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RESEARCH ARTICLE

Improvement in quality of cow's raw milk using novel on-farm milk cooling system

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Abstract: The present study was conducted with an aim to check the improvement in raw milk quality at farm level by novel milk cooling system. Bulk milk cooler was taken as control. Optimization of different precooling temperatures viz., 30°C, 20°C, 10°C and 4°C in novel milk cooling system was carried out based on microbial and physicochemical tests. The raw milk samples were stored at 4°C and analyzed at an interval of 24 h for 72 h. A significant ($p<0.05$) reduction in the aerobic plate count and psychrotrophic count was observed when precooling temperature was decreased from 30°C to 10°C whereas non-significant difference was observed when precooled at 10°C and 4°C. The aerobic plate count and psychrotrophic counts of the raw milk samples ranged from 5.02±0.16 log CFU/ml to 6.87±0.17 log CFU/ml and 1.71±0.13 log CFU/ml to 3.54±0.17 log CFU/ml, respectively throughout the storage period. Whereas, pH and titratable acidity of cow's milk samples ranged from 6.71±0.04 to 6.56±0.08 and 0.14±0.01%LA to 0.16±0.02%LA, respectively throughout the storage period. pH and titratable acidity of all milk samples during storage at 4°C for 72 h remained non-significant. Among the treatments, raw milk precooled at 10°C and 4°C showed lower aerobic plate count and psychrotrophic count with non-significant difference, hence, precooling of raw milk was optimized to 10°C. The better quality of raw milk can be obtained at reduced operating expenses using such novel milk cooling system. The farmers can earn higher incentives from superior quality of raw milk.

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Introduction

In the present scenario of Indian milk collection centre where bulk milk coolers (BMCs) are used, the temperature of blend (milk from morning and evening milk collection in BMC) raw bulk milk reaches about 18 to 20°C when second milking is added to chilled milk (first milking) at 4°C. It takes another one and half hour for a bulk milk (first and second milking) to be chilled to 4°C. Besides the storage temperature, the cooling time to reach storage temperature (4 °C) is also of significance. Keeping milk at elevated temperature for longer time stimulates the microbial proliferation and hence increased microbial load in raw milk. Precooling of milk before it enters the bulk tank by using novel milk cooling system, could restrict the rate of bacterial growth in raw milk thereby gives superior quality of raw milk.

Sameera et al. (2020) evaluated quality of raw milk from two different locations in Hyderabad region, Telangana state, India for a period of six months from January to June. The bacterial count ranged from 7.09 to 8.18 log CFU/ml. Further, the trend of microbial quality of a greater number of milk samples were shifting towards fair, poor and very poor from February to June due to seasonal variation in raw milk quality as affected by variations in milk production practices and ambient temperature with the season. The study concluded that the microbiological quality of most of the milk samples collected from different areas of Hyderabad city were not up to the standards, as evidenced by their high number of microorganisms and also the presence of coliform bacteria. Kakati et al. (2021) assessed the quality of raw milk based on the microbial load, sold in and around Guwahati city of India. All of the raw milk samples had a significantly higher standard plate count and coliform count than the permissible standard. While Dinki and Balcha (2013) evaluated raw milk samples of cattle collected from six different consumers collection centres of Guwahati city, India. It was reported that the mean standard plate count and the mean coliform count of raw milk were 6.38±0.02 and 2.85±0.03 log CFU/ml, respectively. It can be concluded from the above two studies that the raw milk sold in

Guwahati city do not confer to the legal microbiological standard and may pose a high risk of milk-borne illness among consumers.

Psychrotrophic microbes, particularly *Pseudomonas* spp., are found in the microbiota of chilled milk because they can grow at temperatures below their optimal growth temperature. Psychrotrophic counts ranging from 6.00 to 9.00 log CFU/ml in refrigerated raw milk affect cheese quality, since the synthesized thermoresistant enzymes affect the nutritional value, sensory properties and texture. In addition to significantly affecting cheese yields, the enzymes produced by psychrotrophic microbes cause taste alterations, unfavourable clotting times, increased concentrations of free fatty acids and free amino acids, and a shorter shelf-life. Surprisingly, psychrotrophic bacterial growth may represent a serious defect both for fresh or ripened cheeses (Caputo et al. 2015). Poor quality of raw milk is also known to produce inferior quality dairy products with reduced shelf life.

The pH of milk should be between 6.65 and 6.8 to ensure trouble-free processing and high quality of the final product. A lower pH will risk product stability and cause fouling. A higher pH may indicate mastitis-infected milk. As a result, milk that does not fulfil these requirements is not appropriate for UHT processing **(**Tetrapak, 2014). The natural acidity of milk is due to casein, mineral substances, and phosphates. The developed acidity is due to the lactic acid produced by lactose degradation because of microorganisms. The titratable acidity test is used to determine whether milk has a high acidity level that affects its keeping quality and heat stability. The acidity of milk is not a true measure of lactic acid present but in practice, gives a good indication of the quality of milk. The superioir quality raw milk has a relatively steady Titratable Acidity (TA) value ranging between 0.12 to 0.17% lactic acid (Schmidt et al. 1996).

Even though India stood first in milk production, the quality of raw milk produced is poor. This leaves larger impacts on the finished dairy product prepared out of it. Hence there is a necessity to improve the microbial quality of raw milk at the farm level itself by rapid cooling of milk immediately after milking. This can restrict the growth of microorganisms at the initial level. So, the present study was conducted with an objective to see the effect of rapid cooling on microbiological quality of raw milk compared to BMC cooled raw milk at farm level.

Materials and Methods

The novel milk cooling system was installed at Livestock Research Station(LRS) farm of Anand Agricultural University, Anand. The existing milk cooling system *i. e.,* BMC, available at dairy farm was used as a control.

Novel milk cooling system

The different components of milk cooling system were cooling cum storage tank (350 L), plate cooler for pre-cooling of raw milk at different temperature, pump for transferring raw milk from plate cooler to cooling cum storage tank, thermal storage system (500 L) for making required quantity of ice in cooling of raw milk to final storage temperature. Temperature measuring sensors were installed at raw milk inlet to plate cooler, cooling cum storage tank, chilled water supply and return. The piping & instrumentation diagram of novel milk cooling system is given in Figure 1.

Control milk cooling system (BMC)

The different components of existing milk cooling system were buffer tank (Make: Delaval, volume; 20 L), balance tank (150 L), milk transfer pumps, horizontal closed type bulk milk cooler (Make: Delaval, Capacity: 1000 L) and condensing unit of 3 Hp. The piping & instrumentation diagram of control milk cooling system is given is Figure 2.

Precooling of raw milk using plate cooler in novel milk cooling system

The average daily milk production at cow farm was 1250 L. Raw milk collected through machine and manual milking was transferred into novel and control milk cooling system (BMC). In the evening, raw milk was cooled to 4°C in control milk cooling system whereas in novel milk cooling system, raw milk was first, precooled to different temperatures viz., 30°C, 20°C, 10°C and 4°C through plate cooler and then it was transferred to cooling cum storage tank where raw milk was cooled to final storage temperature (4°C) using chilled water. These milks were stored overnight at 4°C in respective milk cooling systems. In the morning, same procedure was followed for raw milk cooling. Hence, raw milk at 34°C received from morning milking was added to previous day evening's raw milk maintained at 4°C.

Both the systems were operated at 50 per cent storage capacity in the evening and remaining 50 per cent in the morning such that system is capable to cool milk as per the standard ISO-5708. For the novel milk cooling system, the required quantity of ice was formed in thermal storage system well before each milk cooling cycle. Trials were conducted for the optimization of precooling temperatures of raw milk based on the storage stability for 72 h at refrigerator temperature (4°C±0.5°C).

Chemicals and glassware

During the entire study, Borosil brand (Borosil Glass Works Ltd., Mumbai, India) of glass wares and analytical grade chemicals were used. The bacteriological media, chemicals and reagents were purchased from Hi-media (Bangalore) and SD chem (India).

Collection of raw milk sample

The milk sample for the analysis was collected in the morning once the blend raw milk temperature reached to 4°C. It was

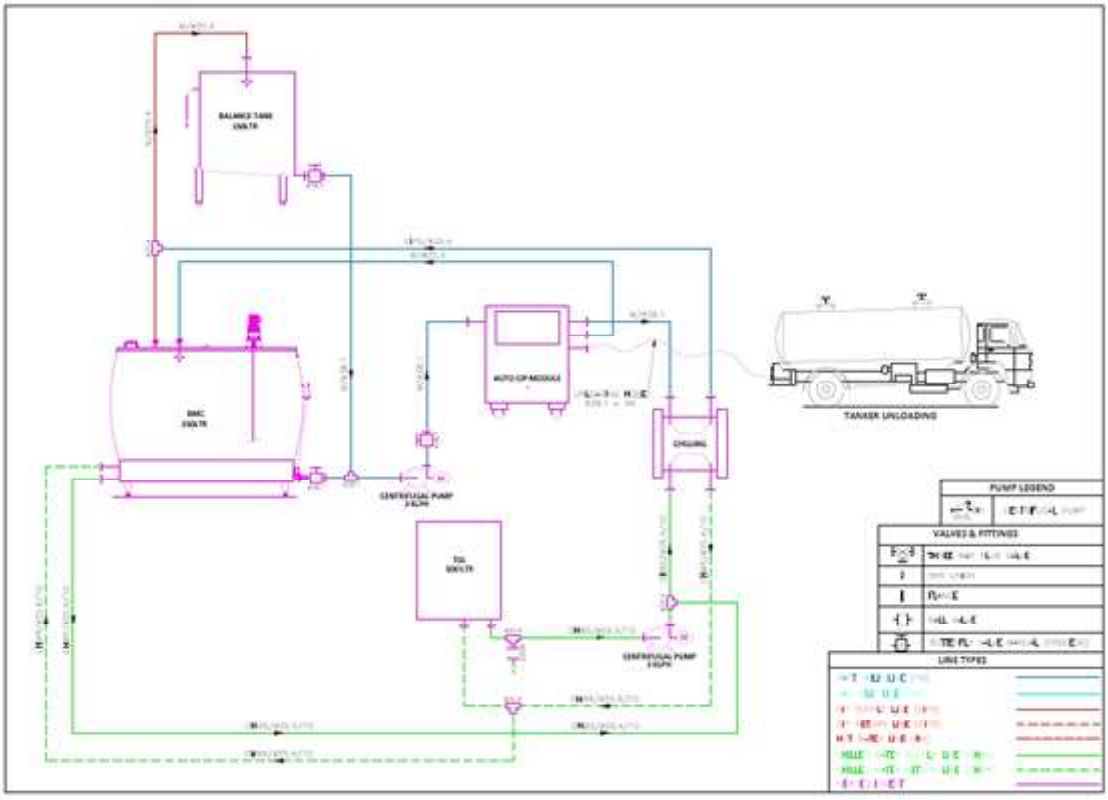

Fig. 1 Novel milk cooling system

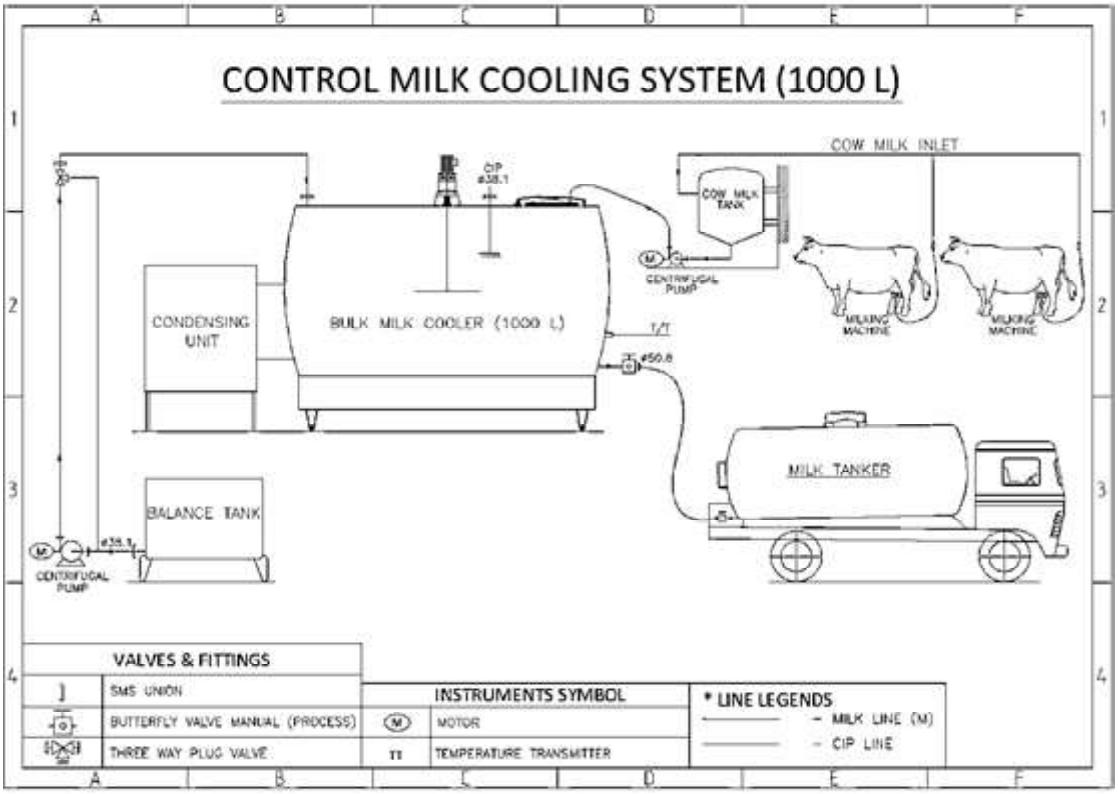

Fig. 2 Bulk milk cooling system

collected from BMC in control milk cooling system and from cooling cum storage tank in novel milk cooling system. Before the collection of samples from BMC/cooling cum storage tank, milk was thoroughly mixed and the outlet of the BMC/cooling cum storage tank was disinfected with the wet alcohol swab. Then initial small volume of milk was allowed to drain and the

representative sample of raw milk was drawn into sterilized container. The samples were then stored in refrigerator maintained at 4±0.5°C to mimic the storage-conditions used in cold chain. The sample was evaluated for microbiological and physico-chemical tests at an interval of 24 h from 0 h to 72 h.

Determination of fat content of raw milk

The fat content was determined by Gerber method as described in BIS (1981).

Determination of solids-not-fat of raw milk

The SNF of raw milk was determined as follow:

$$\text{SNF of raw milk} = \text{Total solids} - \text{Fat}$$

The total solid content of raw milk was estimated by gravimetric method described in lab manual of FSSAI (2015).

Determination of methylene blue reduction time of raw milk

The MBRT was carried out by following the method of ISO 4833-2:2013. The samples showed > 5 h MBR time were considered as excellent quality raw milk.

Determination of aerobic plate count of raw milk

The aerobic plate count was carried out by following the method of IS 5402-2002 & ISO 4833:1991 with slight modifications. The plates containing colonies between 30-300 were considered for the calculations. The counts were expressed as log CFU/ml.

Determination of psychrotrophic count of raw milk

Psychrotrophic count of raw milk sample was performed by following the method suggested by Júnior et al. (2018) with slight modifications. The counts were expressed as log CFU/ml.

Determination of pH of raw milk

The pH of raw milk was measured using digital pH meter (make: chemi line, India) at 25°C temperature.

Determination of titratable acidity of raw milk

The titratable acidity of raw milk was estimated by the procedure described in IS:1479, Part I (1960). Precooling temperature for raw milk in novel milk cooling system was optimized based on the results obtained from the above tests.

Statistical analysis

All the data were subjected to statistical analysis using Completely Randomized Design (CRD) as per the methods described in Steel and Torrie (1980). The significance was tested at 5 per cent level of significance using mean value, co-efficient of variance (C.V.) and critical difference (C.D.).

Results and Discussion

Novel milk cooling system and control milk cooling system

The piping and instrumentation diagram (P&ID) of the novel and control milk cooling system is given in figure 1 and 2, respectively. In novel milk cooling system, piping connections for milk flow was provided from balance tank, milk supply pump, plate cooler and cooling cum storage tank. Plate cooler and cooling cum storage tank were provided with chilled water supply and return lines connected to inlet and outlet of thermal storage system. Piping connections for thermal storage was provided such that chilled water circulates either through plate cooler or cooling cum storage tank. Milk supply pump, chilled water supply pump and condensing unit of vapour compression refrigeration system (VCRS) were connected with three phase power supply. Control panel and temperature display screen were mounted on wall. The system was installed on the roof with good ventilation for proper functioning of the condensing unit. Milk outlet at the bottom of cooling cum storage tank was provided for tanker dispatch of raw milk in the morning.

In control milk cooling system (BMC), milk was transferred from milking machine to buffer tank through piped connection. Milk from buffer tank to bulk milk cooler was transferred through milk transfer pump-1. Whereas milk collected in balance tank from manual milking was transferred to bulk milk cooler through milk supply pump-2. Condensing unit for R-22 was connected with bulk milk cooler. Milk outlet at the bottom of bulk milk cooler was provided for tanker dispatch of raw milk in the morning.

In the novel milk cooling system, the size of the different components was selected according to the volume of milk and cooling demand required in milk collection and cooling during evening and morning at the farm. The thermal storage system was designed for cooling 50 per cent volume of milk (150 L) at a given time. The capacity of the novel system was 300 L. While control system was having capacity of 1000 L. Since BMC was already installed at dairy farm with 1000 L capacity. The remaining surplus milk available at dairy farm was handled by novel milk cooling system. Therefore, two milk cooling systems with different capacity was used in study.

Optimization of precooling temperature of raw milk for the novel milk cooling system

The optimization for precooling temperature of raw milk was carried out based on physico-chemical and microbial analysis. The details of raw milk used for the experiments are given in the Table 1. The milk samples were evaluated for MBR time, aerobic plate count, psychrotrophic counts, pH and titratable acidity (%LA).

The MBR time of all the raw milk sample was > 5 h indicating excellent quality of raw milk.

Of the total volume of milk produced at farm, 80% was collected by machine milking and 20% by manual milking.

Determination of aerobic plate count of raw milk

Aerobic plate count enumerates the total number of microorganisms present in the given sample. Aerobic plate count of the raw milk sample was carried out by pour plate technique. Changes in aerobic plate count of raw milk during storage at 4°C±0.5°C for 72 h is shown in Table 2. It was found that the aerobic plate count of all the raw milk samples increased significantly ($p<0.05$) with the storage period. Also, significant ($p<0.05$) increase in the aerobic plate count was observed in milk samples when precooling temperature increased from 10°C to 30°C. However, non-significant difference in aerobic plate count was observed in milk samples precooled at 10°C and 4°C. The aerobic plate count was the highest in control (5.89±0.15 log CFU/ml) followed by 30°C (5.84±0.22 log CFU/ml), 20°C (5.74±0.23 log CFU/ml), 10°C (5.13±0.16 log CFU/ml) and 4°C (5.02±0.16 log CFU/ml) at 0 h.

Similar results were reported by Malacarne et al. (2013). They evaluated raw milk samples for aerobic plate count during storage at refrigerator temperature. The aerobic plate count of raw milk, when stored at 4-6°C temperature was 5.02±0.37 log CFU/ml at 0 h and 5.12±0.49 log CFU/ml at 48 h storage. In another similar study conducted by Abd Elrahman et al. (2009), 4.80±0.02 log CFU/ml aerobic plate count of fresh raw cow milk was reported. While Tan et al. (2020) reported 5.18 log CFU/ml aerobic plate count of fresh raw cow milk and 7.84 log CFU/ml aerobic plate count of fresh raw goat milk.

Determination of psychrotrophic count of raw milk

Psychrotrophs can grow in raw milk when kept at refrigerator storage temperature. They are sensitive to pasteurization but capable of producing heat stable protease and lipases enzymes. These enzymes remain active in the finished dairy product leading to reduced shelf life of the dairy products. So, it is necessary to detect the presence of psychrotrophs in the given sample of raw milk. Changes in psychrotrophic count of raw milk during storage at 4°C for 72 h is shown in table 3. It was found that psychrotrophic count of raw milk samples increased significantly ($p<0.05$) upon storage at refrigerator temperature(4°C±0.5°C) from 0 h to 72 h. The highest psychrotrophic count was observed in control compared to the treatment samples. A non-significant difference was observed between control and 30°C precooling treatment. Similarly, non-significant difference was observed between 10°C and 4°C precooling treatments. The lowest count was observed when raw milk was precooled at 10°C and 4°C. The psychrotrophic counts of the raw milk samples ranged from

Table 1 Details of raw milk procured for the experiment

Type of milk	Cow
Type of breed	Cross bred of Holstein Friesian, Gir and Kankrej
Type of milking	Machine (80%) and manual (20%)
Frequency of collection	Twice a day (morning and evening)
Fat* (%)	4.51±0.08
SNF* (%)	8.70±0.05
MBR time	> 5 h

*Observation is a mean ± SD of four replicate experiments (n=4)

Table 2 Changes in aerobic plate count of raw milk (log CFU/ml) during storage at 4°C/72 h

Storage time (h), P	BMC (Control)	Precooling, T				Period Average (P)
	4°C	30°C	20°C	10°C	4°C	
0	5.89±0.15	5.84±0.22	5.74±0.23	5.13±0.16	5.02±0.16	5.52
24	6.11±0.13	5.98±0.12	5.98±0.13	5.14±0.32	5.12±0.16	5.67
48	6.46±0.23	6.28±0.11	6.03±0.15	5.18±0.08	5.16±0.15	5.82
72	6.87±0.17	6.83±0.22	6.26±0.13	5.26±0.19	5.21±0.11	6.09
Treatment Average (T)	6.33	6.23	6.01	5.18	5.13	
Source	SEm	CD (0.05)			CV (%)	
T	0.06	0.18				
P	0.06	0.16			3.03	
T×P	0.12	0.37				

Each observation is a mean ± SD of three replicates (n=3)

1.71±0.13 log CFU/ml to 3.54±0.17 log CFU/ml throughout the storage period.

Similar study was conducted by Malacarne et al. (2013). They evaluated raw milk samples for psychrotrophic count during storage at refrigerator temperature. The psychrotrophic count of raw milk was 3.72±1.64 at 0 h and 4.05±1.84 at 48 h of storage at 4-6°C temperature. The psychrotrophic counts were higher compared to present study. In another similar study conducted by Abd Elrahman et al. (2009), 0.84±0.03 log CFU/ml psychrotrophic counts of fresh raw cow milk was reported. While Tan et al. (2020) found 4.96 log CFU/ml psychrotrophic counts of fresh raw cow milk and 5.90 log CFU/ml psychrotrophic counts of fresh raw goat milk.

The wide variation in psychrotrophic count of raw milk, reported by different researchers can be due to the factors like variation in source of raw milk, place of milking, hygiene maintained during milking, cleanliness of milking shed, season of the year, type of milking (manual and machine), etc.

Determination of pH of raw milk

Changes in pH of raw milk during storage at 4°C for 72 h is shown in table 4. The pH of cow milk samples ranged from 6.71 to 6.64 at 0 h of storage in refrigerator (4°C±0.5°C). The non-significant difference in pH of milk was observed for all the raw milk samples including control during storage at 4°C±0.5°C for 72 h. Similar observation was reported by Malacarne et al. (2013). They evaluated raw milk samples for pH during storage at refrigerator temperature. The pH of raw milk was 6.72±0.03 at 0 h and 6.79±0.05 at 48 h of storage at 4-6°C temperature. In another similar study conducted by Abd Elrahman et al. (2009), reported 6.91±0.003 pH of fresh raw milk of cow. While Tan et al. (2020) found 6.59±0.01 pH of fresh raw milk of cow and 6.34±0.00 pH of fresh raw milk of goat.

In our study, the non-significant increase in milk pH values during cold storage at 4°C±0.5°C was the result of two phenomena with conflicting effects: 1) the production of lactic acid by the growing microflora (mainly psychrotrophic bacteria), which decreased milk pH and 2) the dissociation of calcium from the casein micelle, which increased milk pH. Hence, the pH remained in the normal

Table 3 Changes in psychrotrophic count of raw milk (log CFU/ml) during storage at 4°C for 72

Storage time (h), P	BMC (Control)	Precooling, T				Period Average (P)
	4°C	30°C	20°C	10°C	4°C	
0	2.54±0.10	2.40±0.20	2.13±0.10	1.81±0.13	1.71±0.13	2.12^{c}
24	2.62±0.16	2.50±0.09	2.24±0.13	1.86±0.11	1.76±0.19	2.20^{c}
48	2.99±0.14	2.82±0.19	2.33±0.17	1.97±0.18	1.84±0.11	2.39^{b}
72	3.54±0.17	3.25±0.22	2.52±0.13	2.05±0.16	1.98±0.18	2.67^{a}
Treatment Average (T)	2.92^{A}	2.74^{B}	2.30^{C}	1.92^{D}	1.82^{D}	
Source	SEm	CD (0.05)			CV (%)	
T	0.05	0.16				
P	0.05	0.14			6.56	
T×P	0.11	0.32				

Each observation is a mean ± SD of three replicates (n=3)

Table 4 Changes in pH of raw milk during storage at 4°C for 72 h

Storage time (h), P	BMC (Control)	Precooling, T				P Average
	4°C	30°C	20°C	10°C	4°C	
0	6.64±0.08	6.65±0.06	6.66±0.08	6.70±0.07	6.71±0.04	6.67
24	6.60±0.07	6.62±0.08	6.63±0.04	6.69±0.05	6.70±0.07	6.65
48	6.61±0.06	6.59±0.07	6.59±0.09	6.68±0.04	6.67±0.07	6.63
72	6.56±0.08	6.56±0.08	6.57±0.07	6.65±0.08	6.65±0.06	6.60
T Average	6.60	6.60	6.61	6.68	6.68	
Source	SEm	CD (0.05)			CV (%)	
T	0.02	NS				
P	0.02	NS			1.05	
T×P	0.05	NS				

Each observation is a mean ± SD of three replicates (n=3)

Table 5 Changes in titratable acidity (% LA) of raw milk during storage at 4°C for 72 h

Storage time (h), P	BMC (Control)	Precooling, T				P Average
	4°C	30°C	20°C	10°C	4°C	
0	0.14±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.14
24	0.16±0.02	0.15±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.15
48	0.16±0.02	0.16±0.01	0.15±0.02	0.15±0.01	0.14±0.01	0.15
72	0.16±0.01	0.16±0.01	0.16±0.01	0.15±0.01	0.15±0.02	0.15
T Average	0.16	0.15	0.15	0.15	0.14	

Source	SEm	CD (0.05)	CV (%)
T	0.004	NS	
P	0.003	NS	7.35
T×P	0.008	NS	

Each observation is a mean ± SD of three replicates (n=3)

range during initial period of storage and/or until growing psychrotrophs overcomes the buffering capacity of raw milk.

Determination of titratable acidity of raw milk

Titratable acidity indicates the acid produced in the milk in terms of lactic acid. Increased acidity indicates the increased microbial load in the milk. Changes in titratable acidity (% LA) of raw milk during storage at 4°C±0.5°C for 72 h is shown in table 5. In a study, the titratable acidity of all the milk samples remained in the normal range i.e., 0.14 % LA to 0.16 % LA. Increased acidity in milk samples upon storage at 4°C for 72 h was non-significant ($p>0.05$) for all treatments. According to IS 1479-1(1960) standards, acidity of raw milk varies from 0.10 % LA to 0.17 % LA. Any value in excess of 0.17 % LA can safely be thought as developed lactic acid.

Similar study was conducted by Schmidt et al. (1996). They evaluated raw milk samples for titratable acidity during storage at refrigerator temperature. The titratable acidity of raw milk was 0.15±0.01 %LA at 0 h, 0.16±0.01 %LA at 48 h and 0.17±0.02 %LA at 96 h of storage at 6°C temperature. In another similar study conducted by Abd Elrahman et al. (2009), 0.145±0.000 %LA of fresh raw cow milk was reported. While Tan et al. (2020) found 0.16±0.01 %LA of fresh raw cow milk and 0.22±0.01 %LA of fresh raw goat milk.

MBR time, pH and titratable acidity of all the raw milk samples were in the normal range and non-significant difference observed during storage study. From the microbial analysis of precooled raw milk samples stored at 4°C for 72 h, it was found that the microbial count for 10°C and 4°C were lower and remained non-significant among the precooling treatments. Hence, the precooling temperatures for raw milk were optimized to 10°C and 4°C.

Conclusion

Precooling of raw milk using novel system showed the significant improvement in the microbial quality of milk compared to the traditional milk cooling in BMC. Significant ($p<0.05$) reduction in the aerobic plate count and psychrotrophic count was observed from 5.89±0.15 log CFU/ml and 2.54±0.10 log CFU/ml in control sample to 5.13±0.16 log CFU/ml and 1.81±0.13 log CFU/ml in 10°C precooled treatment sample, respectively. Such novel system can help farmers to receive better price of raw milk with improved quality and it could be installed at village cooperative society and/or farm to improve raw milk quality. Further, detailed study can be conducted for the different type of microorganisms present in the raw milk e.g., spore formers, lipolytic, proteolytic, thermoduric, thermophilic.

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