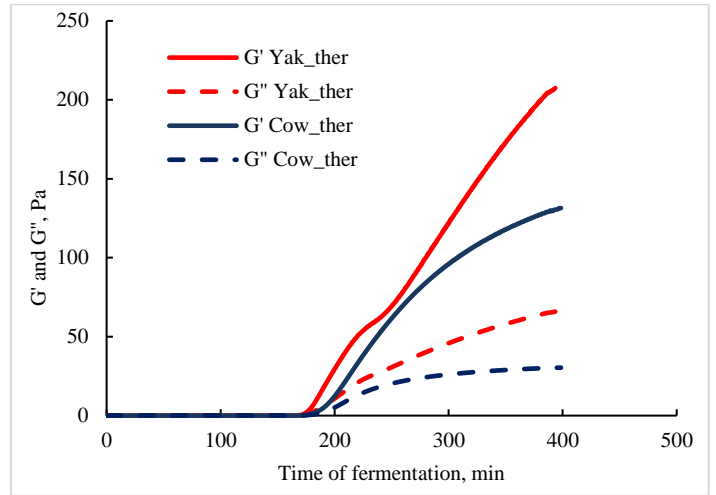


Figure 3 Time-sweep diagram of the fermentation process of cow and yak milk with thermophilic starter cultures. Results are the average of three replicates with standard errors $\leq 0.5\%$

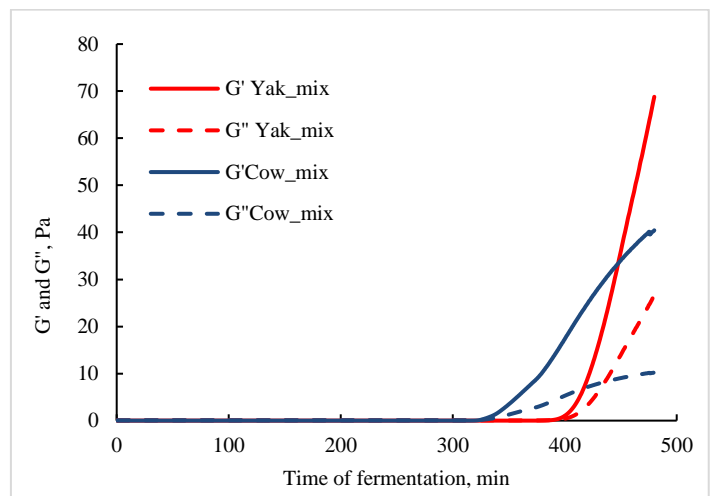


Figure 4 Time-sweep diagram of the fermentation process of cow and yak milk with mixed starter cultures. Results are the average of three replicates with standard errors $\leq 0.5\%$

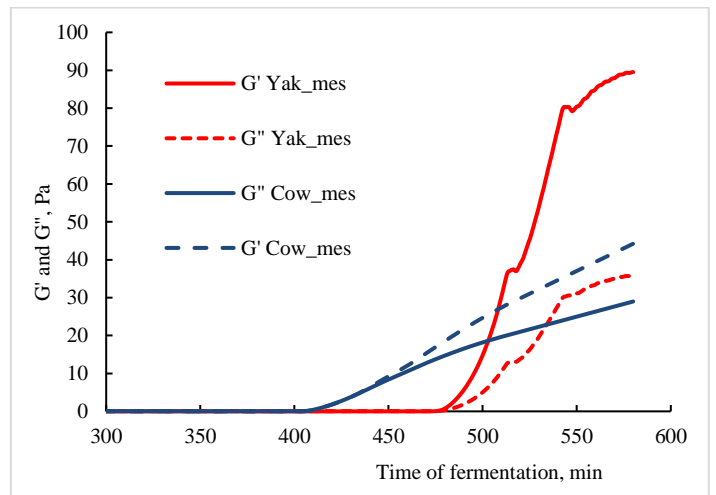


Figure 5 Time-sweep diagram of the fermentation process of cow and yak milk with mesophilic starter cultures. Results are the average of three replicates with standard errors $\leq 0.5\%$

Table 1 Changes of oscillatory shear data of yak and cow milk during fermentation

Samples	T _{gel} , (min)	pH at T _{gel}	G' at T _{end} , (Pa)	T _{end} , (min)
Thermophilic starters				
Yak milk	167±2a	5.95±0.05b	210±2a	400
Cow milk	172±7b	6.00±0.03b	131±11b	400
Mixed starters				
Yak milk	388±2c	6.19±0.01d	69±2c	480
Cow milk	324±7c	6.20±0.01d	40±4d	480
Mesophilic starters				
Yak milk	476±1d	5.59±0.01a	84±1e	580
Cow milk	441±14f	5.60±0.05a	54±13d	580

The values are given as mean ± SD, n = 3. T_{gel} - Time of gel-forming – crossover point of G' and G'' ; G' - elastic modulus;

T_{end} – time of whole fermentation.

Differences between means within each column at p ≤ 0.05 are indicated by different letters

Among all investigated samples, fermentation of cow milk by mesophilic bacteria at 32°C took the longest and was completed in 580 min. Gel formation (G' = G'') was registered at 441 min for cow milk (Fig. 5), corresponding to pH 5.6 (Table 1). In comparison, the structure formation of yak milk fermented by the mesophilic starter culture started at 476 min, which corresponds to pH 5.59. However, the gel strength was not higher than that of milk fermented with thermophilic and mesophilic and mixed starters.

The elastic modulus G' of fermented milk samples was in the same order: ther ≥ mes ≥ mix, gel strength of yak milk was higher with all starters. It should be noted that fermented khainak milk formed also a stronger coagulum with a higher storage modulus than that of cow milk (Elemanova et al., 2022). The higher elastic modulus G' of yak milk can be attributed to its higher protein content (5.1%) when compared with cow milk. The total casein content differs among milk types; for example, yak milk has an average total casein content of 40.2 g·L⁻¹ which is 1.5

times higher than that of cow milk (Li et al., 2010; Walstra et al., 2006; Zhang et al., 2020). Also, the composition of the casein molecules differs between yak and cow milk. Thus yak milk has β-, α_s- and κ-casein concentrations of 20.57 g·L⁻¹, 19 g·L⁻¹ and 8.5 g·L⁻¹ (Li et al., 2010), while cow milk has β-, α_s- and κ-casein concentrations of 13.0 g·L⁻¹, 9.6 g·L⁻¹ and 3.40 g·L⁻¹, respectively (Walstra et al., 2006). According to Zhang et al. (2020), yak milk is characterised by a higher concentration of calcium and a larger size of casein micelles. Colloidal calcium phosphate acts as a stabiliser of casein micelles (Holt, 1992). Therefore, the possible reasons for stronger gel strength in fermented yak milk may be the higher protein and calcium concentration.

Screening of viable bacterial counts in fermented yak and cow milk during storage

The viable bacteria counts of LAB in fermented yak and cow milk samples are shown in Table 2. The number of LAB immediately after the fermentation of yak milk was in the following order: yak_{ther}>yak_{mix}>yak_{mes}. The numbers of the thermophilic LAB in the cow and yak samples after fermentation were 8.23 and 9.12 log CFU·mL⁻¹, respectively. During storage, the number of thermophilic LAB in yak milk declines from 9.12 to <6 log CFU·mL⁻¹ in 72 h, but this change was not statistically significant. The numbers of mesophilic and mixed starter cultures of both samples during storage were near constant. The viability in yak milk remained higher (98.48% – 101.22%) during the storage period than that of cow milk (90.33% – 95.06%). For comparison, Yu et al. (2011) reported that the LAB counts in 12 fermented yak milk samples ranged from 4.30 to 6.90 log CFU·mL⁻¹. It is generally required that probiotic fermented milk should contain at least 8 log CFU·mL⁻¹ of the product (EFSA, 2010). Higher dry substances and protein concentration may affect the viability of the starter bacteria in yak milk.

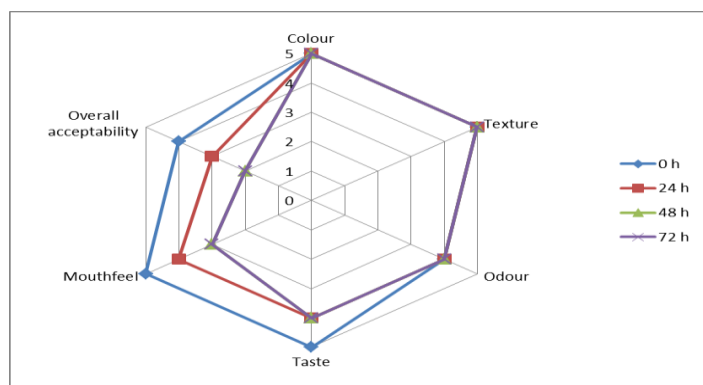
Table 2 The viable LAB from the fermented yak and cow milk samples during storage (log CFU·mL⁻¹).

Days of storage	Yak _{ther}	Cow _{ther}	Yak _{mes}	Cow _{mes}	Yak _{mix}	Cow _{mix}
h	9.12±0.3	8.23±0.03	8.22±0.02	8.89±0.23	8.50±0.01	8.91±0.07
24 h	7.31±0.50	7.08±0.15	8.84±0.07	8.71±0.02	8.49±0.08	8.09±0.24
48 h	6.34±0.02	6.04±0.22	7.99±0.15	8.18±0.20	8.65±0.03	8.10±0.13
72 h	<6	<6	8.32±0.08	8.45±0.19	8.37±0.02	8.05±0.16
Viability, %	0	0	101.22	95.06	98.48	90.33

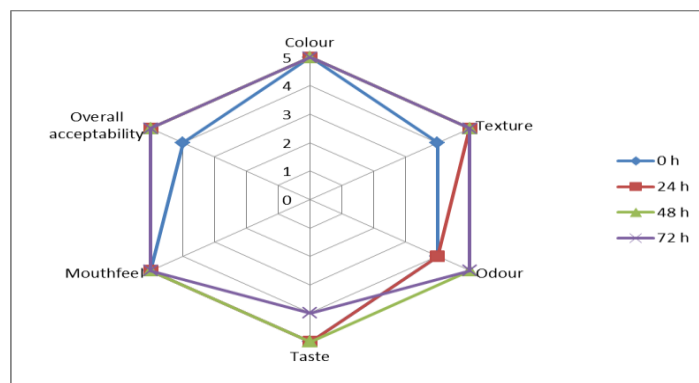
Means (n = 3) ± standard deviations, * _mes- mesophilic; _ther- thermophilic; and _mix - mixed starter cultures.

Sensorial analysis

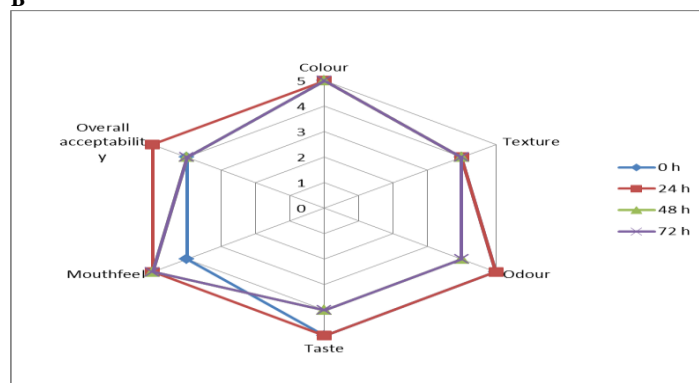
Average sensorial data of fermented yak milk analysed on a 5-point scale are shown in Figure 6. There was no significant difference in the colour or texture of all three samples, but taste and mouthfeel changed during storage in yak milk fermented with the thermophilic starter cultures. Both samples fermented with the thermophilic starter cultures received lower overall acceptability when compared with the other samples. In general, milk fermented with mixed culture was the most favoured by tasters due to its taste and odour. Taste is one of the most important parameters in the acceptance of dairy products (Bezerra et al., 2012; Drake, 2007). The highest average score of sensorial properties after 24 h has yak milk fermented with mixed starter cultures, followed by mesophilic starters, while a relatively low score has the sample with thermophilic starters. Thus, the individual proteins hydrolysed by the proteases specific to the different starter cultures contribute to the flavour and texture.



A



B



C

Figure 6 The sensorial properties of yak milk are fermented with thermophilic (a), mixed (b) mesophilic (c) starter cultures. n = 12, standard deviation ≤ 0.7, standard error ≤ 15%

CONCLUSION

Fermented yak milk is considered an alternative dietary source that could improve human health, but it is used in limited countries and produced on a household scale. The comparison of physicochemical parameters of fermented yak milk and fermented cow milk using three industrial starter cultures was carried out. Classical pH and titratable acidity measurements and modern oscillatory rheological methods such as time-sweep were used to control the fermentation process. Generally, as expected, the acidification of yak milk with thermophilic cultures was faster than with other starter cultures. The gel-forming times of yak milk detected with rheological measurement were in the following order: thermophilic > mixed (meso-thermophilic) > mesophilic starter cultures. Compared with cow milk, samples of yak milk with mixed and mesophilic starters had a longer gel forming time, but higher gel strength. The yak milk with thermophilic starters had the fastest fermentation time and highest gel strength. The viable bacteria counts of fermented yak milk at the end of fermentation were in the following order: thermophilic > mixed (meso-thermophilic) > mesophilic starter cultures. After 72 h of storage, the number of thermophilic starters in yak samples was lower than 6 log CFU·mL⁻¹. However, the viability of mixed and mesophilic starter cultures in yak milk remained higher (98.48% – 101.22%) than that of cow milk (90.33% – 95.06%). Yak milk fermented with mixed starter cultures has the highest average points for sensorial properties, followed by mesophilic and thermophilic starter cultures. The obtained parameters show that mixed meso-thermophilic starter cultures are suitable for the development of new fermented foods with yak milk in terms of sensorial and probiotic properties with suitable gel strength.

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Conflicts of interests: The authors hereby declare that they have no conflict of interest.

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