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Psychrotrophic Spoilage of Raw Milk at Different Temperatures of Storage

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Abstract: Total bacterial count and psychrotrophic count of raw milk samples obtained from farms, milk vendor and milk processing dairies were determined. Growth, proteolytic and lipolytic activities of the psychrotrophs in raw milk stored at the temperatures of 2, 4, and 7°C were analyzed to assess the suitable temperature for storage of raw milk with regard to psychrotrophs. The mean total bacterial count ($\log_{10}$ cfu/ml) and psychrotrophic counts of raw milk samples collected from farms, milk vendors and processing dairies were 5.38 ± 0.10, 6.53 ± 0.14 and 6.25 ± 0.08, and 3.66 ± 0.05 4.96 ± 0.06 and 5.03 ± 0.11, respectively. Storage of raw milk at 2°C was found to support significantly lower growth, proteolytic and lipolytic activities of psychrotrophs and better sensory qualities when compared to 4 and 7 °C of storage for the period of up to 14 days. It can be concluded that raw milk should be stored at 2°C before processing to protect the nutritional and sensory qualities of raw milk.

Keywords: Raw milk, psychrotrophs, storage temperature, growth, proteolytic, lipolytic activity

INTRODUCTION

Refrigeration under tropical countries has become essential to maintain the wholesomeness of milk. The refrigeration on farms and in processing plants has considerably improved the quality of raw milk and of dairy products. Unfortunately, the current practices for the collection and refrigerated storage of the raw milk favoured the growth of psychrotrophic bacteria regardless of their optimal growth temperature[19,9].

Pseudomonas species are the most common organisms in raw or pasteurized milk at the time of the spoilage[22,15]. The *Pseudomonas* species like *P. fluorescens*, *P. putida*, *P. fragi*, *P. putrefaciens*, and less frequently *P. aeruginosa* constitute the predominant microorganisms limiting the shelf life of processed fluid milk at 4°C[6]. Significant contamination by Pseudomonads occur due to inadequately sanitized surfaces of milking, storage and transporting equipment. Furthermore, post-pasteurization contamination may happen at the filling operation[5]. Besides their rapid growth in refrigerated milk, *Pseudomonas* species produce heat stable extracellular proteases, lipases and phospholipases. Some enzymes can survive pasteurization and even UHT heat treatments[2]. Proteases are associated with bitterness in milk; gelation of UHT sterilized milk, and reduced yield of soft cheese. Most of the proteases are able to degrade κ, α_{s1} and β caseins[15]. *Pseudomonas* sp. are the primary concern with regard to lipolytic degradation of milk[19]. The lipases produce flavour defects by hydrolyzing triglycerides associated with fat breakdown in cream, butter, cheese and UHT products[4,19].

Proteolytic and lipolytic activities of the psychrotrophs in general and *Pseudomonas* species in particular are valuable tools for the detection of spoilage of refrigerated foods and in assessing the shelf life of the foods[12]

Consequent to the increasing economic constraints in the milk industry there is a demand for a method, which will allow longer storage of milk prior to pasteurization, without significant risk of subsequent detrimental effects. Hence, this study was proposed to detect the spoilage potential of the psychrotrophs at different temperature of storage of raw milk.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis:
Collection of the Samples: Raw milk samples (52 from Dairy farms, 26 from vendors and 35 from processing dairies) were collected in and around

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Namakkal district of Tamilnadu, India, during the month of June to December 2006 when the ambient temperature ranges from 28 to 37 °C. The samples were placed in ice in thermos jar under aseptic precautions and were transported to the laboratory for analysis. The fat percentage of the milk sample was standardized to 3.5 per cent and the SNF content was standardized to 8.5 per cent. The raw milk samples with the psychrotrophic count of 3.30 to 4.65 $\log_{10}$ cfu/ml were selected for study. The samples were stored at 2°C, 4°C and 7°C and analyzed for growth, proteolytic and lipolytic activities, and for sensory evaluation on days 0, 3, 5, 7 and 14. The raw milk samples after pasteurization were used for the flavour analysis by the panel of trained judges. The data were analyzed by ANOVA - two factor with replication using Microsoft Excel 2007 software as per Snedecor, and Cochran[21].

Microbial Counts: The Total Bacterial Count (TBC) and Psychrotrophic Count (PC) were carried out as per the method of Marshall[14]. Plate Count Agar (Himedia Laboratories, Mumbai, India) was used for further plating.

Measurement of Growth of Psychrotrophs: The growth of the psychrotrophs was assessed by their increase in their population at various temperatures by plating on the standard plate count agar.

Determination of Proteolytic Activity: Total nitrogen (TN), Non-protein nitrogen (NPN) and Non-Casein nitrogen (NCN) were estimated as per AOAC[1] by Kjeldahl method. Calculations for content of true protein (TP) and CN were (TN - NPN) x 6.38 and (TN - NCN) x 6.38, respectively. CN/TP was calculated as (CN/TP) x 100 per cent. Decrease in CN/TP was used as an index of proteolysis[12].

Determination of Lipolytic Activity: The free fatty acid (FFA) content was determined using the copper soap method of Shipe *et al.*[20] as modified by Ma *et al*[12] and results were expressed in meq FFA/kg milk. Increase in FFA was used as an index of lipolysis.

Sensory Evaluation: Raw milk samples were subjected to sensory evaluation like colour, odour and taste on the days 0, 3, 5, 7, and 14 of storage after pasteurization by the panel of trained judges.

RESULTS AND DISCUSSIONS

Total Bacterial Count (TBC) of Raw Milk: The mean TBC ($\log_{10}$ cfu/ml) of milk samples collected from farms, milk vendors and processing dairies were 5.38 ± 0.10, 6.53 ± 0.14, 6.25 ± 0.08 respectively (Table 1). There was a significant ($P< 0.01$) difference between the TBC of milk samples collected from farm and processing dairies. TBC was not significant between milk vendors and processing dairy samples. The mean TBC of farm milk samples is in close agreement with the findings of Kuzin *et al*[10] and Prabha and Shankar[17]. The mean TBC of Samples from processing dairies is in close agreement with the findings of Lee SueJan and Lin Chin Wen[11].

High TBC in the processing dairy and milk vendor samples might be attributed to the long interval between the milking and sampling and also prevalence of favourable temperature for bacterial multiplication.

Psychrotrophic Count (PC) of Raw Milk: The mean PC ($\log_{10}$cfu/ml) of milk samples collected from farms, milk vendors and processing dairies were 3.66 ± 0.05, 4.96 ± 0.06, and 5.03 ± 0.11 respectively. There was significant ($P<0.01$) difference between the PC of the farm milk samples and milk vendor samples. The PC of milk vendor samples and processing dairy samples do not differ significantly. Among the three sources, milk from the processing dairy had three times more psychrotrophs (13.28 per cent) than that of 4.16 and 4.85 per cent in farm and milk vendor samples, respectively. Griffiths *et al.* (1987) reported similar findings for farm bulk tank milk. Similar findings were reported by Lee Sue Jan and Lin Chin Wen[11] for bulk tank milk. High PC of the vendor milk samples may be attributed to the long time handling of milk at ambient temperature like milking and transport. The high count of the milk samples from processing dairies may be due to maintenance of low temperature during transport and/or storage of raw milk.

Out of the total bacterial population of raw milk samples from farm, milk vendor and processing dairy sources, 4.16, 4.85 and 13.28 per cent, respectively, were psychrotrophs. The psychrotrophic bacteria accounted for 10 to 50 per cent of the standard plate count in raw milk[3].

Growth, Proteolytic and Lipolytic Activities of Psychrotrophs in Raw Milk: Mean growth ($\log_{10}$cfu/ml), proteolytic (CN/TP) and lipolytic (meq/Kg) activities of psychrotrophs at 2°C, 4°C and 7°C in raw milk were studied and the results are presented in Table 2.

Growth of Psychrotrophs at Different Temperature of Storage: Raw milk when stored at 2°C the growth of the psychrotrophic bacteria was not significant between day 0 and day 3. But it was significant between day 5 ,7, and 14. At 4°C and at 7°C of storage the growth of the psychrotrophs was significant

Table 1: Mean (SE) total bacterial and psychrotrophic counts of raw milk

S.No.	Source of milk	Total bacterial count (log10 cfu/ml)	Psychrotrophic count (log10 cfu/ml)	Psychrotrophs (%)
1	Farm milk	$5.38^{a} \pm 0.10$	$3.66^{a} \pm 0.05$	4.16
2	Milk vendors	$6.53^{b} \pm 0.14$	$4.96^{b} \pm 0.06$	4.85
3	Processing dairies	$6.25^{b} \pm 0.08$	$5.03^{b} \pm 0.11$	13.28

Means bearing different superscripts in a column differ significantly (P<0.01)

Table 2: Growth, proteolytic and lipolytic activities of psychrotrophs in raw milk

Temperature of storage	Count of Psychrotrophs					Proteolysis (CN/TP)					Lipolysis (meqFFA/Kg)				
	Incubation period in days														
	0	3	5	7	14	0	3	5	7	14	0	3	5	7	14
2 °C	$4.04^{Aa} \pm 0.19$	$4.10^{Aab} \pm 0.20$	$4.53^{Abc} \pm 0.19$	$4.63^{Ac} \pm 0.16$	$5.05^{Ad} \pm 0.19$	$81.94^{Aa} \pm 0.27$	$81.50^{Aab} \pm 0.21$	$80.69^{Ab} \pm 0.23$	$78.73^{Ac} \pm 0.18$	$78.08^{Ad} \pm 0.21$	$0.14^{Aa} \pm 0.01$	$0.15^{Ab} \pm 0.01$	$0.16^{Ac} \pm 0.01$	$0.18^{Ad} \pm 0.01$	$0.23^{Ae} \pm 0.02$
4 °C	$4.04^{Aa} \pm 0.19$	$4.53^{Bb} \pm 0.19$	$5.01^{Bc} \pm 0.19$	$5.50^{Bd} \pm 0.19$	$5.99^{Be} \pm 0.19$	$81.94^{Aa} \pm 0.27$	$80.30^{Bb} \pm 0.26$	$78.58^{Bc} \pm 0.26$	$77.84^{Bd} \pm 0.25$	$74.57^{Be} \pm 0.24$	$0.14^{Aa} \pm 0.01$	$0.16^{Bb} \pm 0.01$	$0.18^{Bc} \pm 0.01$	$0.22^{Bd} \pm 0.01$	$0.27^{Be} \pm 0.02$
7 °C	$4.04^{Aa} \pm 0.19$	$5.43^{Cb} \pm 0.23$	$6.02^{Cc} \pm 0.23$	$6.61^{Cd} \pm 0.23$	$7.19^{e} \pm 0.23$	$81.94^{Aa} \pm 0.27$	$79.89^{Bb} \pm 0.26$	$77.43^{Cc} \pm 0.25$	$76.65^{Cd} \pm 0.25$	$74.16^{Ce} \pm 0.24$	$0.14^{Aa} \pm 0.01$	$0.16^{Bb} \pm 0.01$	$0.20^{Cc} \pm 0.01$	$0.25^{Cd} \pm 0.01$	$0.30^{Ce} \pm 0.02$

Means bearing different superscripts in row (lowercase) and column (uppercase) differ significantly (P<0.01)

(P<0.01) between day 0, 3, 5, 7 and day 14. The increase in the psychrotrophic population to 5.99 log_{10}cfu/ml in raw milk at 4°C was observed on day 14. Similarly, the growth of psychrotrophs in raw milk at 7°C was highest on day 14 (7.19 ± 0.23). These findings are close agreement with the reports of Pierami and Stevenson[16], Eneroth *et al*[5], Griffiths *et al*[7] and Ma *et al*[12]. Continuous growth of psychrotrophs was observed throughout the 14 days study period.

There was a significant difference in the growth of psychrotrophs between 2°C, 4°C and 7°C throughout the 14 days of storage of raw milk.

Proteolytic Activity of Psychrotrophs at Different Temperature of Storage: There was no significant difference between the proteolytic activity (CN/TP) of psychrotrophs on day 0 and day 3 at 2°C and there was a significant (P< 0.01) difference between day 5, 7 and day 14. The proteolytic activities differed significantly between day 0, 3,5,7 and 14 of storage of raw milk at 4°C and 7°C.

There was significant difference in the proteolytic activities of psychrotrophs between the temperatures of 2, 4 and 7 °C of storage except that in day 3 of storage at 4 and 7°C. The proteolytic activity of psychrotrophs at 4 °C and 7 °C was 74.57 ± 0.24 and 74.16 ± 0.24 respectively on day 14. Similar results were reported Guerrero *et al*[8] for the refrigerated raw milk.

Lipolytic Activity of Psychrotrophs at Different Temperature of Storage: The lipolytic activity of psychrotrophs was observed throughout the storage period. There was no significant difference between the lipolytic activity of psychrotrophs on day 0 and day 3 at 2°C and there was significant (P< 0.01) difference between day 5, 7 and day 14. The lipolytic activity differed significantly between 0, 3, 5, 7 and 14 days of storage at 4 and 7°C of storage of raw milk. There was significant difference between 2, 4, and 7 °C of storage except that on day 3 at 4°C and 7°C.

The lipolytic activity corresponding to the psychrotrophic load of raw milk at 2, 4 and 7°C was 0.23 ± 0.02, 0.27 ± 0.02 and 0.30 ± 0.02 meq/Kg respectively on day 14. These findings are in close agreement with the reports of Ma *et al*[12] at 4 °C.

Sensory Threshold for Proteolytic and Lipolytic Activities.: About 33.33 per cent of the panelist could detect the bitter off-flavours when the proteolytic activity was in the range of 79.89 to 78.58 and 100 per cent of the panelists could detect the bitter flavour when the CN/TP was in the range of 77.43 to 76.65. About 4.04 per cent decrease in CN/TP to cause bitter off- flavors in pasteurized fluid milk[13].

Only 50 per cent of the panelist could sense the rancid flavour of the lipolysis when the free fatty acid levels were between 0.18 to 0.20 meq/kg. All the panelist could detect the rancid flavour at the level of 0.25 meq/kg. The best estimated detection threshold for off-flavours caused by lipolysis in 2 per cent fat milk caused by native milk lipases was in the range of 0.25 to 0.35 meq FFA/kg of milk(18). In our study the sensory threshold of 0.25 (meqFFA/kg) was not reached up to 14 days when the milk is stored at 2°C.

Conclusion: Milk is stored at the temperature of below 5 °C in the dairy industry before processing. This temperature supports the growth of psychrotrophs in raw milk. In this study as the temperature of storage of milk is reduced from 7°C to 2°C there was a significant reduction in the Psychrotrophic growth, Proteolytic activity and lipolytic activities. By keeping the milk at 2°C the shelf life of milk can be further increased to 2 more days. It can be concluded that the raw milk should be stored at 2°C before processing into value added products like cheese.

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