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RESEARCH ARTICLE

Quality and functional attributes of vacuum-packed yak milk *mozzarella* cheese as influenced by storage

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Abstract: The fresh m*ozzarella* cheese was developed from yak milk by using direct acidification method, packaged under vacuum atmosphere and stored at 4±1°C for 35 days. The effect of storage was evaluated on proximate composition, physicochemical, functional, microbiological and sensory properties of yak milk *mozzarella* cheese at defined interval. Among the proximate composition, the moisture content of cheese varied from 48.17% at day 0 to 47.00% at the end of storage. As the storage period progressed, a non-significant differences ($p>0.05$) was observed for the fat, protein and ash content. Storage time significantly ($p<0.05$) affected the physicochemical properties of cheese sample, including pH, titratable acidity [lactic acid (% by weight)], tyrosine value (µg/g) and total free fatty acids (µm/g). A significant increase ($p<0.05$) in functional properties (meltability, free oil formation and stretchablity) of cheese sample was recorded as the storage progressed. A significant increase in the standard plate count and, yeast and mould count was also observed during progress of storage. The sensory evaluation of cheese sample revealed that colour & appearance, flavour, overall acceptability was non-significant ($p>0.05$) while, body & texture was significant ($p<0.05$) during storage. Therefore, it was concluded that the vacuum-packed yak milk *mozzarella* cheese sample could be stored up to 28 days with optimum organoleptic attributes, functional properties and without significant changes.

Keywords: Yak milk *mozzarella* cheese; vacuum packaging; quality attributes; functional properties; storage stability

Introduction

Mozzarella is the most widely available un-ripened cheeses in the market. It is an Italian, highly valued and fresh stretched curd cheese that be linked to the pasta *filata* group (El Owni and Osman, 2009). It involves skilfully stretching the curd in hot water to provide a smooth texture and lively surface (Kosikowski, 1982). It is regulated by law as well as a member of the European Protected Designation of Origin (European Communities, 1996). Traditionally, *mozzarella* cheese is made from milk of buffalo, which is premium and nutrient dense cheese world-wide (Sameen et al. 2008; Vogt et al. 2015). Other milks may also be utilized for the production of *mozzarella* cheese, such as cow's, goat's, and sheep's in many countries. Yak milk is superfood and produced in considerable amount especially in Himalayan region of Ladakh, Jammu & Kashmir, Arunachal Pradesh, Sikkim, Himachal Pradesh, Uttarakhand (Singh et al. 2023a). Yak milk cheese production can help highlanders in sustaining their nutrition and boosting their economic activities despite the region's difficult climatic conditions (Singh et al. 2023b). Commercial yak cheese production exist in the yak rearing countries like Tibet, China, Bhutan, Nepal and Russia having a huge demands in the market. However, in India this sector is not well developed due to dwindling yak population. Yak milk is a good source of cheese making owing to its high casein-fat ratio, total solids, larger fat globules and have special qualities as well as it is also beneficial to human health (Zhang et al. 2017; Zhang et al. 2020; Singh et al. 2023a, b). Recently, in India, cheddar style-yak milk cheese (Singh et al. 2023b), yak milk *paneer* (Singh et al. 2022a) and yak milk *ghee* (Singh et al. 2022b) was developed by using yak milk. Increasing demand for *mozzarella* cheese is being driven by the diversification of pizza parlours and fast food chains worldwide (Bhattarai and Acharya, 2010). Since it melts and stretches easily,

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and primarily used in the fast food industry as well as in cheese-based salad dressings (Vogt et al. 2015). It is therefore imperative to standardise the process that uses yak milk and improves the quality of local cheese. It can be a boost to the highland pastoral nomads and yak dairy industries. As far as the use of yak milk in *mozzarella* cheese production is concerned, no research has been carried out in India. In light of yak milk's compositional differences as compared to cow and buffalo milk, it would be interesting to investigate how it is processed and the quality of its *mozzarella* cheese. Therefore, in the present study, yak milk *mozzarella* cheese was developed and was evaluated the effect of storage on proximate composition, physicochemical, functional, microbiological and sensory properties of the vacuum-packed yak milk *mozzarella* cheese during refrigerated storage.

Materials and Methods

Materials

The fresh yak milk (dry matter 16–18%, fat 5.50–7.50%, solid-non-fat 10-11% and protein 3.75–4.25%) was obtained from the Nyukmadung farm of ICAR-National Research Centre on yak, Dirang situated at Nyukmadung, Arunachal Pradesh, India, between latitude of 27°25.948' North and longitude of 092°08.658' East at an altitude of 2750 meters above mean sea level. Immediately, milk was brought to the laboratory under refrigerated conditions for cheese preparation. The microbial rennet (Meito®) commercially produced in granular form from *Mucor pusillus* var. *Lindt* was obtained from M/s Meito Sangyo Co., Ltd., Tokyo, Japan for yak milk *mozzarella* cheese preparation. Analytical grade chemicals and reagents used for various laboratory analyses were procured from standard firms. The commercially available rectangular plastic packaging material of 127µm thickness for vacuum packaging was purchased from Swiss Pac Pvt. Ltd., Gujrat, India.

Preparation of yak milk mozzarella cheese

The yak milk *mozzarella* cheese preparation was carried out according to Guinee et al. (2002) and Kosikowski (1982) with some modifications (Fig. 1). The fresh yak milk was at first filtered and then standardised to a casein-fat ratio of 0.80 by using skim milk. The standardized milk was pasteurised at 72±1°C for 15 sec in a stainless-steel vat and then cooled down to 4-8°C. The pH of yak milk (6.46) was adjusted to 5.2-5.4 by using 25% citric acid, then the temperature was raised to 30±1°C. This was followed by the addition of rennet @25mg/L milk, mixed effectively and then the milk was left undisturbed at 30±1°C and waited for 30-45 min or until a firm set was reached. After coagulation, set curd was cut into small cubes (1-1.5 cm^3 in size) using sterile cheese knives (horizontally and vertically). The curd was left undisturbed for healing for 5-10 min, after healing the cooking was done from 31-41°C with in 40-45 min under simultaneous stirring. After cooking, the whey was drained through strainer. And cheese *coagulum* was taken out and submerged in 82±1°C water and manual stretching was done until forming a shining, smooth and homogenous mass. Thereafter, it was moulded into balls of 200-250 g in size and placed in to chilled brine solution (7.5%w/v non-iodised salt) for 2 h and subsequently surface dried for 4 h under refrigerated conditions (7±1°C). The weight of the prepared

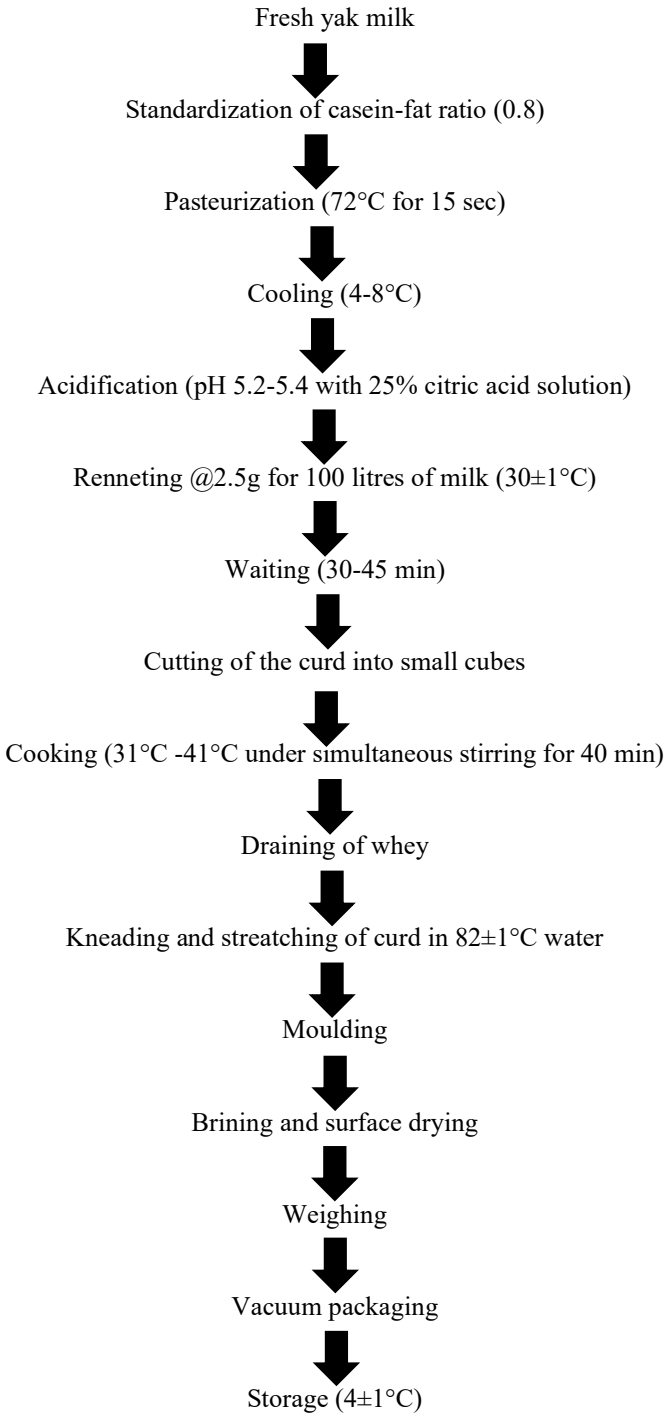

Fig 1. Process of preparation of yak milk *mozzarella* cheese by direct acidification

cheese was recorded and packed under vacuum nylon packs (127µm thickness) by using vacuum packaging machine (Model: QS500VSV4; Make: Sevana Electrical Appliances Pvt. Ltd., Kerala, India) and stored at 4±1°C for 35 days. Samples of cheese were drawn and evaluated for proximate composition, physicochemical, functional, microbiological, and sensory properties at defined intervals. The experiment was repeated twice, and the respective analysis were done in triplicate.

Analytical procedures

Mozzarella cheese yield

The *mozzarella* cheese yield (%) was calculated using the equation as given below:

$$Cheese\ yield\ (\%) = \frac{Weight\ of\ cheese\ (kg)}{Weight\ of\ milk\ taken\ (kg)} \times 100$$

Proximate composition

The moisture and ash content of cheese sample was estimated by following the Association of Official Analytical Chemists' approved methods (AOAC, 2005). whereas, the fat content of cheese sample was evaluated by using the Gerber method and total protein content was estimated by determining the total nitrogen using the Kjeldahl method (IDF, 1993) and converting it to protein content by multiplying by 6.38.

Physicochemical properties

The pH values (grated cheese sample and distilled water, 1:2) were recorded electrometrically using a digital pH meter (Make: PC 2700, Oakton®, India). The titratable acidity was determined by the titration method suggested by AOAC (2005) and results were recorded in lactic acid (% by weight). Juffs (1973) method was used to determine the tyrosine value and results were shown in µg/g. Deeth and Fitz-Gerald (1976) extraction-titration method was used to evaluate the total free fatty acids (TFFA) content, and results were shown in µm/g.

Functional Properties

The meltability of cheese sample was evaluated using a prescribed method from Muthukumarappan et al. (1999) with slight modifications. Samples (3.5 cm in diameter and 1 cm in height was tempered at room temperature for 30 min. Thereafter, sample was then heated at 100±1°C for 5 min in hot air oven. After the cooling of melted cheese at room temperature for 5 min, the meltability of cheese was recorded by measuring the final diameter (minimum and maximum) of the cheese discs at 5 different places and expressed as the mean values in cm. Stretchability of cheese sample was evaluated by following the method of Lemay et al. (1994). The average values in cm was measured as streatchbility of cheese. Free oil formation or oiling off property of cheese sample was evaluated by the method of Breen et al. (1964) with the following modification. Discs of 1.5 cm in diameter and 0.5 cm in thickness were cut, melted on filter paper in the oven at 100±1°C for 5 min. The average of free oil formation at four different angles was expressed in cm.

Microbiological analysis

Microbiological analysis of the sample was done using standard methods mentioned by the American public health association (APHA, 2005). In order to prepare the media, careful attention was paid to the manufacturer's instructions (Plate Count Agar for standard plate count, Potato Dextrose Agar for yeast and mould count, and Violet Red Bile Agar for coliform count). The plates showed 30–300 colonies were counted, and their number was multiplied by reciprocal of dilution. The microbial count was recorded as $\log_{10}$CFU/g of the sample.

Sensory analysis

A twenty four (n-24) semi-trained members (aged between 20 to 60 years) composed of scientists, administrative staff and other employees from ICAR-NRC on Yak, Dirang were selected as the sensory panellists for assessing the yak milk *mozzarella* cheese. The properties of the developed product were defined, and panellists were familiarised with the Sensory Performa before performing the analysis. The cheese was cut uniformly and pieces were tempered for 20-30 min at room temperature (20±2°C) then served individually to each panellists. The coded samples were evaluated for their sensory traits like colour & appearance, flavour, body & texture and overall acceptability using a hedonic scale of 9-point intensity varied from extreme dislike (score = 1) to extreme like (score = 9).

Statistical analysis

Statistical analysis was done using the one-way analysis of variance (ANOVA). The ANOVA was done by using Statistical Package for the Social Sciences (SPSS) software package trial version 22.0 (IBM SPSS Inc. Chicago IL, USA). The statistical significance at 5% level was considered significant. Post-hoc analysis was done using Duncan's multiple range test. The data were recorded in the form of average ± standard deviation.

Results and discussion

The obtained result showed the average yield of 15.09% for yak milk *mozzarella* cheese in the present study. The effect of the storage period on the proximate composition of cheese sample is summarized in Table 1. The moisture content (%) of cheese varied from 48.17±0.89 at day 0 to 47.00±0.61 at the end of storage. Various factors may contribute to the variation in moisture content in cheese, including preparation methods (El-Owni and Osman, 2009), cooking temperatures (McSweeney, 2007), and compositional differences (size of fat globules and casein micelles

characteristics) of yak milk (Zhang et al. 2020). Guinee et al. (2002) stated that having a low pH during storage causes the protein network to become unstable, which causes more moisture to be released. A non-significant differences ($p>0.05$) was recorded for the fat, protein and ash content during the progress of storage period. The fat content (%), protein content (%) and ash content (%) was 24.24±0.40, 22.64±0.55 and 2.96±0.22 on day 0 and 24.76±0.51, 23.27±0.94 and 3.14±0.19 on day 35, respectively. Smaller variations in the proximate composition of cheese might be due to variations during storage conditions. The compositional differences of yak milk influences the proximate composition of cheese (Singh et al. 2023a). Table 2 showed physicochemical properties of cheese sample during storage. Storage period ($p<0.05$) had a significant effect on pH and TA of cheese sample. The pH and TA value varied from 5.45±0.04 to 5.62±0.03 and 0.39±0.03 to 0.52±0.04, respectively, during the storage time. This would be due to biochemical changes occurred during storage and proximate composition of cheese (Sameen et al. 2008). Storage period had a significant effect on the tyrosine value of cheese sample ($p<0.05$). It was increased from 101.50 µg/g to 322.33 µg/g in the first 28 days of storage, and then began to decrease after the 28th day of storage period. In cheese sample, tyrosine level has been found to increase because enzymes and microorganisms hydrolysed proteins during storage (Singh et al. 2012). The results are in line with the study of Singh et al. (2022a) who also reported the increase in tyrosine value of yak milk *paneer* during storage. There was significant increase in free fatty acid (FFA) content of cheese sample during storage. At day 0 of storage, the FFA value was 1.69µm/g and at the end of storage period (day 35), it was increased to 2.45µm/g. It might be due to slower rate of lipolysis under mentioned storage conditions.

Functional characteristics such as meltability, free oil formation (FOF) and stretchablity are presented in Table 3. Meltability is the ability of cheese to melt uniformly, smoothly, and homogeneously (cheese shred should not be visible) without releasing oil or becoming watery (Johnson, 2000). The initial meltability of cheese sample on day 0 was 4.90 cm which significantly increased ($p<0.05$) to 7.53 after 35 days of storage. Similar observations were also observed by Imm et al. (2003) who observed that refrigerated storage of bovine and caprine *mozzarella* cheese (MC) influenced the meltability. The meltability of MC depended on various factors such as water partitioning, rearrangement of protein matrix (McMahon et al. 1999), displacement of the para-casein matrix (Guinee et al. 2001) and the amount as well as distribution of fat in the protein matrix (Imm et al. 2003). Free oil formation, also known as 'oiling off' or 'fat leakage', occurs when free oil separates from the melted cheese and accumulates in pockets or pools, particularly on its surface (Jana et al. 2017). A significant increase ($p<0.05$) in FOF of cheese sample was recorded as the storage progressed (Table 3). Free

Table 1: Effect of storage on proximate composition of yak milk *mozzarella* cheese (Mean±S.D.)

Proximate composition (%)	Storage days					
	0	7	14	21	28	35
Moisture content	48.17 ±0.89^{b}	48.32 ±0.32^{b}	47.93 ±0.56^{b}	47.46 ±0.66^{ab}	47.60 ±0.75^{ab}	47.00 ±0.61^{a}
Fat	24.24 ±0.40^{a}	24.20 ±0.49^{a}	24.35 ±0.54^{a}	24.56 ±0.55^{a}	24.44 ±0.57^{a}	24.76 ±0.51^{a}
Protein	22.64 ±0.55^{a}	22.59 ±0.56^{a}	22.71 ±0.56^{a}	23.02 ±0.45^{a}	22.97 ±0.42^{a}	23.27 ±0.94^{a}
Ash	2.96 ±0.22^{a}	2.92 ±0.20^{a}	2.98 ±0.19^{a}	3.07 ±0.12^{a}	3.04 ±0.18^{a}	3.14 ±0.19^{a}

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

Table 2: Effect of storage on physicochemical properties of yak milk *mozzarella* cheese (Mean±S.D.)

Physicochemical properties	Storage days					
	0	7	14	21	28	35
pH	5.58 ±0.07^{cd}	5.62 ±0.03^{d}	5.60 ±0.02^{cd}	5.56 ±0.02^{bc}	5.52 ±0.05^{b}	5.45 ±0.04^{a}
Titratable Acidity [TA; lactic acid (% by weight)]	0.39 ±0.03^{a}	0.41 ±0.03^{a}	0.42 ±0.04^{a}	0.46 ±0.03^{b}	0.47 ±0.04^{b}	0.52 ±0.04^{c}
Tyrosine value (µg/g)	101.50 ±8.17^{a}	238.44 ±14.08^{c}	274.83 ±20.14^{d}	280.11 ±12.27^{d}	322.33 ±23.68^{e}	214.83 ±5.96^{b}
Total free fatty acids (µm/g)	1.69 ±0.12^{a}	1.81 ±0.16^{ab}	2.01 ±0.15^{bc}	2.13 ±0.27^{cd}	2.33 ±0.21^{de}	2.45 ±0.17^{e}

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

oil started increasing significantly ($p<0.05$) after 14 days of storage in cheese sample, and it continued to increase until 35 days of storage. There are several factors that affect the formation of free oil in *mozzarella* cheese, including fat content, size of fat globules, fatty acid profile, and proteolysis (Tunick, 1994). The stretchability of melted cheese refers to its ability to form fibrous strands that elongate without breaking under tension (Jana et al. 2017). The maximum stretch (35.92 cm) was observed on day 35 of storage which was significantly ($p<0.05$) higher than the value (26.25 cm) obtained on day 1 of storage. According to Rehman et al. (2008), a commercial pizza cheese has a stretch value of 25.27 cm. Whereas, a minimum stretch of 3.0 inches (7.62 cm) of unbroken string is specified for Pizza cheese in the United States (USDA, 2007).

It is important from the perspective of food safety to consider the microbiological quality of cheese, since it is an excellent growth medium for a variety of microorganisms (Dharaiya et al. 2021). Table 4 reports the changes in microbiological properties for cheese sample during storage. The initial SPC (log_{10}CFU/g) in cheese sample was 3.37 on day 0 of storage which was increased to 4.66 on day 35 of storage. The initial average value of YMC count (log_{10}CFU/g) of cheese sample increased from 1.13 to 2.74 after the end of storage period. It indicates storage had significant impact ($p<0.05$) on changes in SPC and YMC counts. The coliform count was absent in cheese sample throughout the storage. During the scalding process, high temperatures reduce the microbial flora associated with contamination and increase the safety of *mozzarella* cheese (Marth and Steele, 2005). According to Han et al. (2015), natural *mozzarella* cheese prepared by direct acidification had a viable count of 5.8 log_{10}CFU/g.

Mozzarella cheese made from yak milk had shown delicious milky flavour, homogenous texture, and a whitish-yellowish colour

Table 3: Effect of storage on functional properties of yak milk *mozzarella* cheese (Mean±S.D.).

Functional properties	Storage days					
	0	7	14	21	28	35
Meltability (cm)	4.90 ±0.24^{a}	5.32 ±0.21^{b}	5.80 ±0.24^{c}	6.27 ±0.48^{d}	6.98 ±0.25^{e}	7.53 ±0.23^{f}
Free oil formation (cm)	2.45 ±0.10^{a}	2.65 ±0.10^{a}	3.00 ±0.14^{b}	3.30 ±0.23^{c}	3.77 ±0.23^{d}	4.35 ±0.22^{e}
Stretchability (cm)	26.25 ±1.57^{a}	28.67 ±2.04^{b}	29.33 ±1.08^{bc}	31.17 ±1.50^{c}	33.50 ±1.70^{d}	35.92 ±1.80^{e}

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$).

Table 4: Effect of storage on microbiological properties of yak milk *mozzarella* cheese (Mean±S.D.).

Microbiological properties	Storage days					
	0	7	14	21	28	35
Standard Plate Count (Log_{10}CFU/g)	3.37 ±0.26^{a}	3.84 ±0.06^{b}	4.13 ±0.07^{c}	4.25 ±0.04^{c}	4.51 ±0.02^{d}	4.66 ±0.03^{e}
Yeast & Mould Count (Log_{10}CFU/g)	1.13 ±0.21^{a}	1.88 ±0.10^{b}	2.01 ±0.12^{b}	2.38 ±0.09^{c}	2.64 ±0.07^{d}	2.74 ±0.04^{d}
Coliform count (Log_{10}CFU/g)	ND	ND	ND	ND	ND	ND

n=6, *mean with different superscripts in a row differs significantly ($p<0.05$). ND-Not detected

Table 5: Effect of storage on sensory properties of yak milk *mozzarella* cheese (Mean±S.D.).

Sensory properties	Storage days					
	0	7	14	21	28	35
Colour & Appearance	8.33 ±0.76^{a}	8.35 ±0.91^{a}	8.17 ±0.78^{a}	8.02 ±0.80^{a}	8.23 ±0.83^{a}	NP
Flavour	8.27 ±0.71^{a}	7.79 ±0.99^{a}	8.04 ±0.82^{a}	7.73 ±0.98^{a}	8.19 ±0.89^{a}	NP
Body & Texture	8.25 ±0.75^{b}	8.17 ±0.86^{b}	8.13 ±0.77^{b}	7.15 ±1.04^{a}	8.29 ±0.86^{b}	NP
Overall Acceptability	8.19 ±0.82^{a}	8.30 ±0.82^{a}	8.13 ±0.72^{a}	7.91 ±1.02^{a}	8.29 ±0.86^{a}	NP

n=24, *mean with different superscripts in a row differs significantly ($p<0.05$). NP-Not Performed

during sensory evaluation by the sensory panellists. Samples of cheese were evaluated periodically for various sensory properties including colour and appearance, flavour, body and texture, and overall acceptability. The sensory evaluation of cheese sample revealed that colour & appearance, flavour, overall acceptability was non-significant ($p>0.05$) while, body & texture was significant ($p<0.05$) during storage (Table 5). It was evident from Table 5, the sensory scores was higher at the beginning and it was lower in later part of storage period. It is because *mozzarella* cheese, unlike most other cheeses, is not ripened or aged, and its sensory properties reduce with the progress of storage time (Yazici et al. 2010; Sulieman et al. 2013). The sensory evaluation of cheese was discontinued on day 35 of storage period due to development of off flavour and mouldy surface on cheese sample. Changes in physicochemical, biochemical, and microbiological properties in cheese during storage might explain this trend.

Conclusion

In the present study, *mozzarella* cheese was developed by using yak milk for the first time in India. In order to determine the shelf-life of vacuum-packed yak milk *mozzarella* cheese (YMMC), the cheese sample was analysed for proximate composition, physicochemical, functional, microbiological and sensory properties during storage. Based on the obtained results, it was found that storage significantly influenced the moisture content, meltability, free oil formation, stretchablity, physicochemical and microbiological properties of YMMC. The cheese was relished by the sensory panellists throughout the storage and could be stored up to 28 days with optimum organoleptic attributes, functional properties and without significant changes. Further research is also needed to fully understand the textural, rheological behaviour and other preparation methods for YMMC. Taking part in yak cheese business could be lucrative for highlanders and provide them with financial assistance.

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