Existence of Spore Forming Bacteria in Raw Milk

M.A.A. Pubudu Kumari[1]* and K.F.S.T. Silva[2]
[1]Department of Agricultural Systems, Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama[2]Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

Abstract

Heat processing techniques are used to destroy problematic microorganisms present in the raw milk. Most of the vegetative bacterial cells are easily destroyed during the pasteurization temperature but, heat resistant spores can survive and create problems than vegetative cells affecting the shelf life, flavour and quality of fluid milk. Therefore, the present study was conducted to find out the existing population level of spore-forming bacteria in milk, to identify the best management practices to ensure low contamination of raw milk under the present situation. Thirty farmers were randomly selected from extensive, semi - intensive and intensive systems according to their management practices. From each category, ten farmers were selected from Polonnaruwa, Medirigiriya and Katuwanwila chilling center areas, that belong to Milco (Pvt) Ltd. Samples were collected at the point of milking. All samples were analysed for mesophilic, psychotropic and proteolytic spore forming bacteria. Results were analyzed using one-way ANOVA. Mean psychotropic, mesophilic and proteolytic spore count of raw milk samples at farmer level were 2.38, 2.93 and 2.47 $\log_{10}$ cfu/ml, respectively. Spore forming bacterialcountswere significantly different ($p<0.05$) in extensive and semi intensive systems from intensive system. Main findings suggest that due to the routine management practices applied in extensive and semi-intensive dairy farming in Polonnaruwa area systems appear to cause high level of contamination of raw milk especially with problematic psychotropic, mesophilic and proteolytic spore forming bacteria.

Key words: Management systems, Spore-forming bacteria, Thermal processing

Introduction

Quality of milk largely depends on the presence of its micro flora as milk presents a highly suitable medium for growth of bacteria. Although, high temperature treatments like Ultra High Temperature (UHT) process are used to obtain commercial sterile products and spoilage of these products occasionally can be observed. During the UHT process, most of the vegetative bacteria are destroyed but heat-resistant spores can survive. Spores can get into the milk at different level and the presence of spore creates problems than vegetative cells affecting shelf life, flavour and quality of fluid milk (Scheldeman *et al.*, 2004).

Bacillus and *Clostridium* species are the commonly found spore-forming bacteria in milk that have been contaminated by soil, water, plant product, food utensils, food handlers, air, dust and animal feed (Jay, 2001). The total number of these bacteria in a product depends on the initial load in raw milk, the processing temperature employed and the extent of post processing contamination (Kumar and Raju, 1989). These organisms are associated with defects such as spoilage of UHT and pasteurized milk (Jay, 2000), sweet curdling and bitty cream caused by proteinase, lipase and phospholipase enzymes (Janstova *et al.*, 2004). An important characteristic of *Bacillus* spp. is the ability of its vegetative cells to produce thermo-resistant extra cellular enzymes (Janstova *et al.*, 2004). Due to their proteolytic and lipolytic activity they can affect the nutritional and sensory attributes such as flavour, texture of the products even if viable bacteria are not present.

***Corresponding author:** a.pubudu@yahoo.com

Adequate information is not available regarding the aerobic mesophilic, psychotropic and measophilicproteolytic spore-forming bacteria count in milk produced in different management systems under tropical climates, *i.e.* Sri Lanka. Hence, this study was conducted to enumerate the spore-forming bacteria in raw milk under three management conditions, and to identify the best management system for low-level of raw milk contamination under available situation

Materials and Methods

The present study was carried out in the laboratory of Condensed Milk Factory, Milco (Pvt) Limited at Gallela. Two hundred samples of raw, fresh, whole cow milk were collected from three chilling centre areas including Polonnaruwa, Medirigiriya and Katuwanwila where milk is processed at Condensed Milk Factory in Gallela. Thirty farmers were randomly selected for the experiment and those were classified based on their management practices as extensive, semi-intensive and intensive. From each category, ten farmers were selected from three chilling centre areas for the study. Samples were collected as the clusters of three framers and milk was collected at the point of milking. All samples were transported and kept at 0 °C until microbial analysis was completed.

All samples were analysed for the measophilic spore forming bacteria count (MSFBC), psychotropic spore forming bacteria count (PSFBC) and proteolytic spore forming bacteria count (ProSFBC) according to the procedure described by Laird *et al.* (2004).

Differences of the means of bacterial counts among three management systems were determined using one-wayANOVA performing Duncan's Multiple Range Test for mean separation with the help of SAS version 9.1.3 statistical package (SAS Institute Inc., Cary, NC, USA)

Results and discussion

Regardless to the management system, the mean PSFBC at the point of milking was 2.38 $\log_{10}$cfu/ml. This observation was lower than the values reported by Chung and Cannon (1971), who reported 0.30 to 2.84 $\log_{10}$ cfu/ml. The mean MSFBC of milk was 2.93 $\log_{10}$ cfu/ml and this value was lower than the values reported by Kumar and Raju (1989) that was 3.20 $\log_{10}$ cfu/ml. The ProSFBCof raw milk was 2.46 $\log_{10}$cfu/ml. The higher counts of spore-forming bacteria in raw milk samples may be attributed to the contamination of raw milk with soiled bedding, fodder, roughages, dung, dust and unhygienic conditions during milking.

As shown in Table 1, bacteria count of measophilic, psychotropic and proteolytic spore formers have similar pattern of presence based on management system at farmer level.

The ubiquitous nature of aerobic spore-forming bacteria leads to numerous points of potential entry into raw milk (Magnusson *et al.*, 2007). Cow's teats become soiled with dung, mud and bedding material such as straw or sand. In extensive system, teats and

Table 1. Mean values of measophilic spore forming bacteria count (MSFBC), psychotropic spore forming bacteria count (PSFBC) and proteolytic spore forming bacteria count (ProSFBC) in raw milk at farmer level under three management systems.

Management System	Mean MSFBC ($\log_{10}$cfb/ml)	Mean PSFBC ($\log_{10}$cfb/ml)	Mean ProSFBC ($\log_{10}$cfb/ml)
Extensive	$3.06^{a} \pm 0.01$	$2.53^{a} \pm 0.03$	$2.69^{a} \pm 0.02$
Semi Intensive	$3.05^{a} \pm 0.02$	$2.45^{a} \pm 0.04$	$2.61^{a} \pm 0.025$
Intensive	$2.74^{b} \pm 0.03$	$2.20^{b} \pm 0.03$	$2.15^{b} \pm 0.045$

Mean values showing different superscript are different ($p<0.05$)

udder are not washed & wiped before milking. Counts of bacteria and spores in bedding for dairy cattle have been found to correlate with the counts found in the bulk tank milk (Magnusson *et al.*, 2007). If cow is bedded on sand and pasture, teats are washed but apparently clean teat surface can have a higher number of spore formers and those can contaminate the milk (Bramley and Mckinnon, 1990). The aerobic thermoduric bacteria on teat surface are almost entirely *Bacillus* spores with spore counts ranging from 10^2-10^5 per teat depending on environmental conditions. Therefore, high number of spore forming bacteria in extensive system may be due to the soiling of udder and teats with bedding materials, dust mud and faeces. In semi-intensive system, most of the time farmers wash the udder and teats before milking but they do not wipe it with a towel. Therefore, the microorganisms present in teats and udder directly mix with the milk. As a result of that, there are high numbers of spore-forming bacteria similar to the extensive system and semi-intensive system.

In extensive system, cleaning of milking equipment is not practiced compared to the intensive system. Milking equipment can act as reservoirs or entry points for potentially highly heat resistant spores into raw milk. Farmers take milk to one milking bucket and then pour to transporting vessels. Therefore, high level of spore forming bacteria in extensive system may be due to the improper cleaning of milking equipment.

Aerial contamination is also possible in extensive system compared to intensive system. In extensive system, dusty and unclean environment is present. Most of the farmers collect milk to the bucket with a large opening. This may increase the possibility of milk contamination with aerial contaminants. In addition, most of the time, milk is poured to transporting vessel within the milking area leading to the increment of aerial contamination.

Contamination of spore-forming bacteria from water supplies is also possible when farmers rely on untreated water. A wide variety of saprophytic microorganisms derived from soil, or vegetation may be present, including *Bacillus* spores (Bramley and Mckinnon, 1990) contaminating the milk with water supplies. Therefore, this also may be a reason for the high number of spore forming bacteria present in raw milk.

Condition of the floor may largely affect the contamination of spore forming bacteria in milk. In extensive system, possibility of teats and udder is soiled with the bedding materials, mud and dung is high. Availability of water for routine practices may affect the contamination of raw milk with spore forming bacteria. Because, in extensive system washing of udder, teats, hands of milker and milking equipment cannot be observed. This may cause the high number of spore-forming bacteria in extensive system compared to the other two systems.

The results suggest that the routine management practices applied in extensive and semi intensive dairy farming in Polonnaruwaarea, caused a high level of contamination of raw milk especially with the problematic spore forming bacteria.

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