

# Quality assessment of buffalo milk *Chakka* prepared from different starter culture

Varsha Vihan, VPSingh, Pramila Umaraw(✉), Akhilesh K Verma, Chirag Singh and Shardanand Verma

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**Abstract:** *Chakka* is a soured dairy product that is produced by removing the whey from fermented milk (dahi, known as Indian yogurt). The starter culture plays a significant role in determining the overall elemental quality of fermented dairy products. The present study was carried to find out the suitability of three different cultures for development of *chakka* viz. T1 (1.5% Mixed microbial culture), T2 (1.5% bacterial culture, STI-13 freeze-dried Lactic culture), and T3 (1.5% yeast cultures). Among the different culture various quality characteristics, physico-chemical parameters and sensory characteristics were assessed for their ability to form *chakka*. Curd prepared from the *Lactobacillus* culture (T2) was found most suitable for preparation of *chakka* as T2 delivered highest yield of curd mass, protein, least pH value and moisture content compared to microbial culture and yeast culture. Sensory attributes of *chakka* also varied significantly ( $P \leq 0.05$ ) among cultures. All sensory attributes rated higher for T2 (*Lactobacillus* cultures). Therefore, result of the study concluded that T2 (1.5% *Lactobacillus*) culture was most appropriate for formulation of *chakka*.

**Keywords:** *Chakka*, Cultures, pH, Proximate composition, Sensory

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Department of Livestock Products Technology  
College of Veterinary and Animal Sciences  
Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250 110, Uttar Pradesh

Pramila Umaraw (✉)  
Email: [pramila1303@gmail.com](mailto:pramila1303@gmail.com)

## Introduction

Milk is highly nourishing and perhaps a fundamental food for human diet. With a production of 209.96 million tonnes in 2020–21, India is presently a leading producer of milk. Approximately 65% to 70% of the total amount of milk produced is sold as liquid milk, and 14% is used to make dairy products (ICFA, 2019). Fermented products comprise on an average 30% of the dietary worldwide (Borresen et al. 2012). In India, according to estimates 6.9% of all the milk produced is used to make dahi (Joshna et al. 2021). Among the fermented dairy products, traditional to India; curd/dahi, srikhand, lassi, mishti dahi and butter milk are major products.

Curd, also known as Dahi is the well liked traditional fermented milk product, which is developed from lactic acid fermentation of milk. Similar to milk, curd is very nutrient-dense and a crucial component of a balanced diet. Curd is a super-food that promotes healthy digestion, acts as a natural laxative, and is packed with essential nutrients like calcium, vitamin B-2, vitamin B-12, potassium, and magnesium. Among different types of milk, buffalo milk is more readily used in curd production and contributes 35% of total milk production (BAHS, 2019). Buffalo milk produces higher yield of curd because of higher total solids content especially fat and protein. Buffalo curd complements the required curd traits with its firm solid texture and white color. It also has lower percent of syneresis because of its higher fat and total solid content. Naturally occurring constituent act as stabilizer in curd that lower the exclusion of whey from curd mass. Therefore, curd prepared from buffalo milk is more superior in texture and body.

In order to better serve consumers, dahi varieties have also been developed. Other products, like *chakka*, are also prepared using it. By draining the whey from curd, a white to pale yellow semi-solid product with good texture and consistency is known as *chakka*. *Chakka* has an acidity of 0.7 to 1% and has a sour flavour (lactic acid). It has superior nutritional qualities and is a good source of proteins, vitamins, minerals, and calcium. It also has all the same health advantages as dahi (Sarkar, 2008). According to reports, *chakka* had a higher protein efficiency ratio (PER) than

pure casein (Joshna et al. 2021).

The present investigation was carried out to find out the suitability of various cultures for development of *chakka*. Mixed culture, bacterial culture (STI-13 freeze-dried lactic culture), and yeast cultures were assessed for their ability to form desirable curd and curd mass on the basis of physico-chemical parameters and sensory characteristics.

## Materials and Methods

Raw buffalo's milk was purchased from nearby village dairy plant, featuring properties like: pH = 6.7, titratable acidity = 0.14%, moisture = 84.1%, ash = 0.69%, fat = 5.37% determined with help of milk lab equipment's. All chemicals and reagents used in the study were of analytical grade and were obtained from standard firms (Himedia, SRL and CDH etc.). For packaging of samples Low density polyethylene (LDPE) of 200 gauge was purchased locally from Meerut market.

**Culture:** In the study three types of cultures were used for inoculation and were encoded as:

T1: milk + 1.5% mixed culture;

T2: milk + 1.5% freeze dried bacterial culture

T3: milk + 1.5% yeast culture.

The mixed culture produced by back sloping method was utilized. Freeze-dried lactic culture was bought from CHR Hensen, Denmark. In 50 ml of distilled water, 0.4 g of STI-13 freeze-dried lactic culture was dissolved to create the active culture. The mixture was then placed in a bottle, sealed, and refrigerate until use. While for yeast culture commercially available dry yeast culture was obtained from local market. The yeast culture was activated by mixing measured amount of dry yeast @1.5% with ½ cup (app. 125 ml) of water (at a temperature range between 37 to 43°C). Stirred till dissolved and then used.

## Preparation of Chakka

*Chakka* was prepared using the techniques outlined by Ronak et al. (2016) with a few minor modifications. In brief, 1500 ml of buffalo's raw milk was heated to 85°C±5 for 20 minutes, followed by cooling to 43°C±2, followed by distribution in three portions (500 ml milk per portion): the first portion was inoculated with 1.5% traditional culture and named T1, the second portion was inoculated with 1.5% freeze-dried lactic culture (bacterial culture) and named T2 and the third portion was inoculated with 1.5% yeast culture and named T3. Then, incubation was allowed to be carried out at 37±2°C for 5-6 hours. For each curd type, the obtained curd was then hanged in muslin cloth at 4°C overnight and whey was allowed to drain (Fig. 1 & 2).

## Analytical procedure

Curd yield (%)

Percent curd yield of the *chakka* was estimated as per Murphy et al. (1975) by the following formula:

$$\text{Curd yield} = \frac{\text{weight of curd obtained g}}{\text{weight of milk g}} \times 100$$

## pH and Titratable acidity

Using a pestle and mortar, ten grams of samples were mixed for one minute with 50 ml of distilled water. By dipping an aliquot of the sample into the combined glass electrode of a digital pH metre (Esico (Model-1012), Microprocessor based pH system), the pH of the sample was determined (Trout et al. 1992). While, the titratable acidity in terms of percent lactic acid was determined by method as described by Shelef and Jay (1970).

Spontaneous whey separation (%)

The spontaneous whey separation (SWS) was assessed by the drainage method under refrigeration temperature as per Isanga and Zhang (2009) by employing the following equation

$$\text{SWS (\%)} = V1/V2 \times 100$$

Where,

V1- volume of expelled whey collected after draining

V2- volume of curd sample

Chakka mass yield:

Percent chakka mass yield was estimated as per Murphy et al. (1975) by the following formula:

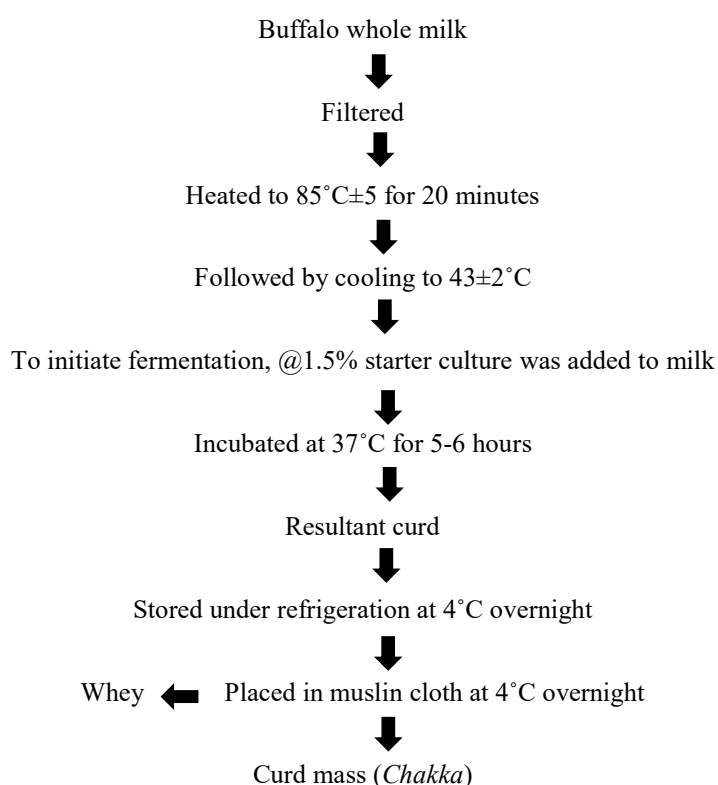
$$\text{Chakka mass yield} = \frac{\text{weight of chakka obtained g}}{\text{weight of curd g}} \times 100$$

## Proximate analysis

The proximate analysis of *chakka* was done following methods described by AOAC (1995) for moisture using hot air oven (Meta-Lab Scientific Industries), fat with SOCS PLUS (SCS-06-AS DLS TS, Pelican Industries, Chennai) and ash (muffle furnace) while automatic digestion and distillation unit (KEL PLUS-KES 12L R TS, Pelican Industries, Chennai) was used for protein estimation following methodology given in AOAC (2000). Total carbohydrates percent was calculated using Atwater values by difference as follows using Atwater values:

$$\text{TC \%} = [100 - (\text{moisture} + \text{protein} + \text{fat} + \text{ash})]$$

## Sensory evaluation



**Fig. 1** Flow chart for preparation of *chakka*

The sensory evaluation of *chakka* prepared were analyzed by experienced panelists from the Department of Livestock Products Technology and College of Veterinary and Animal Sciences, SVPUAT- Meerut, Uttar Pradesh. A nine-point descriptive scale was used for analysis of taste, aroma, texture and overall accept-

ability of *chakka*, where 9 was extremely desirable, 5 was neither like nor dislike and 1 was for extremely undesirable.

### Statistical analysis

The whole experiment was repeated thrice with duplicate sampling (six observations) for all studied parameters except sensory evaluation where number of observations were 21 (7x3). The data obtained was analyzed for ANOVA using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). While, level of significance was evaluated by Duncan’s multiple range test and homogeneity test at (P≤0.05) level.

## Results and Discussion

### Quality characteristics of curd

#### Curd yield

The microorganisms of starter culture play a very crucial role in production of curd with characteristic flavour and acidity. The three starter cultures in the present study mixed, bacterial and yeast culture did not show significant (P≥0.05) difference in the curd yield (Table 1). The mean ± S.E. values for curd yield of T1, T2 and T3 were recorded as 90.04±1.09 (T1), 92.49±1.05 (T2) and 91.48±1.45 (T3), respectively.

#### pH and acidity of curd

Addition of microorganisms in milk lead to aggregation of milk proteins due to acidification. The inoculation of different cultures produced curd with varying pH (Table 1). The pH values of mixed and bacterial culture did not differ significantly (P≥0.05) which might be attributed to the presence of *Lactobacillus*. The mixed culture is primarily cocktail of *Streptococcus* and *Lactoba-*

**Table 1:** Quality characteristics of curd made from different cultures

Parameters	T1	T2	T3
Curd yield (%)	90.04±1.09	92.49±1.05	91.48±1.45
pH	4.83 <sup>a</sup> ±0.02	4.84 <sup>a</sup> ±0.03	5.88 <sup>b</sup> ±0.03
Acidity (% lactic acid)	0.875 <sup>c</sup> ±0.026	0.748 <sup>b</sup> ±0.033	0.478 <sup>a</sup> ±0.014

Means values bearing small letters (a, b, c) groups wise indicate differ significantly (P≤0.05) n=6; T1: curd made with mixed microbial culture; T2: curd made with lactobacillus culture; T3: curd made with yeast culture.

**Table 2:** Quality characteristics of whey made from different cultures

Parameters	T1	T2	T3
Volume of whey (ml)	20.49 <sup>a</sup> ±0.57	18.35 <sup>a</sup> ±0.46	36.24 <sup>b</sup> ±1.39
pH	4.80 <sup>a</sup> ±0.02	4.92 <sup>a</sup> ±0.01	5.62 <sup>b</sup> ±0.10
Acidity (% lactic acid)	0.915 <sup>c</sup> ±0.020	0.760 <sup>b</sup> ±0.019	0.395 <sup>a</sup> ±0.018
SWS (%)	22.78 <sup>b</sup> ±0.75	19.82 <sup>a</sup> ±0.35	39.57 <sup>c</sup> ±1.10

Means values bearing small letters (a, b, c) groups wise indicate differ significantly (P≤0.05) n=6; T1: curd made with mixed microbial culture; T2: curd made with lactobacillus culture; T3: curd made with yeast culture

**Fig. 2** Curd mass (*chaka*) prepared by using various culture



Mixed culture curd mass



Bacterial culture curd mass



Yeast culture curd mass

*cillus* both of which act on lactose to produce lactic acid. The T3 evinced significantly ( $P \leq 0.05$ ) highest pH values among all.

Acidity of curd plays very crucial role in its structure and firmness which is a desirable characteristic of good quality curd (Table 1). All the samples exhibited significant differences ( $P \leq 0.05$ ) in acidity values of which T3 recorded the lowest value and T1, the highest. The difference in acidity might be attributed to fermentation process in which mixed and bacterial cultures utilise lactose in milk while *Saccharomyces cerevisiae* does not utilise lactose (Roostita and Fleet, 1996). The titratable acidity for a good product is around 0.85-0.9% (Jay et al. 2008).

#### Quality characteristics of whey made from different cultures

##### Volume of whey (ml)

Volume of whey produced during curd formation depends on factors such as composition of milk, starter culture and processing conditions (Gyawali and Ibrahim, 2016). T1 and T2 showed insignificant ( $P \geq 0.05$ ) difference in the volume of whey produced while, T3 recorded significantly ( $P \leq 0.05$ ) highest volume (Table 2). This might be related to our previous results of curd acidity where it is evident that due to low acidity the structure of curd was not firm. Firm structure has higher water holding capacity due to the branched protein network which entraps water molecules. Due to loose structure, higher whey off was obtained in T3. Weak yoghurt gel showed higher permeability and tend to release higher whey volumes (Lee and Lucey, 2003).

##### pH and acidity of whey

The pH of whey obtained from T3 recorded significantly ( $P \leq 0.05$ ) higher values than T1 and T2 whereas, pH of T1 and T2 differed non-significantly ( $P \geq 0.05$ ) (Table 2). Lower pH value in T1 and T2 might be implicated to higher lactose degradation by bacterial cultures present in mixed and bacterial culture leading to lactic acid accumulation. The acidity of whey of all samples were recorded and expressed as percent lactic acid. All the samples evinced significantly ( $P \leq 0.05$ ) different acidity that were recorded as  $0.915 \pm 0.02$ ,  $0.760 \pm 0.019$  and  $0.395 \pm 0.018$  for T1, T2 and T3, respectively. Overall, T1 unveiled highest acidity, followed by T2 and T3 recorded least percent acidity. T1 was incubated with

mixed culture which contained various bacterial population of which *Streptococcus* grows rapidly during initial phase and is largely responsible for acid production (Jay et al. 2008). This acid production occurs at higher rate than that produced later by *Lactobacillus* sp. this might be implicated to the lower pH of T1 followed by T2.

##### Spontaneous whey separation (SWS)

The spontaneous appearance of serum from milk gel due to acidification is known as Whey off or spontaneous whey separation. It is a very important aspect of yoghurt or dahi production which is vital for its consumer appeal and quality. In the present experiment spontaneous whey separation from all three samples were significantly ( $P \leq 0.05$ ) different (Table 2). T3 recorded the highest whey off followed by T1. T2 evinced the least whey off. A strong and firm gel network prevents wheying off. Dahi or yoghurt gels are formed due to interaction of denatured whey protein with the casein micelles forming a cross link network. As the pH of milk falls and reaches casein isoelectric point (4.6) the structure gets firm. The high whey off in T3 might be attributed to its weak structure due to low acidity and high pH. Lee and Lucey (2004) have reported that unstable network causes greater spontaneous whey separation due to gel network.

##### Physicochemical characteristics and proximate composition of curd mass (*chakka*) prepared from different cultures

##### Curd mass yield

The curd mass yield made from different cultures evinced significant ( $P \leq 0.05$ ) differences (Table 3). Overall, lactic culture (T2) unveiled the highest yield of curd mass which might be due to the least whey off, followed by mixed culture curd (T1). The least yield by yeast culture curd (T3) might be due to lower coagulation of milk proteins and high whey off.

##### pH and acidity of chakka

Two concepts that relate to acidity in food analysis are titratable acidity and pH. Each of these quantities has been analytically

determined in a unique manner, and each offers a unique perspective on the quality of the food. The pH values for T1, T2 and T3 showed significant ( $P \leq 0.05$ ) difference (Table 3). T2 recorded the lowest ( $P \leq 0.05$ ) pH values followed by T1. The low value of T1 and T2 might be due to the formation of lactic acid when milk changes to curd. The findings were supported by the reports of Daeschel (1993) who noted the ability of lactic acid bacteria to form lactic acid, thereby declining the fermenting medium pH value. Thus, decline in pH makes the medium acidic which is not favorable for the endurance of spoilage organism. Akabanda et al. (2014) also reported that low pH is essential for coagulation and prohibition or depletion of growth of unpremeditated microflora in curd/yoghurt production. Yeast cultured curd mass T3 reported highest ( $P < 0.05$ ) pH which might be attributed to the ability of yeast culture to exploit other organic acids, enhancing the pH of the medium (Rekha and Vijayalakshmi, 2008).

While pH is important for determining a microorganism's ability to grow in a specific food, titratable acidity is a better predictor of how organic acids in the food impact flavour than pH. The difference in acidity was significantly ( $P \leq 0.05$ ) evident in all samples (Table 3). T1 presented the highest acidity and T3 the least. Treatment T3 evinced lower percent lactic acid which might be due to utilization of more lactose as their ability to produce carbon dioxide and ethanol (Gadaga et al. 2007) along with lactic acid production. Fast acidification is a pre-eminence of starter cultures for development of fermented milk products. For dairy fermentation process, the rapid acidifying culture are therefore good agents as prime starter culture, while culture with poor acidification utilized

as adjunct cultures relying on other characteristics (Ayad et al. 2004). T1 evinced more acidity followed by T2 as lactic acid bacteria (LAB) present in culture ferment the carbon source available in raw products into lactic acid with concomitant pH depletion (Kandasamy et al. 2018). The findings are also very well supported by the report of Rekha and Vijayalakshmi (2008) who reported increase in titratable acidity of fermented soy milk during fermentation is due to production of acid.

### Proximate composition

The T3 showed significantly highest moisture (%) among all, whereas T2 recorded least moisture value (Table 3). The moisture content of T1 was non-significantly higher than T2. The present study was in agreement with the study of Everard et al. (2011) which stated that curd moisture was affected by total solids of milk. In the study, it was observed that decreased Protein: Fat ratio was associated with improved total solids in milk which led to reduced curd moisture and increased curd yield. The result also concurred with the finding of Ozer (2006) that reported higher total solids in strained yogurt.

Casein, the reserve protein of milk that gives curd its white colour, is the main ingredient in curd. The curd's primary biological value is its high protein content (typically 10-12%), which varies slightly depending on the curd variety. All samples recorded significantly ( $P \leq 0.05$ ) different protein percent, where T2 showed the highest protein content followed by T1 and T3 reported the lowest protein content (Table 3). The higher protein content of T2 and T1

**Table 3.** Physicochemical characteristics and proximate composition of curd mass (*chakka*) prepared from different cultures

Parameters	T1	T2	T3
Curd mass yield (gm)	73.77 <sup>b</sup> ±0.65	77.05 <sup>c</sup> ±0.78	52.02 <sup>a</sup> ±1.41
pH	4.74 <sup>b</sup> ±0.02	4.46 <sup>a</sup> ±0.03	5.63 <sup>c</sup> ±0.04
Acidity (% lactic acid)	1.063 <sup>c</sup> ±0.025	0.836 <sup>b</sup> ±0.016	0.51 <sup>a</sup> ±0.017
Moisture (%)	61.46 <sup>a</sup> ±1.55	59.05 <sup>a</sup> ±0.76	69.66 <sup>b</sup> ±0.33
Protein (%)	16.10 <sup>b</sup> ±0.38	17.72 <sup>c</sup> ±0.55	14.32 <sup>a</sup> ±0.30
Fat (%)	13.57 <sup>b</sup> ±0.34	14.06 <sup>b</sup> ±0.44	12.46 <sup>a</sup> ±0.30
Ash (%)	1.08 <sup>b</sup> ±0.02	1.12 <sup>b</sup> ±0.01	1.00 <sup>a</sup> ±0.02
Carbohydrates (%)	5.79 <sup>b</sup> ±1.37	5.57 <sup>b</sup> ±0.75	2.58 <sup>a</sup> ±0.23

Means values bearing small letters (a, b, c) groups wise indicate differ significantly ( $P \leq 0.05$ ) n=6; T1: curd made with mixed microbial culture; T2: curd made with lactobacillus culture; T3: curd made with yeast culture.

**Table 4:** Sensory evaluation of curd mass (*chakka*) prepared from different cultures

Parameters	T1	T2	T3
Colour and appearance	7.93 <sup>b</sup> ±0.20	8.29 <sup>b</sup> ±0.18	6.29 <sup>a</sup> ±0.15
Taste	7.71 <sup>b</sup> ±0.14	8.32 <sup>c</sup> ±0.09	5.50 <sup>a</sup> ±0.18
Aroma	7.57 <sup>b</sup> ±0.44	8.36 <sup>b</sup> ±0.21	5.71 <sup>a</sup> ±0.41
Texture	6.93 <sup>ab</sup> ±1.16	8.50 <sup>c</sup> ±0.12	6.04 <sup>a</sup> ±0.15
Overall acceptability	7.64 <sup>b</sup> ±0.18	8.25 <sup>c</sup> ±0.09	5.54 <sup>a</sup> ±0.23

Means values bearing small letters (a, b, c) groups wise indicate differ significantly ( $P \leq 0.05$ ) n=21; T1: curd made with mixed microbial culture; T2: curd made with lactobacillus culture; T3: curd made with yeast culture.

might be implicated to the presence of *Lactobacillus* species which were present in mixed and bacterial cultures. The protein content in fermented milks is increased due to protein arising from *Lactobacillus helveticus* (Rekha and Vijayalakshmi, 2008). Similarly, Hou et al. (2000) also observed increased protein content in fermented milk with Bifidobacteria.

Overall, T2 showed the highest Fat (%) that differed non significantly ( $P \geq 0.05$ ) from T1 while T3 showed significantly the lowest fat (%) (Table 3). Lower fat content in T3 than T1 and T2 might be due to more loss of fat content in whey off as T3 evinced highest spontaneous whey separation value. Samanta, et al. (2015) stated that curd have more nutritional values than milk. Although there is no increase in fat content of milk during the process of fermentation.

The estimation of ash content of *chakka* revealed that T2 showed the highest ash (%) that differed non significantly ( $P \geq 0.05$ ) from T1, whereas T3 exhibited significantly ( $P \leq 0.05$ ) the lowest ash (%) (Table 3). The carbohydrates (%) of *chakka* recorded the highest values in T1 which was comparable to T2 and T3 showed the lowest carbohydrate (%) value. No significant ( $P > 0.05$ ) differences were observed in carbohydrate content between T1 and T2, the findings were supported by the reports of Samanta et al. (2015) that reported no increase in carbohydrates during fermentation of milk. The significantly lower content of carbohydrates in yeast culture curd (T3) might be due to their capability to amend carbohydrates into ethanol and carbon dioxide ( $\text{CO}_2$ ), is determined by Louis Pasteur in the 1860s.

#### Sensory evaluation of curd mass (*chakka*) prepared from different cultures

The data presented in Table-4 revealed significant difference in sensory scores of colour and appearance, taste, aroma, texture and overall acceptability among cultures. Among the three cultures, T2 showed fairly high sensory scores of 8.25 and above, followed by T1 sensory scores in the range of 6.93 to 7.93 indicating acceptability by the sensory panelists and T3 showed lowest sensory score ranging from 5.5 to 6.29 indicating non acceptability. Granata and Morr (1996) reported that the physical stability, texture, aroma and taste of yogurt are related to pH changes. T2 exhibited highest colour and appearance score as compared to T1, however the difference was not significant ( $P \leq 0.05$ ). T3 exhibited the lowest appearance score among all cultures. The lower appearance score for T3 might be due to the appearance of large pores and runny structure.

Significant difference ( $P \leq 0.05$ ) was observed in sensory taste scores among cultures. T2 exhibited highest taste score at 8.32 followed by T1 whereas, T3 exhibited the significant ( $P \leq 0.05$ ) lowest taste score. Fermentation of milk, yeast culture breaks down the native protein of milk and generated some bitter peptides molecules, alcohol as well as carbon dioxide which leads to de-

creased sensory attributes than the *Lactobacillus* and mixed culture. Sharma et al. (2020) also reported that during the fermentation yeast culture produce alcohol as well as carbon dioxide.

Aroma is one of the most important sensory parameters for dairy products. Dairy products are rich in small chain fatty acids which are responsible for its characteristic flavour. The flavour of fermented dairy products depends largely on the fast acidification of raw materials that prevents the growth of harmful microorganisms, and imparts desired characteristic aroma, texture and taste of the final product (Akabanda et al. 2014). Bacterial culture (T2) exhibited highest flavor score followed by mixed culture (T1) although the difference was not significant. Lactic acid bacteria improve the nutritional value, taste, aroma and texture of fermented foods, including dairy products (yogurt, cheese buttermilk and fermented milk), sour dough bread, fermented beverages, fermented vegetables and meat (Leroy and De Vuyst, 2004; Landete, 2017). Yeast culture (T3) exhibited lowest sensory score showed significant difference ( $P \leq 0.05$ ) as compared to T1 and T2. Texture score was found to be highest in T2. T3 exhibited lower texture scores as compared to T1 and T2. Acidity can also cause souring in the final product, which is a characteristic of fermented foods. Mostly, lactic acid bacteria ferment the available carbon sources in raw food into lactic acid and at the same time lower the pH value, resulting in significant effects, such as removing undesirable organisms and improving sensory properties and texture, as well as imparting health benefits. The overall acceptability score differed significantly ( $P \leq 0.05$ ) among different cultures. T2 recorded highest overall acceptability followed by T1 and T3 exhibited lower scores. The higher acceptability of T2 might be attributed to the overall good appearance, well developed flavor, firm structure and appealing taste due to proper fermentation.

#### Conclusion

Utilizing mixed culture, bacterial culture and yeast culture for developing *chakka* evinced significant changes in quality characteristics. Inoculating milk with 1.5 % of mixed, bacterial and yeast culture respectively produced curd, whey and *chakka* of varying quality. The results indicate that the highest curd yield, *chakka* yield, protein content as well as overall acceptability was observed in T2 (bacterial culture inoculation).

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