Total viable, psychrotrophic and thermoduric bacterial population of market samples of pasteurized milk

Vishnu Suresh[1], Ligimol James[1], Beena A.K.[1], Aparna Sudhakaran V.[1], and Divya M.P.[2]

[1] Department of Dairy Microbiology, College of Dairy Science and Technology, Kerala Veterinary and Animal Science University, Thrissur, Kerala, India-680651
[2] Department of Dairy Chemistry, College of Dairy Science and Technology, Kerala Veterinary and Animal Sciences University, Thrissur, Kerala, India-680651

Date submitted:13/01/2021 *Date accepted: 23/03/2021* *Page(s): 16-19*

Abstract

Pasteurization, the process of heating every particle of milk to at least 63° C for 30 min or 72° C for 15 sec or to any temperature-time combination which is equally efficient is one of the major thermal preservation processes adopted by the dairy industry. The microbial groups of high relevance in terms of quality and safety aspects of pasteurized milk are the pasteurization surviving thermodurics and the low temperature growing psychrotrophic bacteria. So a study was conducted to determine the standard plate, psychrotrophic and thermoduric bacterial counts of market samples of pasteurized milk available in the local market and to derive correlations in between them. A total of forty market samples of pasteurized milk belonging to five different brands were assessed for their standard plate (SPC), psychrotrophic (PC) and thermoduric (TC) counts. The mean (±standard error) SPC, TC and PC of the pasteurized milk samples were 4.64 ± 1.04, 2.9 ± 0.64 and 2.63 ± 1.3 log CFU/ml respectively. All the samples contained thermoduric organisms whereas psychrotrophic organisms were present in 87.5% of them only. Standard plate count was found to be significantly ($p \leq 0.05$) positively correlated with TC and PC. Statistically significant ($p \leq 0.05$) positive correlation was observed between PC and TC also. Among the different correlation coefficients obtained the highest was observed between SPC and PC ($r = 0.824$). Significant differences ($p \leq 0.01$) were observed in between the mean values of SPC, TC and PC of the different brands of pasteurized milk samples tested.

Keywords: Pasteurized milk, standard plate count, thermoduric count, psychrotrophic count

Introduction

Pasteurization is the major thermal process the dairy industry relies on to safeguard the safety of its produce. It is well recognized as an efficient process for ensuring the elimination of all the pathogenic organisms present in raw milk. As the spoilage organisms are also getting simultaneously reduced, this process also addresses the quality as well as the safety aspects of pasteurized milk. Food Safety and Standards Authority of India (FSSAI) defines pasteurization as a microbicidal heat treatment aimed at reducing the number of any pathogenic micro-organisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. It also stipulates that the pasteurization conditions shall be designed to effectively destroy the organisms *Mycobacterium tuberculosis* and *Coxiella burnetii* [1]. Taking into consideration the rationale of this thermal process and the fact that the recommended storage condition for pasteurized milk is 'under refrigeration', the microbial groups of high relevance in pasteurized milk are the pasteurization surviving (thermodurics) and the low temperature growing psychrotrophic bacteria. The survivors (thermodurics) and the post-pasteurization contaminants together constitute the standard plate count, an index of good manufacturing practices of pasteurized milk. The thermoduric count is often used as an indicator of the effectiveness of sanitation and hygiene adopted during production and processing [2]. Presence of psychrotrophic population in pasteurized milk is mainly attributed to post pasteurization contamination though some spore forming psychrotrophic surviving organisms are also reported. Many of these psychrotrophic organisms are well recognized for their ability to produce heat stable proteolytic and lipolytic enzymes [3]. The fact that many of these enzymes retain their activity even after the conventional heat treatment of milk is an area of special concern in terms of shelf life of the products prepared using it. Psychrotrophic bacteria, the most common spoilage organisms of the heat treated milk and dairy products are traced to post-pasteurization contamination of the products [4]. Common spoilages caused by this microbial group include change of flavour, undesirable coagulation of milk proteins, increased concentration of free fatty and amino acids as well as significant negative effects on yields of dairy products [5]. Majority of psychrotrophic bacteria that cause milk and dairy product spoilage are non pathogenic. However some studies reported the involvement of psychrotrophic organisms in disease outbreaks associated with consumption of pasteurized milk [6, 7] and the possibilities of them acting as opportunistic human pathogenic bacteria [8, 9]. So the thermoduric organisms together with the psychrotrophic ones, if present in pasteurized milk can influence its shelf life as well as safety. As studies on thermoduric count (TC) and psychrotrophic count (PC)

Milk Science International (74) 2021 P. 16-19
ISSN 2567-9538; https://doi.org/10.48435/MSI.2021.3

of pasteurized milk are very scanty an attempt was made to understand the per cent contribution of these two types of microflora to the standard plate count (SPC) of market samples of pasteurized milk. For this, High Temperature Short Time (HTST) pasteurized milk samples collected from the local market (Mannuthy, Thrissur district, Kerala, India) over a time period of three months from September, 2019 were assessed for their SPC, TC, PC and the data are reported in this study.

Materials and Methods

Collection of pasteurized milk and enumeration of SPC, PC and TC: A total of forty (eight samples each of five brands assigned with blind sample codes H, I, J, K ,L) High Temperature Short Time (HTST) pasteurized milk samples collected from the local market (Thrissur, Kerala, India) over a time period of three months were used for this study. Samples of pasteurised milk in 500ml Low Density Polyethylene (LDPE) sachets which were well within their expiry date were purchased from retailers and transported to research laboratory at 4°C in ice boxes packed with ice packs avoiding any temperature abuse and damage to the product. Upon arrival at the laboratory the samples were stored at 4°C till analysis which was completed within 2 hours of sample collection. For the analysis, the samples were mixed thoroughly, cut open aseptically and 5 ml of milk was transferred directly into sterile test tubes without letting the sample to touch the sides of the test tube. Samples of the same brand were collected in one week interval. SPC was determined by pour plating appropriate dilutions using plate count agar (PCA, HiMedia Laboratories Pvt. Ltd., Mumbai) and subsequent incubation at 37° C for 48 h as per Bureau of Indian Standards [BIS, 10]. In the case of PC, the samples were pour plated as in the case of standard plate count but the incubation was done at 7° C for 10 days according to American Public Health Association standard methods [APHA, 11]. The TC of the samples was determined by subjecting them to laboratory pasteurization. For this, five ml of thoroughly mixed samples was aseptically transferred directly into sterile test tubes. The test tubes were closed tightly using cotton plugs and heated in a water bath at 63° C for 30 min (timed from the moment the water reached the desired temperature) ensuring that the entire sample of milk in the tubes was below the water level of the water bath and that the temperature of milk reached 63° C within five minutes. One more tube containing the same amount of milk at the same level and a thermometer inserted was kept along with to serve as the temperature control to ensure that the pasteurization temperature was attained internally also. At the end of heating, the tubes were immediately transferred to ice bath and chilled to 7° C. Appropriate dilutions were prepared and the dilutions were pour plated using PCA according BIS. The plates were incubated at 37° C for 48 h [12] and the colonies developed were counted.

Statistical Analysis: For statistical analysis, all the counts were transformed to $\log_{10}$ CFU/ml and analysed using SPSS, version 26 (IBM, New York, US). The SPC, TC and PC of the samples were subjected to Spearman correlation coefficient analysis. Summary statistics and cumulative frequency distribution of the counts were analysed. The pasteurised milk sample counts were subjected to one way ANOVA and correlation coefficient analysis to find significant differences in between the brands and the relationship between the counts respectively.

Results and Discussion

The mean (± standard error) SPC, PC and TC of the pasteurized milk samples were 4.64 (± 1.04), 2.63 (± 1.3) and 2.9 (± 0.64) log CFU/ml respectively (Table 1). In exponential terms the counts were 4.4 x10^4 (± 1.1 x 10^1), 4.3 x 10^2 (± 2 x 10^1), 7.94 x 10^2 (± 4.4), CFU/ml, thus PC and TC constituting 0.98% and 1.8% of the viable population. This is comparable to an early report of 0.98% contribution of thermoduric population to standard plate count of pasteurized milk samples collected from the same locality [13]. The slight increase observed in the current study could be attributed to the difference in the study design adopted. These values are much lower than the 53 and 39.5% reported for PC and TC of pasteurized milk by Mahari and Gashe [14]. The SPC ranged from 2.3 (sample PM-H4) to 7.52 (sample PM-I3) log CFU/ml (Fig. 1). As the pasteurization survivors are only 1.98% of the SPC, 98% of the microflora of the tested pasteurized milk is contributed by post pasteurization contaminants. This is well supported by the earlier report of post-pasteurization contaminants as the major contributor towards the total plate count of pasteurized milk marketed in the same area [13]. Considering that only six of the tested samples contained thermodurics capable of growing under refrigerated conditions (data not shown) it can be inferred that psychrotrophic population of the tested samples was mainly constituted by post pasteurization contaminants and that majority of the post pasteurization contaminants were incapable of growing under refrigerated storage.

Psychrotrophic population were present in 35 out of the 40 samples, constituting to 87.5% of the samples tested. This detection rate lies between the 62.9% reported by Citak [15] and 98.1% by Mahari and Gashe [14]. Presence of psychrotrophic organisms except for some psychrotolerant sporeformers in pasteurized milk is attributed to post pasteurization contamination (PPC). Considering the significant role the psychrotrophic bacteria play in limiting the quality, safety and shelf life of pasteurized fluid milk it is critical to put in place effective measures to prevent post pasteurization contaminations. Presence and growth of microorganisms introduced by PPC is extensively reported in pasteurized milk samples exhibiting reduced shelf life. Besides due to their ability to grow at low temperatures and produce a variety of enzymes these psychrotrophic organisms are capable of causing flavor, odor, and body defects and thereby can ultimately affect the perception and willingness of consumers to buy pasteurized milk [16]. Thermoduric bacterial population was found in all the samples and the counts ranged from 1.7 (sample PM-H4) to 4.32 (sample PM-I1) log CFU/ml.

Table 1: Total and brand wise mean values and standard deviations of standard plate count (SPC), psychrotrophic bacteria (PC), and thermoduric bacteria (TC) each presented as log CFU/ml

Brand		SPC	PC	TC
H	Mean	3.73^{c}	1.36^{c}	2.57^{ab}
	SD	0.6	1.18	0.72
I	Mean	6.13^{a}	4.28^{a}	3.40^{a}
	SD	0.67	0.61	0.53
J	Mean	4.17^{ab}	2.05^{ab}	2.36^{c}
	SD	0.42	1.29	0.36
K	Mean	4.43^{ab}	2.63^{b}	2.93^{abc}
	SD	0.73	0.53	0.46
L	Mean	4.72^{b}	2.81^{b}	3.22^{bc}
	SD	0.86	0.62	0.53
Total	Mean	4.64	2.63	2.9
	SD	1.04	1.3	0.64
F value		14.331*	11.376*	5.225*
p		≤ 0.01	≤ 0.01	0.002

Figures are the mean ± standard deviation of eight values
*** significant at (p ≤ 0.01) 1% level**
[a-e] Means with different superscripts vary significantly within the column

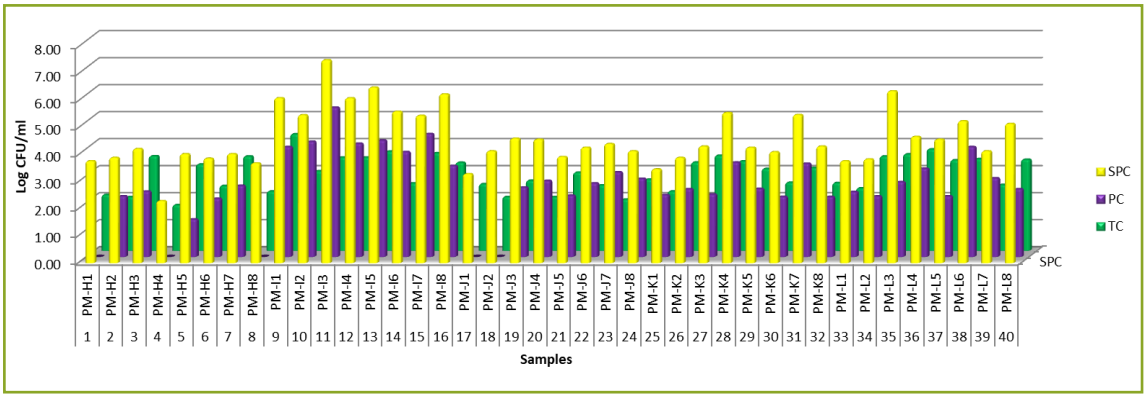

Figure 1: Standard plate (SPC), psychrotrophic (PC) and thermoduric counts (TC) (Log CFU/ml) of the pasteurized milk (PM) samples. The letters H,I,J,K,L are blind brand codes and the numbers indicate nth sample of the same brand.

This is in agreement with the observation of presence of thermoduric population in all the fifty samples of pasteurized milk by Kikuchi [17]. It is worth mentioning that the thermoduric bacterial population obtained in this study are really hardy in nature as they could survive the pasteurization process twice; once during the processing and then the laboratory pasteurization. Raw milk is considered to be the principal source of thermoduric organisms in pasteurized milk [18]. High thermoduric counts are also attributed to chronic/persistent cleaning failures within the milking system [19]. Considering these aspects, extraordinary attention is warranted in this direction also. The FSSAI stipulated 'm' and 'M' values of aerobic plate counts (APC also known as standard plate count; SPC) of pasteurized milk are 4.5 log CFU/ml (3 x 10^4 CFU/ml,) and 4.7 log CFU/ml (5 x 10^4 CFU/ml) respectively classifying the product as 'satisfactory', acceptable' and 'unsatisfactory' quality subjected to the sampling plan. Being a process hygiene criteria, APC indicate the acceptable functioning of the production process and is not used as a requirement for releasing the products in the market .As this is an indicative contamination value, pasteurized milk samples graded as 'unsatisfactory' indicate that corrective actions are to be carried out to maintain the hygiene of the process in compliance with food law. Under such cases of non-compliance measures should be taken to check and improve the hygienic practices adopted during processing.Out of the 40 samples tested 23 (57.5%) were having counts lower than the stipulated 'm' value whereas for 13 (32.5%) samples the counts were more than the 'M' value and for four samples it was in between 'm' and 'M'. Out of the 13 samples having counts higher than 4.7 log CFU/ml, eight were belonging to the same brand 'I' hinting the necessity of initiating corrective actions in this processing facility. Interestingly three out of the six pasteurized milk samples exhibiting the presence of pasteurization surviving psychrotrophic organisms were belonging to this particular brand (data not shown). Non-conformance to the existing national standards is a major concern in the case of pasteurized milk all over the world. Pasteurized milk samples not adhering [20, 21] and adhering [22, 23] to the stipulated respective national standards are reported in other studies. A comparison was also made in between the microbial counts of the different brands tested. Three of the brands exhibited mean standard plate counts lower than 4.5 log CFU/ml (Table 1) ml and can be graded as of 'satisfactory' quality in terms of this count. One of the brands namely 'H' exhibited the lowest SPC and PC whereas the lowest TC was exhibited by the brand 'J'. 37.5% and 25% of the samples belonging to these brands were devoid of psychrotrophic population. Among the different brands, only one brand 'I' had its mean PC higher than the mean TC. Significant differences ($p \leq 0.01$) were observed in between the mean SPC, PC and TC of the different brands of pasteurized milk samples tested. The highest SPC, TC and PC were exhibited by brand 'I' and were found to be significantly higher ($p \leq 0.01$) than that of the other brands. Upon analysing the correlation coefficients of the pasteurized milk samples, SPC was found to be significantly positively ($p \leq 0.05$) correlated with TC and PC (Table 2). Statistically significant ($p \leq 0.05$) positive correlation was observed between PC and TC also. This could be of relevance as it offers the possibility of extrapolating TC to PC and thereby shorten the decision time considering the longer incubation period required for the enumeration of psychrotrophic population. Among the different correlation coefficients obtained the highest was observed between SPC and PC ($r = 0.824$). It could be well attributed to the ability of the psychrotrophic organisms to grow in both mesophilic and psychrophilic range which allows them to contribute equally to both the SPC and PC. Though there are many studies reporting statistically significant correlation between psychrotrophic bacteria, total bacterial count [24, 25] and SPC, thermoduric count [26,27] of raw milk, no such studies are reported on pasteurized milk.

Table 2: Correlation coefficients between standard plate count (SPC), thermoduric count (TC) and psychrotrophic count (PC) of pasteurized samples

Samples		SPC	TC	PC
Pasteurized milk n=40	SPC	1.000	0.570*	0.824*
	TC		1.000	0.514*
	PC			1.000

*** significant ($p \leq 0.05$) at 5% level**

Conclusion

Results of this study provide an insight into the microbiological quality of market samples of pasteurized milk in terms of the per cent contribution of thermoduric and psychrotrophic population to the total viable bacterial population of pasteurized milk. Presence of microorganisms capable of growing under the recommended storage condition of pasteurized milk can have serious implications. Apart from the economic concerns of spoilage of milk, the possibility of some of these organisms being pathogenic can lead to severe food safety issues. In this study, SPC was found to be well correlated with other two parameters tested. The study also revealed that odds are high that pasteurized milk with high TC can have high PC also. Considering the Indian dairy scenario of production of milk by masses rather than mass production, the tropical climate and existence of large variety of climates, large scale regional specific studies are warranted to have a detailed microbiological profiling of pasteurized milk.

Acknowledgement

The authors thank the Kerala Veterinary and Animal Sciences University for the financial support.

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