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Enumeration of Thermoduric and Thermophilic Spores in Commercial Repacked Milk Powder

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Abstract: The present study was carried out to enumerate the thermoduric and thermophilic spores in commercial repacked milk powder. A total of 30 dried milk powders, 10 each of Skim Milk Powder (SMP), Semi Skim Milk Powder (SSMP) and Full Cream Milk Powder (FCMP) purchased from market of Hyderabad, Sindh were evaluated for microbiological quality characteristics of Thermoduric Count (TDC) and Thermophilic Spore Count (TPSC). Thermoduric count, ($5.15\times10^{2}\pm2.6\times10^{1}$cfu/g) was significantly ($p<0.05$) higher in FCMP compared to SMP $2.7\times10^{2}\pm4.7\times10^{1}$ and SSMP $1.6\times10^{2}\pm3.1\times10^{1}$. TPS count enumerated from FCMP ($8.68\times10^{2}\pm4.1\times10^{1}$cfu/g) and SSMP ($7.75\times10^{2}\pm1.74\times10^{1}$) were relatively similar ($p<0.05$), but significantly different ($p<0.05$) from SMP ($4.06\times10^{2}\pm5.9\times10^{1}$cfu/g). The overall average count of TD, ($3.17\times10^{2}\pm3.7\times10^{1}$cfu/g), TPS ($6.83\times10^{2}\pm7.3\times10^{1}$), were detected higher (3.7 folds), (6.83 folds), (18.4 folds) compared to ISI standard respectively. Although, TPS indicates the unhygienic condition of dried milk powders with higher risk level for human health. While TD count appeared in higher concentration level may reveal significant influence on the quality of the final product.

Key words: Thermoduric, theremophilic, repacked, milk powder

INTRODUCTION

Milk is a major part of food consumption and plays a prominent role in the Pakistani diet and comes second to cereals in the level of per capita consumption (Anonymous, 2007-08). Moreover, due to alarming rate of increase in human population, rapid urbanization and rising per capita income levels, forced the increase in demand of fresh milk and milk products (Younis, 2003). The milk provides a highly nutritious substrate that can support the wide variety of bacteria for their growth and reproduction (Phillips and Griffiths, 1990). The contamination role of thermoduric and thermophilic spores bacteria during the production of milk powder has been well documented. The thermoduric and thermophilic can have significant economic consequences when they exceed specification limits and may result in down grading of the products (Ronimus *et al*., 2005). Because these have ability to produce extremely heat resistant spores and thus are significant source of pre- and post pasteurization (White *et al*., 1993). With this many studies have provided evidence of thermoduric and thermophilic spores growth during the manufacturing of milk powders. The other contamination source in milk powders are reuse of by products such as buttermilk and permeate from milk ultra filtration ingredients added to the process such as lactose and recycle loops in manufacturing plants (Hill and Smythe, 2004). As these powders find their utilization in dairy sector, for yoghurt, tea, ice-cream Zand cheese making or for reconstitution purposes, the presence of micro organisms even in low numbers may cause potential hazards and/or defects in the derived products (Yadav *et al*., 1993). Since, no work has been reported on any aspect of thermoduric and thermophilic spore counts in milk powders in the province of Sindh. Thus, present study has been designed for determination of thermoduric and thermophilic spore counts in commercial repacked milk powders.

MATERIALS AND METHODS

Collection of milk powder samples: A total of thirty samples of milk dried i.e 10 form each category (skim, semi skim and full cream) were collected in a sterilized sample bottles from the randomly selected milk powder shops of Hyderabad and brought to the Laboratory of Dairy Microbiology, Department of Dairy Technology. Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, for the evaluation of microbial quality characteristics. However, among the thirty samples, six samples showed either spreaded colonies and/or heavily contaminated. Thus rejected and not included in the present study.

Preparation of test samples: Milk powder (10 g) was diluted in warm (45°C) sterile diluents i-e peptone water solution (90 ml) to make primary dilution (10^{-1}). Then a series up to 10^{-5} dilution was prepared by transferring primary dilution (1 ml) into test tube containing sterile diluents (9 ml) to obtain 10^{-2} dilution and repeating the operations with sterile diluents (9 ml) using the 10^{-2} and further dilutions to obtain 10^{-3}, 10^{-4} and /or 10^{-5}.

Enumeration of thermoduric and thermophilic spore counts (colony count technique at 55°C): Thermoduric and thermophilic count was enumerated according to the method of Marshall (1993). Milk powder (10 g) was reconstituted in peptone water diluents (90 ml) and heated (80°C or 100°C) for 10 or 30 minutes to eliminate the vegetative cells. Heat treated sample (1 ml) of 10^{-1}, 10^{-2} and /or 10^{-3} dilution was transferred into petri dishes (in duplicate) through sterile automatic pipette (1000 µl) and warmed (45±1°C) sterile nutrient or milk starch agar medium (15 ml) was mixed with inoculums. The mixture was allowed to solidify and incubated (55°C) for 48 h. Parallel to that control plates were also prepared using medium (15 ml) to cheek the sterility. The dishes containing more than 10 and/or fewer than 200 colonies were selected and counted using colony counter. The result was calculated using preparation of test samples.

RESULTS

Thermoduric Count (TDC): Thermoduric count of SMP, SSMP and FCMP was evaluated and the results are presented in Fig. 1. A wide variation was observed in TD counts in all types of dried milk powders examined in the present study. The concentration of TD count in SMP ranged between 1.1×10^2 to 4.9×10^2 cfu/g and averaged $1.65\times10^2\pm4.7\times10^1$ cfu/g. While in case of SSMP, the TD counts were observed in between 3.2×10^1 to 2.7×10^2 cfu/g with mean value of $1.6\times10^2\pm3.1\times10^1$ cfu/g. Where ever, TD count in FCMP varied between 3.9×10^2 to 5.9×10^2 cfu/g and averaged $5.15\times10^2\pm2.6\times10^1$ cfu/g. Moreover, the results of statistical analysis (Analysis of Variance, ANOVA) showed significant difference ($p<0.05$), in TD counts in SMP, SSMP and FCMP. It was further observed that TD count of FCMP ($5.15\times10^2\pm2.6\times10^1$ cfu/g) was significantly ($p<0.05$) higher than SMP ($1.65\times10^2\pm4.7\times10^1$ cfu/g) and SSMP ($1.6\times10^2\pm3.1\times10^1$ cfu/g). While there was no significant difference ($p>0.05$) in TD counts observed between SMP and SSMP (Table 1).

Table 1: Thermoduric Counts (cfu /g) in different dried milk samples compared to ISI standards

	Thermoduric Count (TDC, (cfu/g)	
Sample	Observed (a)	Deviation in folds from ISI standard (b) = (x) ÷ (a)
SMP	270	+3.7
SSMP	160	+1.98
FCMP	515	+5.03
Mean	317	+3.60

a = Observed Values
x = (Standard Value of ISI, 1993) = $\leq1.0\times10^2$ cfu/g)
ISI = Indian Standards Institute

Thermophilic Spore Count (TPSC): SMP, SSMP and FCMP were evaluated for thermophilic spore count and the results are presented in Fig. 2. A wide variation was observed in TPS counts in all types of dried milk powders examined in the present study. The concentration of TPS count in SMP ranged between 2.1×10^2 to 6.8×10^2 cfu/g and averaged $4.06\times10^2\pm5.9\times10^1$ cfu/g. While in case of SSMP, the TPS counts were observed in between 2.0×10^2 to 1.8×10^3 and averaged $7.75\times10^2\pm1.7\times10^1$ cfu/g. TPS count in FCMP varied between 7.0×10^2 to 1.1×10^3 cfu/g and averaged $8.68\times10^2\pm4.1\times10$ cfu/g. Moreover, the results of statistical analysis (ANOVA) showed significant difference ($p<0.05$), in TPS counts in SMP, SSMP and FCMP. Further LSD comparison of means showed that TPS count of FCMP ($8.68\times10^2\pm4.1\times10$ cfu/g) was significantly ($p<0.05$) higher than SMP ($4.06\times10^2\pm5.9\times10$ cfu/g). While there was no significant difference ($p>0.05$), in TPS counts observed between SSMP ($7.75\times10^2\pm1.7\times10$ cfu/g) and FCMP ($8.68\times10^2\pm4.1\times10$ cfu/g) Table 2.

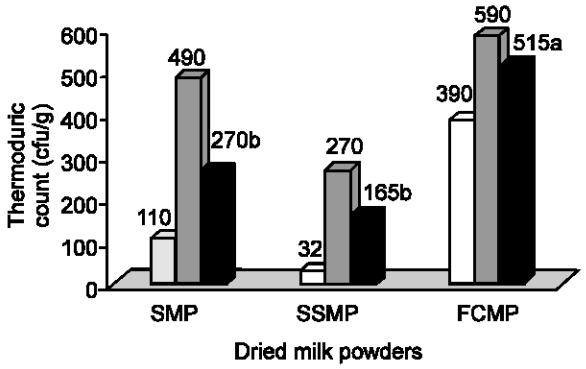

Fig. 1: Graph shows minimum, maximum and mean values of thermoduric counts (cfu/g) in skim milk, semi skim milk and full cream milk powders

SE± = 51, LSD (0.05) = 106
SMP = Skim Milk Powder
SSMP = Semi Skim Milk Powder
FCMP = Full Cream Milk Powder
CFU = Colony Forming Unit

DISCUSSION

Thermoduric count of FCMP ($5.15\times10^2\pm2.6\times10^1$ cfu/g) was significantly ($p<0.05$) higher than SMP ($2.7\times10^2\pm4.7\times10^1$ cfu/g) and SSMP ($1.6\times10^2\pm3.1\times10^1$ cfu/g). While there was no significant difference ($p>0.05$) in TD counts observed between SMP and SSMP. Moreover, the mean (3.7×10^1) of TD counts in the present study is higher than values (1.8×10^1 cfu/g) of skimmed milk powder. SM (3.8×10^1cfu/g), in Whole Milk Powders (WM) reported by Ronimus *et al.* (2005). The reason of thermoduric growth is processing, if they are present in raw milk their growth accelerate at the time of pasteurization, because temperature of pasteurization is favorable for the growth of thermoduric bacteria (Murphy *et al.*,1999). The transit time between the silo milk and spray drier is typically 20-

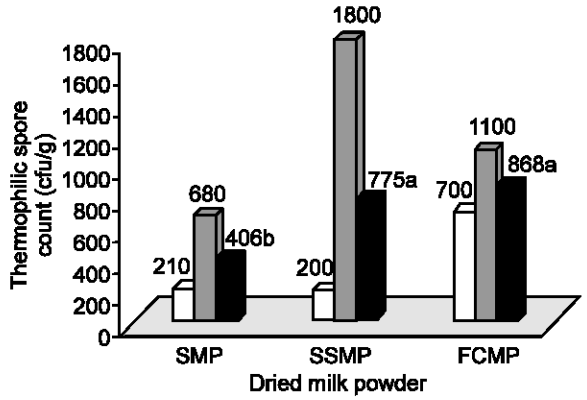

Fig. 2: Graph shows minimum, maximum and mean values of thermophilic count (cfu/g) in skim milk, semi skim milk and full cream milk powders

Table 2: Thermophilic Spore counts ((cfu/g) in different dried milk compared to ISI standards

	Thermophilic Spore Count (TPSC cfu/g)	
	Observed	Deviation in folds
Sample	(a)	(b) ÷ (a)
SMP	406	+4.06
SSMP	775	+7.75
FCMP	867	+8.67
Mean	683	+ 6.83

a = Observed Values

x = (Standard Value of ISI, 1993) = $\leq 1.0\times10^2$ cfu/g)

ISI = Indian Standards Institute

30 min there is obviously bacterial growth is clearly associated with processing and bio transfer to the end product (Flint *et al*., 2006; Wirtanen *et al*., 1996 and Stadhouders *et al*., 1982).

The thermophilic spore of FCMP ($8.68\times10^2\pm4.12\times10^1$ cfu/g) was significantly ($p<0.05$) higher than SMP ($4.06\times10^2\pm5.92\times10^1$ cfu/g). While there was no significant difference ($p<0.05$) in TPS counts observed between SSMP ($7.75\times10^2\pm1.744\times10^1$ cfu/g) and FCMP ($8.68\times10^2\pm4.12\times10^1$ cfu/g). However, the average TPS count ($6.83\times10^2\pm7.3\times10^1$ cfu/g) obtained in present study is lower than the mean value reported by Recukert *et al.* (2005) i.e SSMP ($3.2\times10^4\pm3.4\times10^3$cfu/g) and FCMP, ($2.4\times10^4\pm5.1\times10^3$cfu/g). If the spores are present in raw milk that rapidly grow, when they obtain favorable temperature during milk processing (pasteurization). The other evidence provided i.e foulant, it is a major source of thermophilic contamination in a full scale milk powder plant (Scott *et al*., 2007).

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