

# Evaluation of quality and shelf-life of yak milk phrum during refrigerated storage

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## Abstract

Phrum is an acid-coagulated yak milk product popular in the West Kameng district of Arunachal Pradesh, India. However, the available research data on this milk product is very limited. Therefore research was undertaken to study the characteristics of fresh yak milk phrum and its quality changes during refrigerated storage. Results have shown that yak milk phrum has a higher yield, protein, fat, and highly acceptable sensory scores compared to cow milk paneer. During refrigerated storage, yak milk phrum showed a significant increase in titratable acidity, thiobarbituric acid reactive substances value, standard plate count, psychrophilic, lactobacillus, and yeast and mold counts. Sensory scores of yak milk phrum for appearance, flavor, body, texture, and juiciness declined significantly during the refrigerated storage. The shelf-life of yak milk phrum was nine days under refrigerated storage ( $4\pm 1^\circ\text{C}$ ) with well acceptable quality attributes.

**Keywords:** Yak milk, Phrum, Quality, Refrigerated temperature, Shelf-life

## Introduction

Yak (*Bos grunniens* or *Poephagus grunniens* L.) is a high-altitude animal thriving at very low temperatures where low atmospheric oxygen concentration and poor feed resources are prevalent. Despite the challenging living conditions, yak serves as the sole source of income for the yak herdsman utilizing its valuable products. Yak milk is a highly nutritious product rich in fat, protein, essential minerals, and healthy polyunsaturated fatty acids like conjugated linoleic acid and omega-3 fatty acids [1,2;3]. The composition of yak milk includes around 5.07% fat, 9.16% Solids Not Fat (SNF), 3.47% protein, and 2.78% casein [4]. Yak milk is creamy white in color, having thick consistency and peculiar wet yak hair odor or sweat odor which is more prevalent in the winter season. People have to get accustomed to yak milk, the main reason being its peculiar flavor. Preparation of phrum, an acid-coagulated milk product, from yak milk is an excellent way to overcome this problem. Yak milk phrum can be consumed like any other paneer prepared from cow or buffalo milk and it does not require any special acclimatization. Traditionally yak milk phrum is prepared with full fat resulting in rich flavor and soft consistency. Paneer is an ubiquitous product in India, having great demand among consumers in the national and international markets. Paneer is a well-known traditional dairy product prepared by acid coagulation of hot milk. About one percent of the total milk produced in India is converted into paneer. Traditionally, yak milk phrum is prepared by using the juice from a citrus fruit called *thung* or whey obtained from the preparation of *chura* or *churpi*. Citric acid is seldom utilized by *brokpas*, the yak herdsman, for making traditional

phrum in yak rearing tracts of West Kameng and Tawang districts of Arunachal Pradesh. Mostly yak milk phrum is prepared with full-fat milk resulting in rich flavor, soft body, and texture. It is considered luxurious food, offered to Buddha on festival occasions, and gifted to lamas for preparing delicious dishes. Yak milk phrum has a great demand in the yak rearing states of India, which is utilized in various culinary preparations. A study conducted by Kandeepan et al. concluded that well-acceptable low-fat paneer with improved body and texture can be prepared with 1% fat in yak milk [5]. A study was also undertaken to compare sensory quality attributes of full fat, low fat, and dietary fiber-enhanced low fat paneer prepared from yak milk [6;4]. It was found that guar gum as dietary fiber can be successfully incorporated in low-fat yak milk paneer at the level of 0.15% with improved body texture and juiciness. Because of the high moisture content, paneer cannot be stored for more than one day at room temperature. But low-temperature preservation can improve the shelf-life of paneer by several days by delaying the deteriorative changes. Also, low-temperature preservation can ensure a continuous supply of highly nutritious products even during a scarce period.

Refrigerated storage extends the shelf-life of paneer but some of the physicochemical, microbiological, and sensory changes gradually affect the storage quality of the product. Few attempts have been made to study different shelf-life parameters during the storage of paneer prepared from various sources of milk. Arora and Gupta observed that the paneer made from 4, 5, and 6% fat milk could be stored for no more than 6 days at  $10^\circ\text{C}$  and at least 120 days at  $-32^\circ\text{C}$  without much deterioration in its quality [7]. Agnihotri and Pal indicated that good quality paneer from goat milk (Barbari) can be prepared and stored safely only for 3 days under refrigeration before it is marketed [8]. Karadbhajnne and Bhojarkar concluded that among citric acid, ascorbic acid, lactic acid, and tartaric acid used for the preparation of paneer from buffalo milk, ascorbic acid is the best coagulant for providing the best texture properties as well as chemical and organoleptic test [9]. Vandana Bali et al. found that bacteriocin extracted from *Enterococcus faecium* bs 13 significantly increased the shelf-life of paneer upto 15 days [10]. Ritesh Ranjhan Singh et al. reported that a 0.6% addition of turmeric slows down the chemical, microbial and textural changes during storage, especially in refrigerated temperature below  $5^\circ\text{C}$ , and extend the self-life of cow or buffalo milk paneer upto 15 days [11]. However scientific information on the storage stability of yak milk phrum is not available. Therefore, a study was undertaken to evaluate the quality characteristics of yak milk phrum during refrigerated storage ( $4\pm 1^\circ\text{C}$ ).

## Materials and methods

**Source of milk:** Yak milk was obtained from the Institute's farm of

National Research Centre on Yak, Nyukmadung, West Kameng District, Arunachal Pradesh, India.

**Phrum preparation:** A standardized formulation and processing steps were followed to prepare phrum from yak milk [4]. The yak milk was stored in a refrigerator at  $4\pm 1^\circ\text{C}$  for 6 hours until phrum preparation. The yak milk (6% fat and 10% SNF, Solids Not Fat) was heated to  $90^\circ\text{C}$  for 15 seconds and cooled to  $76^\circ\text{C}$ . Then 1% Citric acid boiled and kept at  $70^\circ\text{C}$  was added to it. The whole content was allowed to cool to  $70^\circ\text{C}$ , where, the coagulum gets settled within 10 minutes. The coagulum was then transferred to a muslin cloth. The whey was allowed to drain and the coagulated mass was pressed with weight (about 5 times the weight of the coagulum) for 10 minutes. The blocks were immersed in potable chilled water at  $4^\circ\text{C}$  for 3 hours. Excess water was drained and the product was packaged in  $80\mu$  low-density polyethylene (LDPE) bags and stored at  $4^\circ\text{C}$ . The LDPE bags were sterilized in ultraviolet (100–280nm) light chamber for 30 minutes before utilization for packaging phrum.

**Sample preparation for analysis:** Ten batches of freshly prepared phrum were examined for various physicochemical and sensory quality parameters. Five batches of phrum were also examined in duplicates for changes in physicochemical, microbiological, and sensory properties during 0, 3, 6, 9, 12, and 15 days of storage at  $4\pm 1^\circ\text{C}$ . Each time three packets of 100 g were randomly selected for shelf-life study, one each for physicochemical, microbiological, and sensory properties. Samples from the packets for microbiological examination were taken first followed by a sample for pH determination. The remaining parts of the samples were used for sensory evaluation.

**Analytical procedures:** The quality of phrum was evaluated by assessing the yield, proximate composition [12], physicochemical properties, microbiological assay [13], and sensory attributes.

#### Physicochemical characteristics:

**Yield:** The yield of the phrum was calculated as the percent weight of the final product to the initial weight of milk taken for the preparation of phrum.

**Proximate composition:** Percent moisture, ether extract, protein, and ash contents in phrum were determined according to [12] methods. Moisture was determined by drying the sample in an oven at  $102\pm 2^\circ\text{C}$  to constant mass. Ether extract in phrum was estimated by Soxhlet extraction of above-dried samples with petroleum ether. Protein content was determined using micro-kjeldahl distillation. The lactose content was also determined by the method described by [12].

**pH:** For pH determination, a 10 g sample was triturated with 90 ml double distilled water and measurements were made at room temperature ( $25\pm 2^\circ\text{C}$ ) using a combined glass electrode of a digital pH meter (pH Tutor, Eutech Instruments, India). The titratable acidity was measured as percent lactic acid by [12] method.

**TBARS (2-Thio barbituric acid reactive substances) value:** The distillation method of [14] was followed to estimate the TBARS value. A 10 g sample was used to extract the distillate. Then 2-Thiobarbituric acid mixed with glacial acetic acid was used to develop a pink color in the distillate of yak milk phrum. The absorbance of the color developed was recorded at 538 nm using a spectrophotometer (Scanning mini SPEC, model SL 177, Elico Ltd, Hyderabad). The absorbance was multiplied by a factor of 7.8 and the TBARS value was expressed as mg malonaldehyde/kg of the sample [15].

**Microbiological quality:** All the microbiological parameters of yak milk phrum were determined as per the methods described by APHA [13]. Readymade media from Hi-Media Laboratories (P) Ltd, Mumbai, India were used for the enumeration of different microbes. Preparation of

samples and serial dilution of yak milk phrum were done near the flame in a horizontal laminar flow unit (Model YSI-188, Yarco Sales (P) Ltd., New Delhi, India) which was pre-sterilized by ultraviolet radiation, observing all possible aseptic precautions. Sterile peptone water (0.1%) was used as a diluent for making serial dilutions. Plate count agar (M091) was used to enumerate the total plate count and psychrotrophic count. In the case of Lactobacilli count, MRS agar (M 6411) was used with glycerol. The Potato Dextrose agar (M096) medium acidified with a 10% sterilized tartaric acid solution was used for yeast and mold count. Violet Red Bile agar (VRBA, M049A) was used for the coliform counts. After the incubation period of 48 hours at  $37\pm 1^\circ\text{C}$  for standard plate count, and coliform count, and 7 days at  $25\pm 1^\circ\text{C}$  for yeast and mold count, the number of colonies was multiplied with the reciprocal of the dilution and expressed as  $\log_{10}$  cfu/g.

**Sensory evaluation:** Phrum samples fresh (control) as well as stored, were examined for changes in appearance, flavor, body & texture, and juiciness by the panelists. A sensory panel was used to study the perceived differences in the phrum samples by descriptive profile scoring of sensory attributes. The sensory panelists ( $n=10$ ) consisted of scientists and research assistants of the ICAR-National Research Centre on Yak, Dirang, West Kameng District, Arunachal Pradesh, India. Panelists were trained for sensory analysis of milk products and were well acquainted with the different sensory attributes. They were briefly told about the nature of the experiment without disclosing the identity of the samples. The tested samples were stored for 15 days at  $4\pm 1^\circ\text{C}$  and the samples were drawn at the interval of 0, 3, 6, 9, 12, and 15 days for sensory evaluation. About 100g of sample was examined at each interval and the experiment was replicated thrice. Samples were warmed ( $40\text{--}45^\circ\text{C}$ ) using a microwave oven (LG electronics India (P) Ltd., Mumbai) for 1 min and served to the panelists. Each panelist received two cubes from each sample of phrum in a randomized order. Each session included samples from all groups of phrum. Panelists were provided with filtered water to cleanse their pallets between samples. Panelists evaluated samples for appearance, flavor, body & texture, and juiciness using an eight-point descriptive scale. The scale used for appearance was: 1 = extremely poor, 2 = very poor, 3 = moderately poor, 4 = slightly poor, 5 = fair, 6 = good, 7 = very good, and 8 = excellent. The scale used for flavour was: 1 = extremely bland, 2 = very bland, 3 = moderately bland, 4 = slightly bland, 5 = slightly intense, 6 = moderately intense, 7 = very intense, and 8 = extremely intense. The scale used for body & texture was: 1 = extremely firm, 2 = very firm, 3 = moderately firm, 4 = slightly firm, 5 = slightly soft, 6 = moderately soft, 7 = very soft, and 8 = extremely soft. The scale for juiciness was: 1 = extremely dry, 2 = very dry, 3 = moderately dry, 4 = slightly dry, 5 = slightly juicy, 6 = moderately juicy, 7 = very juicy, and 8 = extremely juicy. Panelists reported scores to the nearest half-point. Panelists' scores were subjected to statistical analysis. Tests were conducted under white fluorescent lights in partitioned booths.

**Statistical analysis:** The data generated for different quality characteristics were compiled and analyzed using SPSS version 10.0 for Windows; SPSS, Chicago, Ill., U.S.A. The data were subjected to analysis of variance (one-way ANOVA for storage quality parameters), least significant difference [16], and Duncan's multiple range test [17] for comparing the means to find the difference between storage periods and their interaction for various parameters in different experiments. The smallest difference ( $D_{5\%}$ ) for two means to be significantly different ( $P<0.05$ ) was reported.

## Results and discussion

**Product characteristics:** Freshly prepared yak milk phrum had a creamish white surface and soft, spongy texture. Unlike boiled yak milk, freshly prepared phrum had no peculiar smell or taste. The cut surface was smooth and slightly moist. The body of the yak milk phrum was soft with a smooth texture and no air pockets. Masud et al. indicated the sensory quality of buffalo milk paneer prepared at a coagulation temperature of 95°C to be fairly white in color, fairly soft in body, coarse and fibrous in texture, and sweet and nutty in flavor. The body of the yak milk phrum was comparable to the above study [18].

**Yield:** The results of the yak milk phrum indicated an average yield of 21.67±0.56% (Table 1). [19] reported a yield of paneer as 15.20% for cow milk and 21.40% for buffalo milk. Buffalo milk paneer prepared at a coagulation temperature of 95°C resulted in a 21.54% yield [18]. Singh and Kanawjia reported a paneer yield of 20.52% from buffalo milk with 2% citric acid as coagulant [9]. The paneer prepared from goat milk gave a yield of 16.41% [8]. The yield of hard cheese from yak milk was reported as 13.85-15.50% which showed that yak milk phrum is comparatively a profitable entrepreneurship [20; 21]. The cheese making process differs from phrum making as discussed in materials and methods section. The yak cheese is made with pasteurized milk, mesophilic starter culture, and calf rennet enzyme with 1,150 international milk-clotting units/g. Cheese is packed into blocks and ripened at 6°C for 90 d [21]. The yield was much higher than the yield of paneer reported from cows and comparable to the buffalo milk paneer yield (21.8%) [22]. The higher yak milk phrum yield compared to the paneer yield data available for cow or goat milk could be related to fine protein matrix formation and fat retention in yak phrum. The yield of paneer was reported to be influenced by breed, total solids, level of proteins, fat content in milk, amount of moisture retained in paneer, and losses of milk solids in whey [23].

**Proximate composition:** The mean values of moisture, protein, fat, and ash contents of yak milk phrum were 52.41±0.99%, 18.35±0.17%, 23.30±0.60%, and 3.41±0.45% respectively (Table 1). The paneer from cow milk with 3.5% fat indicated 55.97% moisture, 18.98% fat, 20.93% protein, 2.01% lactose, and 1.45% ash [24]. Kumar et al. reported that paneer prepared from buffalo milk with 6% fat showed 50.98% moisture, 27.97% fat, 14.89% protein, 2.63% lactose, and 2.08% ash

[25]. Buffalo milk paneer prepared at a coagulation temperature of 95°C resulted in 20.58% protein, 23.5% fat, 2.92% ash, 52.94% total solids, and 46.81% moisture [18]. Karadbhajne and Bhojarkar reported moisture, fat, and protein content of paneer prepared from buffalo milk as 51.50%, 25.88%, and 20.75% respectively with 2% citric acid as a coagulant [9]. Buch et al. indicated that paneer prepared from 5.5% fat standardized cow and buffalo mixed milk had 51.56% moisture, 26.88% fat, 18.41% protein, 1.53% ash, and 1.62% lactose [26]. Agnihotri and Pal reported the proximate composition of goat milk paneer as 46.94% moisture, 19.99% protein, 26.95% fat, and 1.93% ash [8]. A study conducted on the preparation of paneer from reconstituted milk with 2% citric acid as a coagulant resulted in 57.4% moisture, 19.1% fat, 18.1% protein, and 1.9% ash contents [27]. In a study conducted on market samples of paneer, the gross chemical composition revealed 51.43% moisture, 26.8% fat, 17.67% protein, 2.07% lactose, and 1.89% ash [28].

The moisture content was comparatively lower than the values reported for cow milk paneer (55.26%) or buffalo milk paneer (54.10%) [22; 29]. The lower moisture content of the yak milk phrum was attributed to the higher fat content of the milk. The protein and ash content of the yak milk phrum was similar to the values reported for paneer from cow or buffalo milk as mentioned below research findings. The protein content of the cheddar cheese from yak milk was 22.51% [21], which was comparatively higher than the protein content of yak milk phrum as recorded in the present study. The fat content of the product was related to the fat content of the yak milk. The fat content in the yak milk phrum contributed to the soft texture of the product. The fat content of yak milk cheddar cheese was indicated as 32.05% [21], that was greater than the fat content of yak milk phrum in the current research. As reported by Zhang et al. [21], the moisture content of yak milk cheese was comparatively lower (31.28%), while the ash content was comparatively higher (4.38%) than the moisture and ash content of the yak milk phrum as per the current study. The results of ash content of yak milk phrum were higher than the ash contents of cow and buffalo milk paneer, reported as 2.53% and 2.67% respectively [30]. The lactose content of yak milk paneer (2.43%) was within the range of lactose content of cow and buffalo milk paneer with values of 2.52% and 2.33% respectively as indicated by [30].

**Sensory attributes:** The sensory evaluation results of fresh phrum showed appearance, flavor, body & texture, and juiciness scores of 7.50±0.06, 7.50±0.06, 7.20±0.03, and 7.45±0.01 respectively (Table 1). The appearance of the yak milk phrum was dark creamy white, which was much preferred by the sensory panel. The wet hair odor or the sweat odor of yak that was prevalent in the fresh milk was completely absent in the phrum prepared by the acid coagulation method. A highly acceptable fine nuance of the yak milk flavor present in the phrum was perceived by the sensory panel. The body and texture of the yak milk phrum were softer due to the fat globules dispersed in the milk. The body of the phrum was not very firm due to which the sliceability was very good. Masud et al. reported sensory scores of 6-8 on a 9-point hedonic scale for buffalo milk paneer prepared at a coagulation temperature of 95°C [18]. A study conducted on the sensory quality of paneer made from reconstituted milk using 2% citric acid as coagulant revealed a mean sensory score of 7.7 for appearance, 7.6 for flavor, 7.6 for body and texture, and 7.6 for overall acceptability [27]. The sensory scores of yak milk phrum were comparable to the appearance and flavor scores reported in the above study.

### Quality changes during refrigerator storage:

**Physicochemical characteristics:** The pH of the yak milk phrum differ

**Table 1: Physicochemical and sensory characteristics of yak milk phrum**

Parameters	Mean±SE
<b>Physicochemical quality #</b>	
Yield (%) ###	21.67±0.56
Moisture (%)	52.41±0.99
Protein (%)	18.35±0.17
Fat (%)	23.30±0.60
Ash (%)	3.41±0.45
Lactose (%)	2.43±0.50
<b>Sensory attributes ####</b>	
Appearance	7.50±0.06
Flavor	7.50±0.06
Body and texture	7.20±0.03
Juiciness	7.45±0.01

#n=10, ##n=5, ###n=50

\*Based on 8 points descriptive scale

**Table 2: Physicochemical characteristics of yak milk phrum at refrigerated storage (4±1°C)**

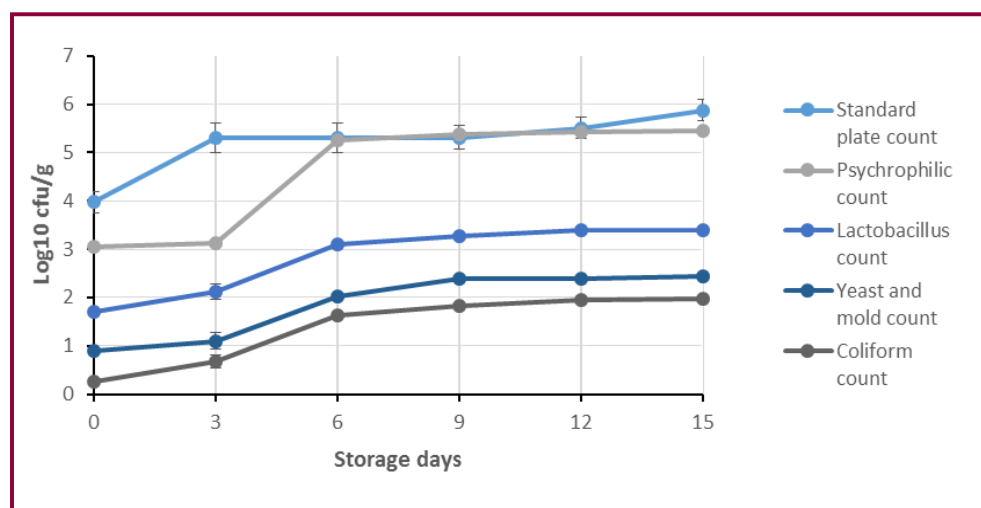
Parameters	Storage period (days)					
	0	3	6	9	12	15
pH	5.25±0.09	5.25±0.09	5.13±0.08	5.13±0.08	5.13±0.09	5.13±0.09
Titrateable acidity (% LA)	0.46±0.01 <sup>c</sup>	0.46±0.01 <sup>c</sup>	0.46±0.01 <sup>c</sup>	0.46±0.01 <sup>c</sup>	0.49±0.01 <sup>b</sup>	0.54±0.01 <sup>a</sup>
TBARS (mg MDA/kg)	0.03±0.01 <sup>d</sup>	0.03±0.01 <sup>d</sup>	0.03±0.01 <sup>d</sup>	0.07±0.01 <sup>c</sup>	0.14±0.01 <sup>b</sup>	0.47±0.01 <sup>a</sup>

n=10

Means with different superscripts in the same row indicate a significant difference (P&lt;0.05)

significantly ( $p<0.05$ ) during the refrigerated storage period (Table 2). However, there was a fall in pH which was due to acidity development in phrum. Arora and Gupta reported no significant change in the pH of the paneer during storage at 10°C for 6 days [7]. Agnihotri and Pal reported a non-significant increase in pH from 5.91 to 5.99 during 7 days of refrigerated goat milk paneer. The titrateable acidity of the yak milk

phrum increased significantly ( $p<0.05$ ) beyond 12 days of refrigerator storage. The increase in titrateable acidity might be attributed to acidic metabolites released due to the growth of microbes during the storage period [8]. Ritesh Ranjhan Singh et al. reported a significant increase in the acidity of paneer prepared from cow and buffalo milk and subsequent storage at refrigerated temperature for 15 days. The acidity

**Figure 1: Microbiological quality changes in yak milk phrum during refrigerated (4±1°C) storage**

values ranged from 0.162 to 0.414 during the storage period [11]. In a study conducted on market samples of paneer, the gross chemical composition revealed 0.77% lactic acid [28]. Buch et al. stated that the acidity (% lactic acid) of stored paneer varied from 0.55 on day 0 to 0.63 on day 12. The percent lactic acid of yak milk phrum reported in this study is lower than the above finding [26].

The TBARS value of yak milk phrum increased significantly ( $p<0.05$ ) after 9 days of refrigerator storage (Table 2). The increase in TBARS value was due to the oxidation of milk lipids and the release of by-products such as malonaldehyde. However, the level of lipid oxidation was within the permissible limit of 0.1-1mg/kg during the storage period [14].

Ritesh Ranjhan Singh et al. reported a gradual increase in the peroxide value of refrigerated paneer from 0.486 to 2.43 [11].

**Microbiological characteristics:** The microbial counts of yak milk phrum increased significantly ( $p<0.05$ ) during the storage at refrigerated temperature (Table 3 & Figure 1). The standard plate count of yak milk phrum increased significantly ( $p<0.05$ ) after 3 days of refrigerator storage at 4±1°C. The increase in microbial growth might be attributed to the utilization of abundant carbohydrate, protein, and lipid sources present in the phrum. Sachdeva and Singh reported that fresh paneer had a total plate count of  $10^1$  to  $10^3$  cfu/g, whereas for spoiled samples counts ranged from  $158 \times 10^4$  cfu/g to  $45 \times 10^6$  cfu/g of paneer [31].

**Table 3: Microbiological characteristics of yak milk phrum at refrigerated storage (4±1°C)**

Parameters	Storage period (days)					
	0	3	6	9	12	15
Standard plate count (Log cfu/g)	3.98±0.22 <sup>b</sup>	5.30±0.31 <sup>a</sup>	5.30±0.31 <sup>a</sup>	5.31±0.24 <sup>a</sup>	5.50±0.22 <sup>a</sup>	5.87±0.22 <sup>a</sup>
Psychrophilic count (Log cfu/g)	3.05±0.04 <sup>c</sup>	3.12±0.07 <sup>c</sup>	5.26±0.01 <sup>b</sup>	5.38±0.06 <sup>a</sup>	5.42±0.01 <sup>a</sup>	5.45±0.01 <sup>a</sup>
Lactobacillus count (Log cfu/g)	1.70±0.01 <sup>d</sup>	2.13±0.16 <sup>c</sup>	3.09±0.01 <sup>b</sup>	3.28±0.01 <sup>a</sup>	3.40±0.01 <sup>a</sup>	3.40±0.01 <sup>a</sup>
Yeast and mold count (Log cfu/g)	0.90±0.04 <sup>c</sup>	1.10±0.18 <sup>c</sup>	2.02±0.01 <sup>b</sup>	2.38±0.01 <sup>a</sup>	2.40±0.01 <sup>a</sup>	2.44±0.01 <sup>a</sup>
Coliform count (Log cfu/g)	0.25±0.01 <sup>d</sup>	0.68±0.14 <sup>c</sup>	1.64±0.01 <sup>b</sup>	1.83±0.01 <sup>a</sup>	1.95±0.01 <sup>a</sup>	1.97±0.01 <sup>a</sup>

n=10

Means with different superscripts in the same row indicate a significant difference (P&lt;0.05)

**Table 4: Sensory attributes of yak milk phrum at refrigerated storage (4±1°C).**

Attributes <sup>a</sup>	Storage period (days)					
	0	3	6	9	12	15
Appearance	7.50±0.01 <sup>a</sup>	7.46±0.04 <sup>a</sup>	7.38±0.06 <sup>a</sup>	6.79±0.10 <sup>b</sup>	5.63±0.06 <sup>c</sup>	ND
Body & Texture	7.20±0.03 <sup>a</sup>	7.17±0.04 <sup>a</sup>	7.15±0.03 <sup>a</sup>	6.04±0.04 <sup>b</sup>	4.96±0.04 <sup>c</sup>	ND
Flavor	7.50±0.01 <sup>a</sup>	7.50±0.04 <sup>a</sup>	7.38±0.06 <sup>a</sup>	6.21±0.10 <sup>b</sup>	5.08±0.05 <sup>c</sup>	ND
Juiciness	7.45±0.01 <sup>a</sup>	7.43±0.02 <sup>a</sup>	7.33±0.06 <sup>b</sup>	6.01±0.01 <sup>c</sup>	4.96±0.04 <sup>d</sup>	ND

n=50  
<sup>a</sup>Based on 8 points descriptive scale  
 ND=Not detected since the product was spoiled on day 15  
 Means with different superscripts in the same row indicate a significant difference (P<0.05)

Agnihotri and Pal [8] reported a significant increase in SPC from the initial microbial load of 3.94 cfu/g to a 7<sup>th</sup>-day value of 6.08 cfu/g during refrigerated storage of goat milk paneer [8]. Vandana Bali et al. indicated that in paneer samples, a slight increase in the microbial count was observed up to 5 days followed by a sharp increase in the count after 10 days of the storage period, which could be due to the initiation of spoilage of paneer at 4°C [10]. Ritesh Ranjhan Singh et al. reported a significant increase in the SPC of refrigerated paneer from 30×10<sup>3</sup> to 475 ×10<sup>3</sup> cfu/g [11].

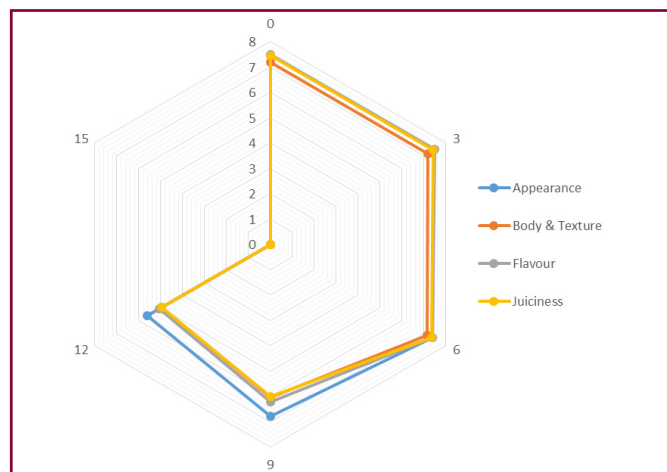
The psychrophilic count increased significantly after 6 days of refrigerator storage (Table 3). Agnihotri and Pal [8] reported a significant increase in psychotropic bacteria from the initial microbial load of 2.22 cfu/g to the 7<sup>th</sup>-day value of 4.26 cfu/g during refrigerated storage of goat milk paneer. The lactobacillus count of yak milk paneer increased significantly after 3 days of refrigerator storage.

The yeast and mold count increased significantly (p<0.05) after 6 days of refrigerator storage (Table 3). Agnihotri and Pal reported a significant increase in yeast and mold count from the initial microbial load of <1.00 cfu/g to a 7<sup>th</sup>-day value of 2.60 cfu/g during refrigerated storage of goat milk paneer [8]. Ritesh Ranjhan Singh et al. reported a significant increase in yeast and mold count of refrigerated paneer from 24 ×10<sup>3</sup> to 275.5 ×10<sup>3</sup> cfu/g [11].

The increase in lactobacillus count and yeast and mold count was related to the increase in titratable acidity of the yak milk phrum during storage. However, the values were well within the limits prescribed for paneer from cow or buffalo milk [32]. The Indian standards specification for paneer (IS: 10484-1983) indicates that the bacterial count per gram of paneer sample is 10×10<sup>5</sup>, while the fungal count per gram is 250. Food Safety and Standard Regulations [33] indicated microbiological parameters for paneer as total plate count not more than 50,000 cfu/g, coliform count not more than 90 cfu/g, and yeast and mold count not more than 250 cfu/g. A higher mesophilic, coliform, yeast, and mold content of the paneer is attributed to the post-milking contamination. Dhole et al. evaluated seventy samples of fresh paneer from seven vendors of Ahmednagar City for microbiological quality. The average coliform count in the market samples of paneer was found in the range of 12.6 × 10<sup>3</sup> to 23.2 × 10<sup>3</sup> cfu/g [34]. Vishweshwaraiah and Anantkrishnan reported good grade paneer having 5,000-50,000 cfu/g standard plate count and satisfactory grade paneer with <10 cfu/g coliform count [35]. Sachdeva and Singh observed the microbiological characteristics of paneer stored at 8–10°C and reported that the fresh paneer samples showed an initial count ranging from 2.3×10<sup>4</sup> to 9.0×10<sup>4</sup> cfu/g. The total plate count of the spoiled samples ranged from 1.58×10<sup>6</sup> to 4.5×10<sup>7</sup> cfu/g. The initial yeast and mold count of fresh samples varied over a narrow range of 3.5×10<sup>2</sup> to 5.2×10<sup>2</sup> cfu/g, while at the time of spoilage it ranged from 5.3×10<sup>3</sup> to 6.3×10<sup>4</sup> cfu/g [31].

Singh and Singh analyzed the market samples of paneer collected from Agra city, India and found comparatively lower total plate count (6.51 log<sub>10</sub> cfu/g), coliform count (3.05 log<sub>10</sub> cfu/g), yeast and mold count (2.99 log<sub>10</sub> cfu/g), Enterococcus count (2.73 log<sub>10</sub> cfu/g) and Micrococcus count (2.03 log<sub>10</sub> cfu/g) for laboratory-made samples against 18.00, 10.39, 7.54, 5.05 and 5.07 log<sub>10</sub> cfu/g, respectively for market samples. They concluded that the poor bacteriological quality of market samples was mainly due to the use of poor quality milk, and unhygienic practices during the manufacturing, handling, and storage of products [36]. A study conducted on the microbiological quality of market samples of paneer indicated a total viable count of 52,18.95×10<sup>5</sup> cfu/g, coliform count of 98,242.57×10<sup>2</sup> cfu/g, and yeast and mold counts of 11,920.94 cfu/g [28].

The standard plate count, coliform counts, and yeast and mold counts of the yak milk phrum are within the acceptable microbial limits compared to the above studies. Since phrum is obtained by direct acidification at elevated temperature, its microflora arises mainly from post-processing contamination namely from air, utensils, workers, handling conditions, packaging materials, and raw milk. The level of microbial contamination in raw yak milk, prevailing low temperature as well as hygiene during processing were the main reasons for an increased shelf-life of 9 days for the phrum produced in this study unlike 7 days for buffalo milk paneer [37]. Kanawjia et al. also reported the shelf-life of paneer as only 6 days at refrigerated with the freshness of the product lost after 3 days [38]. Thippeswamy et al. reported that hurdle technology adopted paneer with modified atmosphere packaging (MAP) extended the shelf-life from 1 to 12 days at room temperature (30±1°C) and 6 to 20 days at refrigeration (7±1°C) temperature [39].

**Figure 2: Sensory quality changes in yak milk phrum during (4±1°C) storage**

**Sensory attributes:** The sensory scores of the yak milk phrum decreased significantly during the storage period (Table 4 & Figure 2). The appearance, body and texture, flavor, and juiciness scores decreased significantly ( $p < 0.05$ ) after 9 days of storage period. The appearance of the product during storage showed a slight change towards pale creamish white on the surface, but it remained acceptable. Agnihotri and Pal reported a significant decrease in color scores from an initial value of 4.72 to a 7<sup>th</sup>-day value of 3.90 on a 5-point hedonic scale during refrigerated storage of goat milk paneer [8].

The characteristic fresh phrum flavor was changed towards bland on day 9 but lactic flavor was predominant in the samples on 15 days of storage period. Surface slime was detected on the 12<sup>th</sup> day of the storage period which gave a slightly greasy feeling in touch by the panelists. The decrease in flavor scores might be attributed to an increase in acidity and lipid oxidation observed during the storage period. Agnihotri and Pal reported a significant decrease in odor scores from an initial value of 4.70 to a 7<sup>th</sup>-day value of 3.60 on a 5-point hedonic scale during refrigerated storage of goat milk paneer [8]. Arora and Gupta observed no significant change in the sensory attributes during the 6-day storage [7]. However, they reported that when the storage period was extended to the 7<sup>th</sup> day, it gave a putrid odor and became unacceptable. Ritesh Ranjhan Singh et al. reported a significant decrease in color and appearance and body and texture scores of paneer prepared from cow and buffalo milk and during subsequent storage at refrigerated temperature [11]. In the present study, the sensory scores decreased significantly on the 9<sup>th</sup> day of storage but the scores were well within the acceptable limit, whereas the scores became below the acceptable limit on the 12<sup>th</sup> day of storage.

The juiciness scores of yak milk phrum decreased significantly after 6 days of storage period. The decrease in juiciness scores might be attributed to the changes in the texture of the phrum during the refrigerator storage. The softer texture of the phrum decreased the amount of mastication and hence lowered juiciness scores.

## Conclusions

It is concluded that the yak milk phrum is rich in protein and fat and gives desirable product yield and sensory scores. The hygienically processed yak milk phrum stored at refrigeration temperature ( $4 \pm 1^\circ\text{C}$ ) showed a shelf-life of 9 days without any significant deterioration on its physicochemical, microbial, and sensory quality.

## Compliance with Ethical Standards

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