



**MICROBIOLOGICAL ANALYSIS OF PASTEURIZED, MICROFILTERED AND UV TREATED MILK SAMPLES**

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**ABSTRACT**

In the thermal method of milk pasteurization, milk is heated to 71.7°C for 15 seconds to destroy harmful bacteria and reduce the numbers of spoilage organisms to extend shelf-life and it doesn't kill thermophilic bacteria. The non thermal processing milk such as microfiltration and UV radiation of milk are serving as an alternative methods for processing milk. The treatments were divided as pasteurized milk (TC - control), rawmilk (T1), microfilteredmilk (T2), UV treated milk at 1.5 kj and UV treated milk at 1.7 kj respectively. The milk samples were subject to microbiological analysis such as standard plate count method and coliform count during 0, 3, 6, 9, 12 and 14 days of storage at 4-5°C. Up to 14 days of storage at 4-5°C, the standard plate count of the milk samples TC, T2 and T3 exceed the limit of the legal limits prescribed by FSSAI, (2011). The UV treated milk sample 1.7 kj (T4) had no coliforms till 14 days of storage. The coliform count of milk samples viz., TC, T2 and T3 exceeded the limit on 6, 3 and 12 days as prescribed by Food Safety and Standard Authority of India. Hence, conclusion can be made that the UV treated samples obtained a low standard plate count and coliform count till 14 days of storage at 4-5°C.

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**INTRODUCTION**

The thermal method of milk pasteurization, by which milk is heated to 71.7°C for around 15 seconds to destroy harmful bacteria and reduce the numbers of spoilage organisms to extend shelf-life and highly developed technique. Pasteurization is the fact that it's a gentle heat treatment and it doesn't kill thermophilic bacteria but this process kill all pathogenic microorganism. Pasteurization process needs more heat energy and leads to subsequent loss nutrients. Now a days, non thermal processing milk such as microfiltration and UV radiation of milk are serving as an alternative methods for processing milk. Microfiltration uses pore size diameters of 0.2–2.0 µm and operation pressures of 0.05–0.2 MPa, allowing the retention of milk particles, such as somatic cells, fat globules, bacteria, and casein micelles (Coimbra and Teixeira, 2010). The UV light acts as a physical method for microbial disinfection and does not generate any chemical residues. Ultraviolet (UV) light causes thymine dimers to form between adjacent thymine's within a single strand of DNA.

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When DNA polymerase encounters the thymine dimer, it does not always incorporate the appropriate complementary nucleotides and leads to formation of mutations that can ultimately kill microorganisms. Further, UV treatment offers some technological advantages in a small-scale production due to its low maintenance cost, low installation cost and low operational cost with minimal energy use. In spite of its many advantages, its low penetration power restricts the area of use in food industry (Ayhan and Halil, 2016). By considering the importance of non thermal processing technologies, the study has been designed to analyze the microbiological properties of pasteurized milk as control and raw milk, microfiltered milk, UV treated milk at 1.5 kj and UV treated milk at 1.7 kj respectively.

**MATERIALS AND METHODS**

The raw milk collected from the farmers in and around Dindigul by the M/s Dindigul Farm Products Pvt Limited, Dindigul, Tamil Nadu, India was utilized in this study. The pasteurized milk was obtained from the M/s Dindigul Farm Products Pvt Limited, Dindigul, Tamil Nadu was utilized in this study. HTST pasteurization equipment manufactured and supplied by M/s Tavaron Engineers, the model no: N-35,

930/171/2013 was utilized by following manufacturer's instruction. The microfiltration of milk was carried out at the M/s Dindigul Farm Products Pvt Limited, Dindigul, Tamil Nadu, India was utilized in this study. The microfiltration unit supplied by M/s Pall Corporation, Newyork, USA was utilized in this study. The commercial Surepure 40 Turbulator TM system (SP-40) designed and manufactured by Surepure AG, Switzerland installed at M/s L.P Dairy, Kanchipuram, Kalavai, Tamil Nadu, India was utilized in this study. The required raw milk for UV treatment was obtained from M/s L.P Dairy, Kanchipuram, Kalavai, Tamil Nadu. The milk samples has been processed 1.5 and 1.7 kj respectively.

The treatments were divided as pasteurized milk (TC - control), rawmilk (T1), Microfiltered milk (T2), UV treated milk at 1.5 kj and UV treated milk at 1.7 kj respectively. The milk samples were subject to microbiological analysis such as standard plate count method and coliform count on 0, 3, 6, 9, 12 and 14 days of storage time.

Standard plate count method was determined by the method described in BIS 5402, 2012. About 10ml of the sample was transferred to 90ml sterile saline. Then, a serial dilution was prepared until the sixth dilution. After that, 0.1ml of the diluted samples was pipette into a plate count agar (PCA) plate. The plates were inverted and incubated aerobically at 37°C for 24 hours. Coli form count (CC) is determined according the method described by BIS 5401, 2012 using violet Red Bile Agar (VRBA). About 10ml of the sample was transferred to 90ml sterile saline. Then, a serial dilution was prepared until the second dilution. After that, 0.1ml of the diluted samples was pipette into a violet red bile agar (VRBA) plate. Plates are incubated at 30°C for 24 hours. Typical dark red colonies (> 0.5 mm in diameter) are considered as coli forms. Coli form counts are reported as counts per gram of buttermilk. The results were subjected to analysis of variance (ANOVA) procedure. The data were analyzed by approved statistical methods of Snedecor and Cochran (1989).

**RESULT AND DISCUSSION**

The results of the control and treatments (T1 to T4) milk samples stored at 4-5°C up to 14 days pertaining to standard plate count (Log10 cfu/ml) are presented in Table 1. From the day 0 to day 14, control and treatments (T1 to T4) milk samples exhibited a significant difference (P < 0.01) among them. Up to 9 days, the trend was similar. On day 14, pasteurizedmilk and microfiltered milk observed a same trend. The UV treated milk at 1.5 kj and UV treated milk at 1.7 kj had a significant change in the standard plate count on day 14. Anjum *et al.*, (2019) studied the standard plate count of pasteurized milk stored at 4°C and reported that the standard plate count on day 12 was 3.72 Log10 CFU/mL. Dan *et al.*, (2010) have also recorded similar reduction in standard plate count from 5.41±0.07 log10 cfu/ml in raw milk to 2.64±0.07 log10 cfu/ml after HTST pasteurization. Petrus *et al.*, (2010) studied the keeping quality of homogenized whole pasteurized (75°C/15 s) milk packed in HDPE bottle and LDPE pouches stored at 4°C up to 21 days and found that the samples stored in HDPE bottle and LDPE pouches had a mesophilic count of 4.00 log cfu/ml and 3.42 log cfu/ml respectively. Wang *et al.*, (2019) evaluated the effectiveness of cold microfiltration to extend the shelf life of milk. Raw skim milk underwent microfiltration at 6 ± 1°C with a ceramic membrane of 1.4-µm pore size, at a transmembrane pressure of 75.8 kPa and a cross

flow velocity of 7 m/s and found that the cold microfiltration effectively reduced the microbial load an average of 3.4 log reduction in vegetative bacteria. Rossitto *et al.*, (2012) studied the effect of UV treatment on the pasteurized milk with 2 per cent fat content by using a research-scale low-power UV unit designated SP-4 (SurePure, Milnerton, South Africa) with a wave length of 0 J/liter, 880 J/liter, 1760 J/liter and the laboratory pasteurization count was 1.90, 0.64 and 0.44 (log CFU per milliliter) on 14 days of storage and control was 1.76 log CFU per milliliter respectively. Food Safety and Standards Regulations (FSSAI,2011) specify the maximum upper limit of the total plate count as 30,000 cfu/g in pasteurized milk in India. The World Health Organization standards and other scientific works, prescribed a limit of total bacterial count 1.3×10<sup>6</sup> cfu/ml for quality milk (WHO, 1989). Institute of Medicine National Research Council of the National Academies, USA considered the shelf-life and safe consumption of milk, most countries have legal limits for the maximum number of bacteria in milk, for example, Canada: 10,000 CFU mL<sup>-1</sup>; USA: 20,000 CFU mL<sup>-1</sup>; European Economic Community: 50,000 CFU mL<sup>-1</sup> (Carol *et al.*, 2013). In the present investigation, up to 14 days of storage at 4-5°C the milk samples TC (pasteurized milk), T2 (microfiltered milk) and T3 (UV treated milk at 1.5 kj) exceed the limit of the legal limits prescribed by Institute of Medicine National Research Council of the National Academies, USA Carol *et al.*, (2013), FSSAI, (2011), WHO, (1989), and Anjum *et al.*, (2019) respectively and in accordance with that of Petrus *et al.*, (2010), Dan *et al.*, (2010), Wang *et al.*, (2019) and Rossitto *et al.*, (2012) respectively. UV treated milk at 1.7 kj has within the prescribed limits by Carol *et al.*, (2013), (FSSAI,2011) and WHO, (1989) respectively.

**Table 1** Standard plate count (log 10 cfu/ml) of various milk samples (n=4) stored at 4-5°C

Days of storage at 4-5°C	TC	T1	T2	T3	T4
0	3.674 <sup>c</sup> ±0.030	5.506 <sup>a</sup> ±0.019	3.959 <sup>b</sup> ±0.007	2.901 <sup>d</sup> ±0.022	2.470 <sup>e</sup> ±0.030
3	3.890 <sup>c</sup> ±0.041	5.752 <sup>a</sup> ±0.022	4.179 <sup>b</sup> ±0.036	3.155 <sup>d</sup> ±0.044	2.637 <sup>e</sup> ±0.035
6	4.003 <sup>c</sup> ±0.027	5.809 <sup>a</sup> ±0.070	4.284 <sup>b</sup> ±0.012	3.414 <sup>d</sup> ±0.038	2.926 <sup>e</sup> ±0.033
9	4.432 <sup>c</sup> ± 0.017	6.073 <sup>a</sup> ± 0.020	4.751 <sup>b</sup> ± 0.015	3.688 <sup>d</sup> ± 0.039	3.154 <sup>e</sup> ± 0.046
12	4.651 <sup>b</sup> ±0.008	6.212 <sup>a</sup> ±0.068	4.751 <sup>b</sup> ±0.015	4.041 <sup>c</sup> ±0.017	3.278 <sup>d</sup> ±0.016
14	4.796 <sup>b</sup> ±0.005	6.374 <sup>a</sup> ±0.001	4.775 <sup>b</sup> ±0.013	4.301 <sup>c</sup> ±0.019	3.531 <sup>d</sup> ±0.031

Means bearing different superscripts differ significantly (P<0.01)

The coliform count results of the control and treatments (T1 to T4) milk samples stored at 4-5°C up to 14 days (cfu/ml) are presented in Table 2. From the day 0 to day 14, control and treatments (T1 to T4) milk samples exhibited a significant difference (P < 0.01) among them. From day 0 to day 9, the coliform count of control and treatments the same trend, whereas ascending order of T1, T2, TC, T3 and T4 respectively. On day 12, the pasteurized milk and microfiltered milk had a similar values. But, the UV treated milk samples had a significant difference with each other on day 12 and 14 respectively.

Anjum *et al.*, (2019) studied the coliform count of pasteurized milk stored at 4°C and reported that the coliform count on day 12 was 1.46 Log10 CFU/mL. Blake *et al.*, (1995) studied the effect of direct steam injection at 100°C on the microbial

quality of milk. The raw milk stored at 7°C had a coliform count of  $1.8 \times 10^2$  cfu/ml and the heat processed milk had a coliform count of (cfu/ml) <10 on day 0 and the count reached  $10^7$  during storage at 7°C for 15 days. Janzen *et al.* (1982) studied the shelf-life of pasteurized fluid milk as affected by age of raw milk and found that the coliform counts remained less than 100/ml, there were no significant relationships between these bacteria counts and age of raw milk (stored at 4.5°C for 0, 2, 4, and 6) or storage time of the pasteurized milk ( at 4.5°C for 0, 4, 8, 12, 16 days). Rossitto *et al.*, (2012) studied the effect of UV treatment on the pasteurized milk with 2.0 per cent fat content with a wave length of 0 J/liter, 880 J/liter and 1760 J/liter and reported that the time (in days) to exceed minimum acceptable level is 7, >35 and >35 days respectively. Bandlaet *al.*, (2012) studied the impact of UV-C processing of raw cow milk treated in a continuous flow coiled tube ultraviolet reactor. The residence time of milk through the UV reactor was increased to 17 s to provide increased UV dose (16.822 mJ cm<sup>-2</sup>), as may be required to achieve greater more than 5-log reductions of *E. coli* in raw cow milk and found that no coliforms were detected in the UV-treated milk during the storage at 4°C for 7 days. UV processing of raw milk can reliably achieve a 3–4 log 10 reduction of initial microbial load measured as standard plate, psychrotrophic, coliform, and thermoduric counts, and extend shelf life up to 14 days (Koutchma and Gail, 2013). Coliforms count is used to monitor the quality of milk, are not a single species of organism. it can be used as an hygienic indicator to reflect the general microbiological quality. The presence of coliforms has long been thought to indicate fecal contamination, however, recent discoveries indicates that only a fraction are fecal in origin, while the majority are environmental contaminants. In the study, UV treated milk sample 1.7 kj (T4) had no coliforms till 14 days of storage. But the other treatments exceeded the limit as prescribed by Food Safety and Standard Authority of India, (FSSAI, 2011). The present results are in accordance with the findings of Anjum *et al.*, (2019), Blake *et al.*, (1995), Janzen *et al.* (1982), Rossitto *et al.*, (2012), Bandlaet *al.*, (2012) and Koutchma and Gail, (2013) respectively.

**Table 2** Coliform count (cfu/ml) of various milk samples (n=4) stored at 4-5°C

Days of storage at 4-5°C	TC	T1	T2	T3	T4
0	0.00 <sup>b±</sup> 0.00	1137.50 <sup>a</sup> ±23.94	1.75 <sup>b±</sup> 0.25	0.00 <sup>b±</sup> 0.00	0.00 <sup>b±</sup> 0.00
3	0.00 <sup>b±</sup> 0.00	1337.500 <sup>a</sup> ±23.94	2.750 <sup>b±</sup> 0.25	0.00 <sup>b±</sup> 0.00	0.00 <sup>b±</sup> 0.00
6	0.50 <sup>b±</sup> 0.50	1800.00 <sup>a±</sup> 40.82	6.00 <sup>b±</sup> 0.41	0.00 <sup>b±</sup> 0.00	0.00 <sup>b±</sup> 0.00
9	6.250 <sup>b±</sup> 0.250	2225.0 <sup>a±</sup> 47.871	11.50 <sup>b±</sup> 0.645	0.00 <sup>b±</sup> 0.00	0.00 <sup>b±</sup> 0.00
12	11.25 <sup>b±</sup> 0.48	2550.0 <sup>a±</sup> 64.55	14.25 <sup>b±</sup> 0.48	1.00 <sup>b±</sup> 0.41	0.00 <sup>b±</sup> 0.00
14	15.25 <sup>b±</sup> 0.48	3300 <sup>a±</sup> 70.71	17.75 <sup>b±</sup> 0.48	3.75 <sup>b±</sup> 0.48	0.00 <sup>b±</sup> 0.00

Means bearing different superscripts differ significantly (P<0.01)

**CONCLUSION**

This investigation was made to find out the effectiveness of microbial reduction through comparing the thermal and non thermal methods of milk processing. The microbiological analysis of pasteurized, microfiltered and UV treated milk samples for standard plate count and coliform count were carried out from day 0 to 14 of storage at 4-5°C. Up to 14 days

of storage at 4-5°C, the standard plate count of the milk samples TC (pasteurized milk), T2 (microfiltered milk) and T3 (UV treated milk at 1.5 kj) exceed the limit of the legal limits prescribed by FSSAI, (2011). The UV treated milk sample 1.7 kj (T4) had no coliforms till 14 days of storage. The coliform count of milk samples viz., TC, T2 and T3 exceeded the limit on 6, 3 and 12 days as prescribed by Food Safety and Standard Authority of India. Hence, conclusion can be made that the UV treated samples obtained a low standard plate count and coliform count till 14 days of storage at 4-5°C.

**References**

Anjum R, Imran J, Barbara R, Shyam S, Muhammad A, Muhammad AA, Muhammad A., Muhammad, I., Tanweer, A. G., Muhammad I.A, Muhammad A, Bahare S, Célia FR., Javad S and Natália M (2019). Measurement of off-flavoring volatile compounds and microbial load as a probable marker for keeping quality of pasteurized milk. *Appl. Sci.*, 9: 2-16.

Ayhan D and Halil IK (2016). Ultraviolet radiation (UV) applications in milk industry. *Int. conference on Eng. and Nat. Sci.*, 10: 2757 - 2760.

Bandla S, Ruplal C, Dennis GW and John H (2012). Impact of UV-C processing of raw cow milk treated in a continuous flow coiled tube ultraviolet reactor. *Agric. Eng. Int: CIGR Journal.*, 14 (2): 86-93.

BIS 5401 (2012). Indian Standard - Microbiology of food and animal feeding stuffs, Indian Standards, New Delhi

BIS 5402 (2012). Indian Standard - Microbiology of food and animal feeding stuffs, Indian Standards Institution, New Delhi.

Blake MR, Weimer BC, McMahan DJ and Savello PA(1995). Sensory and microbial quality of milk processed for extended shelf life by direct steam injection. *J. Food Prot.*, 58(9): 1007-1013.

Carol R, Tanya M, William S, Bernadette D, Stic H, BethLand Joseph A (2013). Review of Institute of Medicine and National Research Council Recommendations for One Health Initiative. *Emerg. Infect Dis.*, 19(12): 1913–1917.

Coimbra JSR and Teixeira JA (2010). (Ed.). Engineering aspects of milk and dairy products. Boca Raton: Taylor and Francis Group, P. 673.

Dan SD, Mihaiu M, Lapusan A, Taulescu C and Ciupa A(2010). Microbiological risk assessment in a milk processing plant in Transilvania. *Bulletin UASVM*, 67 (2): 58-63.

FSSAI, 2011. Food Safety and Standards Regulation. Government of India, New Delhi.

Janzen JJ, Bishop JR, Bodine AB and Caldwell CA (1982). Shelf-life of pasteurized fluid milk as affected by age of raw milk. *J. Dairy Sci.*, 65: 2233-2236.

Koutchma T and Gail B (2013). Shelf life enhancement of milk products. *Food Technol.*, 10.13: 68-69.

Petrus RR, Loiola CG and Oliveira CA (2010). Microbiological shelf life of pasteurized in bottle and pouch. *J Food Sci.*, 75(1): 36-40.

Rossitto PV, Cullor JS, CrookJ, Parko J, Sechi P and Cenci-Goga BT (2012). Effects of UV irradiation in a continuous turbulent flow uv reactor on microbiological and sensory characteristics of cow’s milk. *J. Food Prot.*, 75(12): 2197–2207.

Snedecor GW and Cochran WG (1989). Statistical methods. 9th Edn. The Iowa State University Press, Ames, Iowa.

Wang D, Fritsch J and Moraru CI (2019). Shelf life and quality of skim milk processed by cold microfiltration with a 1.4-µm pore size membrane, with or without heat treatment. *J. Dairy Sci.*, 102(10): 8798-8806.

WHO(1989). Report of Joint WHO/IAEA Collaborative Study. Geneva: World Health Organization, Minor and trace elements in breast milk. 157–159.