PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 8 (5): 704-709, 2009
ISSN 1680-5194

Microbial Quality Assessment Study of Branded and Unbranded Milk Sold in Peshawar City, Pakistan

Hamida Abid[1], Javed Ali[1], Mohammad Waqas[2], Yasir Anwar[1] and Javed Ullah[2]
[1]Food Technology Centre, PCSIR Laboratories Complex, Peshawar-25120, Pakistan
[2]Institute of Food Technology, NWFP Agriculture University, Peshawar-25120, Pakistan

Abstract: Four brands of commercially milk samples and unpasteurized milk, produced by farmers collected from Peshawar City (NWFP). These samples and samples of sterilized milk treated with ultra-high temperature (UHT) process, were microbiallly examined. The average minimum TPC of raw milk were 7.05 x 10^4cfu/ml and maximum were 3.5 x 10^5cfu/ml, minimum average coliforms were 16.65MPN/ml and average maximum 132MPN/ml, maximum fecal coliforms were 7.65MPN/ml and minimum value =0.3MPN/ml, *E. coli* O157:H7 were isolated from all samples except S7, Salmonella were also isolates from all raw milk samples, average maximum yeast and mould were 4.3 x 10^6cfu/ml and minimum were 3 x 10^4cfu/ml. Storage life study of branded milk at t 4°C, 25°C and 35°C for TPC were indicate that after 45 days TPC were increases and at the end of 90 days TPC were decreases, but in some cases it increases. All above microbes can have a hazardous effect on human body, unpasteurized milk sold by farmers showed a very high total viable count which indicates serious faults in production hygiene, unsatisfactory sanitation and unsuitable storage temperature. On contrast, the UHT milk produced by modern dairies showed a very high quality of microbial standard with a very delicate flavor.

Key words: Raw milk, branded milk, microbiological quality, storage temperature, total plate count.

INTRODUCTION

Milk is a major part of human food and plays a prominent role in the Pakistan diet. Approximately 50 % of the milk produced is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as ice cream, butter, khoa, paneer rabri, kheer, barfi and gulabjamin (Anjum *et al*., 1989). Raw milk, as it leaves the udders of healthy animals normally contains very low, numbers of microorganism. Total count usually is less than 10^3cfu/ml and many bacteria usually present (Deman *et al*., 1960; Kleter, 1975). Microorganism associated with foodborn illness may enter the raw milk supply through infected animals, milking personal, or the environmental. Recently, the consumption of raw milk (on the form or as certified raw milk has been implicated in outbreak of foodborn illness (Bryan, 1983; Bryan, 1988). Bacteria related to foodborn illness are destroyed by proper pasteurization. Recent outbreaks of salmonellosis and listeriosis in pasteurized milk have been linked to pos- pasteurization contamination (Bryan, 1983; Jervis, 1988). The prevalence of *E. coli* O157:H7 strains in raw milk is apparently low (Bleem, 1994), but ranges from 0% out of 603 samples (Hancock *et al*., 1994) to 10% out of 115 samples (Padhye and Doyle, 1991). *E. coli* O157:H7 were reported in pasteurized milk (Upton and Coia, 1994). Milk has long been recognized as an agent in the spread of human disease and within a few years it was appreciated that pasteurization was also providing protection against milk borne disease originally the main health concerns associated with milk were Tuberculosis caused by Mycobacterium bovis and M.Tuberculosis and Brucellosis caused by Brucella *spp*. In some parts of the world milk is still a significant source of these infections (Adam and Moss, 1999). Post- pasteurization contamination has been found to contribute most of the bacteria in milk that are capable of growth and subsequent spoilage (Maxcy, 1967; Muir, 1996). The time required for the occurrence of flavour change depends on the number and types of organisms present and these are usually Gram negative, psychrotrophic contaminants belonging to the genera *Pseudomonas Flavobacterium, hromobacterium, Akaligenes* and coliforms. Other factors which will affect rate of deterioration of pasteurized milk include temperature, agitation and dissolved oxygen (Allen; Joseph, 1985). The aim of the present study was to assess the prevalence of microbial contamination load of branded, raw milk and the study of branded milk at different storage temperature for total plate count.

MATERIALS AND METHODS

Samples collection: Four brands of UHT treated milk and 160 fresh milk samples (100ml samples in sterilized glass bottles) from four different spots of the local market were collected and analyzed microbiologically (Aerobic plate count, Total coliforms bacteria, Fecal coliforms bacteria, *E. coli* O157:H7, Salmonella, Yeast and Mold). Duplicate samples

(Branded milk) were collected at the same time and transported to lab. For shelf life studies the branded milk samples were keep on 4°C, 25°C and 35°C over a period of three months in the Food Microbiology laboratory of PCSIR Labs. Complex Peshawar. Tetra packed milk remained in their packaging material until they were evaluated after an interval of 15 days for microbial analysis.

Microbiological analysis: Aerobic plate count was determined by pour plate method as described by Andrews (1992), serial dilution (10^{-1} to 10^{-8}) of the milk samples was made and aliquots of 1ml were added to each duplicate Petri dishes. Plate count agar was added to each Petri dish and incubated at 35°C for 48 hours ±2, after incubation colony was count by colony counter and result was expressed as cfu/ml.

Total Coliforms bacteria and faecal coliforms bacteria count was determined by method as described by Andrews (1992), growth and appearance on Violet Red Bile Agar after an incubation period of 24h at 35°C were used for a presumptive count, and growth and gas production in 2% Brilliant Green Broth were used as the confirmatory test for coliforms. Simultaneously, the faecal coliforms bacteria was obtained on VRBA plates incubated for 24h at 44°C, confirming typical colonies from these VRBA plates with growth and gas production in E.C. Broth over 24h at 44°C.

E. coli was determined by the procedure described by Andrews (1992), Positive tubes of EC medium were used for the determination of *E. coli*. Streak loopful from these tubes on L-EMB agar and incubated the plates for 18-24h at 35°C. The plates were observed for *E. coli* colony (dark centered with or without metallic sheen). The typical colonies were confirmed by biochemical tests and also by kits (*E. coli O157:H7* latex test reagent kit Pro Lab. Canada).

For detection of *Salmonella* spp., 25 g samples of milk were homogenized in 225 mL lactose broth and incubated for 24 h at 35°C, then 1 mL was subcultured in 10 mL of selenite cystine (SC) broth and another 1ml from the same sample to 10ml tetrathionate (TT) broth and incubated for 24 h at 35°C. Then a loopful of SC broth and TT broth was streaked on Hektoen enteric (HE) agar, Bismuth Sulfite (BS) agar and Xylose Lysine Desoxycholate (XLD) agar and plates were incubated for 24–48 h at 35°C. Suspected colonies were further screened biochemically and serologically (Andrews, 1992).

Yeast and Mold was calculated following the FAO method described by Andrews (1992).25 gm of the milk sample will be blended with 225 ml of butter field's phosphate buffer for 2 minutes, and make a serial dilution (10-1 to 10-5). One ml portion of each dilution was pippeted onto separate plates of Potato dextrose agar (PDA). Petri dishes were incubated in the dark at 22-25°C for 5 days.

RESULTS AND DISCUSSION

The microbial counts for 40 samples (10 samples per brand) of UHT milk sold in Peshawar city are shown in Table 1. The results indicate that total plate count, total coliforms bacteria, total fecal coliforms bacteria, *E. coli* O157:H7, salmonella, yeast and mould were absent in these branded samples. The study carried out by (Riadh AL-Tahiri, 2005) on UHT milk showed that Total plate count, Coliform, *Staph.aureus*, Yeast and mould were also absent in analyzed samples of UHT milk. But the studies carried out by Srikandakumar *et al.* (2004), showed that there was no effect of months of milk collection on SPC, TC, FC, and S. aureus but all five brands of milk were found to contain these bacteria. The studies carried out by Zehner *et al.*,1986 and Fenlon *et al.*, 1995 concluded that microbial contamination of pasteurized milk can occur from different sources. Because of this, determining the cause of bacterial contamination is not always straightforward. Though there is often one source of bacteria that cause high counts, high counts of bacteria can also result from a combination of factors like dirty milking equipment, inefficient pasteurization, contamination from the environments, and poor packaging.

The microbial counts for 160 samples (40 samples per area) of fresh milk sold in Peshawar city were shown in Table 2. The results of this work showed that the raw milk samples collected from S-5 have Total Plate Count (TPC) maximum were 4 x 10^5 cfu/ml, minimum value were 3 x 10^5cfu/ml and average value were 3.5 x 10^5cfu/ml. The results of TPC for S-6 were 1.4 x 10^5cfu/ml maximum, 1 x 10^3cfu/ml minimum and 7.05 x 10^4 cfu/ml average, these results indicate the TPC value of S5 were high as compared to S-6.The TPC value of S-7 were showed that maximum count were 3 x 10^5cfu/ml ,this count high as compared to S-6 maximum value but low as compared to S5 count value. The minimum value of S-7 were 2 x 10^3cfu/ml and average count were 1.51 x 10^5cfu/ml. The TPC results of S-8 showed that maximum count were 5 x 10^5cfu/ml, minimum count were 2 x 10^4cfu/ml and average count were 1.1 x 10^5cfu/ml. The study carried out by (Riadh AL-Tahiri, 2005) on unpasteurized fresh farm milk showed that Total plate count were 5×10^5cfu/ml , Coliform were 60×10 cfu/ml, *Staph.aureus* were 3×10^2cfu/ml and Yeast and mould were 15×10^3cfu/ml. The high viable count of fresh farm milk of this work show that this figure can be regarded as a high count even for raw milk as been mentioned by Bramley and Mckinnon (1990) that counts of greater than 10^5 ml for raw milk are indicative of serious faults in production hygiene. High viable counts often indicate contaminated raw materials, unsatisfactory sanitation and unsuitable storage temperature or a combination of these. Milk can be contaminated with different kind of microorganisms due to direct or indirect contact with any source of external contamination during all the steps of

Table 1: Microbiological analysis of branded milk

	Lab.ID of branded samples			
Microbiological parameters studied	S1 (10 samples)	S2 (10 samples)	S3 (10 samples)	S4 (10 samples)
Total Plate Count (cfu/ml)	Nil	Nil	Nil	Nil
Total Coliform bacteria (MPN/ml)	Nil	Nil	Nil	Nil
Total Coliform bacteria (MPN/ml)	Nil	Nil	Nil	Nil
*E.Coli*O157:H7	Nil	Nil	Nil	Nil
Salmonella	Nil	Nil	Nil	Nil
Yeast & Mould (cfu/ml)	Nil	Nil	Nil	Nil

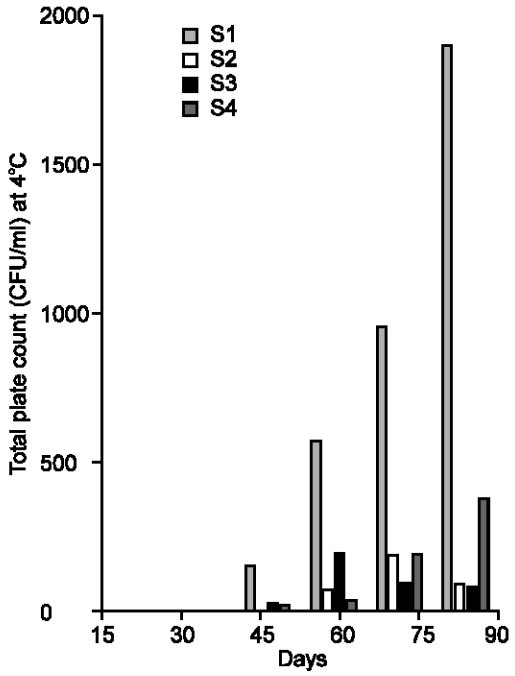

Fig. 1: Effect of temperature on total plate count on branded samples at 4°C

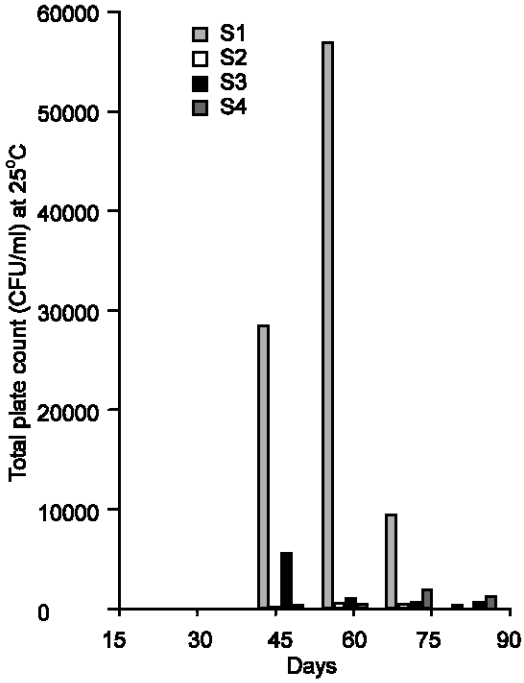

Fig. 2: Effect of temperature on total plate count on branded samples at 25°C

milking, collection, packing and transport. Direct physical contact of milk with unclean surfaces such as those of milking utensils, and the hands of milkers besides environmental factors such as the design and cleanliness of buildings and installations, the adequacy of the water supply, the solid wastes management disposable practice, and the amount of dust in the road side shops are important in so far as they may contribute to the microbial contamination of surfaces with which milk comes into contact.

The Total Coliform bacteria (TC) of S5 were showed a maximum MPN were 110, minimum were 46 and average were 78.The maximum, minimum and average MPN of S-6 were 154,110 and 132 respectively. The TPC results indicate that S-6 were more contaminated as compared to S5.TPC of S-7 indicate that maximum MPN were 24, minimum were 9.3 and average were 16.65.The S-8 raw milk samples showed that TPC were maximum, minimum and average were 46,15 and 30.5 respectively.

The results of total fecal coliforms bacteria (TFC) of raw milk showed the average levels of fecal coliforms bacteria of S1 were 5.8 MPN/ml, S-6 were 7.65MPN/ml, S-7 were $\leq$ 0.3MPN/ml and S-8 were 1.3 MPN/ml. *E. coli* O157:H7 were also isolated from all raw milk samples except S-7, the number of positive isolates of S-5, S-6 and S-8 were 5, 3 and 6 respectively. Our results were a closed agreement of the study carried out by Bei-Zhong *et al.* (2007) on raw buffalo milk of China showed a level of coliforms bacteria and E. coli were 2.42 ± 0.12 and 1.53 ± 0.17 log cfu/ml, respectively. In another study carried out by Desmasures *et al.* (1997), who reported that 84% of samples of French cow milk had coliforms counts <100 cfu/ml and 80% had *E. coli* counts 610 cfu/ml. *E. coli* may be considered an indicator microorganism of faecal contamination and other enteric pathogens. Its occurrence in milk may originate from the external surface of the udder, milking machines, manual milking, handling, and inferior quality of water (Fook *et al.*, 2004).

Table 2: Microbiological analysis of raw milk

Microbiological parameters studied	Total No. Samples tested	40	40	40	40
	Lab.ID	S5	S6	S7	S8
Total Plate Count (cfu/ml)	Maximum	$4x10^5$	$1.4x10^5$	$3x10^5$	$5x10^5$
	Minimum	$3x10^5$	$1x10^3$	$2x10^3$	$2x10^4$
	Average	$3.5x10^5$	$7.05x10^4$	$1.51x10^5$	$1.1x10^5$
Total Coliform bacteria (MPN/ml)	Maximum	110	154	24	46
	Minimum	46	110	9.3	15
	Average	78	132	16.65	30.5
Total Faecal Coliform (MPN/ml)	Maximum	9.3	15	$\leq$ 0.3	2.3
	Minimum	2.3	0.3	$\leq$ 0.3	$\leq$0.3
	Average	5.8	7.65	$\leq$0.3	1.3
E. coli O157:H7	NPI	5	3	Nil	6
	NNI	14	11	Nil	26
	NSI	21	26	Nil	8
Salmonella	NPI	2	1	3	4
	NNI	25	7	18	22
	NSI	13	32	19	14
Yeast & Mould (cfu/ml)	Maximum	$8x10^4$	$6x10^4$	$7x10^4$	$8x10^6$
	Minimum	$2x10^2$	$3x10^1$	$5x10^2$	$6x10^5$
	Average	$4x10^4$	$3x10^4$	$3.5x10^4$	$4.3x10^6$

NPI= No positive isolates, NNI = No negative isolates, NSI= No strain isolate, MPN= Most probable number

Table 3: Total plate count of branded milk at different temperature

		Total Plate Count(cfu/ml)					
Lab.ID	Storage condition	15 days	30 days	45 days	60 days	75 days	90 days
S1	4°C	Nil	Nil	$1.6x10^1$	$6x10^2$	$1x10^3$	$2x10^3$
	25°C	Nil	Nil	$3x10^4$	$6x10^4$	$1x10^4$	$1x10^2$
	35°C	Nil	Nil	$2.3x10^2$	$5.5x10^2$	$5x10^2$	$8x10^1$
S2	4°C	Nil	Nil	Nil	$7x10^1$	$2x10^2$	$1x10^2$
	25°C	Nil	Nil	$1.5x10^1$	$4x10^2$	$4x10^2$	10^1
	35°C	Nil	Nil	$1x10^1$	$3.8x10^2$	$1x10^3$	$3x10^3$
S3	4°C	Nil	Nil	$3x10^1$	$2x10^2$	$1x10^2$	$9x10^1$
	25°C	Nil	Nil	$6x10^3$	$10x10^4$	$6x10^2$	$4x10^2$
	35°C	Nil	Nil	$5x10^2$	$1x10^3$	$1x10^2$	$8x10^1$
S4	4°C	Nil	Nil	$2x10^1$	$3x\ 10^1$	$2x10^2$	$4\ x10^2$
	25°C	Nil	Nil	$4x10^2$	$6x10^2$	$2x10^3$	$1x10^3$
	35°C	Nil	Nil	$3x10^2$	$5x10^3$	$1x10^3$	$2x10^1$

Salmonella were also isolated from the analyzed raw milk samples, the results showed that a number of positive isolates of S5, S6, S7 and S8 were 2, 1, 3 and 4 respectively. The studies carried out by Barbara *et al.,* 2006 were reported that no strain of Salmonella was recovered isolated from milk samples.

Yeast and mould were also determined in raw milk and the results were shown in Table 3. The results showed that the highest average yeast and mould count were found in So i.e. 4.3 x 10^6cfu/ml, the other average count of yeast and mould in S6, S7 and S8 were 4 x 10^4cfu/ml, 3 x 10^4cfu/ml and 3.5 x 10^4cfu/ml respectively. The study carried out by (Riadh AL-Tahiri, 2005) on unpasteurized fresh farm milk showed that Yeast and mould were 15 x 10^3cfu/ml, our results yeast and mould count were high as compared to this finding. But our results were a closed agreement of the study carried out by Pesic *et al.,* 2005, who reported also that milk were contaminated with mould. The big difference in microbial conditions between the UHT milk and the unpasteurized fresh farm milk and this completely agreed with Lewis (1994) that milk is heated for a variety of reasons. The main reasons are to remove pathogenic organisms and to increase shelf -lift up to period of six mouths.

The total plate count (TPC) studies of branded milk were studies at 4°C, 25°C and 35°C for 90 days, the results were shown in Table.3. The result showed that TPC were absent in all branded samples during storage condition at 4°C, 25°C and 35°C up to 30 days. TPC of S1 sample on 45 days at 4°C were 1.6 x 10^1cfu/ml, at 25°C were 3 x 10^4cfu/ml and at 35°C were 2.3 x10^2cfu/ml, on 60 days TPC were increases at all storage temperature and showed that at 4°C were 6 x 10^2cfu/ml, at 25°C were 6 x 10^4cfu/ml and at 35°Cwere 5.5 x 10^2cfu/ml. It observed that on 75 days again TPC were increased at 4°C were 1 x 10^3cfu/ml, but decrease at 25°C i.e. 1 x 10^4cfu/ml and 5 x 10^2cfu/ml at 35°C. Similarly on 90 days again at 4°C TPC of S1 were increases up to 2 x 10^3cfu/ml, but decreased at 25°C and 35°C viz. 1 x 10^2 cfu/ml and 8 x 10^1cfu/ml. The increase in microbial count during freezing was probably caused by the fact that the freezing process may cause rupture of

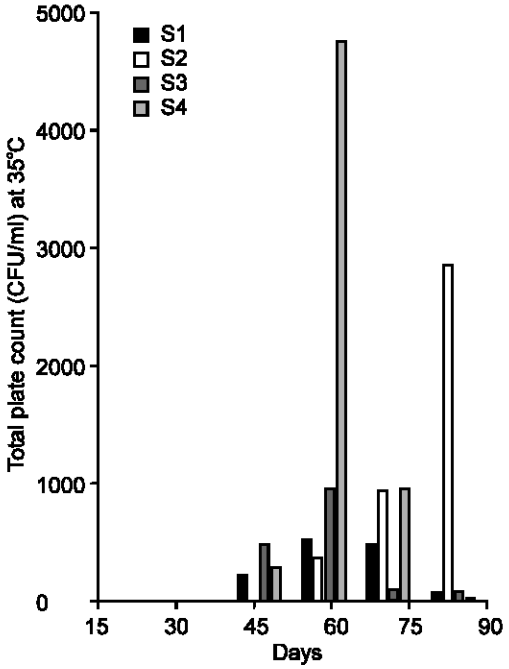

Fig. 3: Effect of temperature on total plate count on branded samples at 35°C

milk macrophages and neutrophils, releasing phagocytized bacteria. Freezing may also disrupt bacterial cell aggregates (Villanueva *et al*., 991; Godden *et al*. 2002).

The S2 branded milk sample on 45 days showed that TPC were absent at 4°C, at 25°C it were 1.5 x 10^1cfu/ml and at 35°C were 1 x 10^1cfu/ml calculated. On 60 days S2 at 4°C were increases up to 7 x 10^1cfu/ml, similar increases were found at 25°C viz. 4 x 10^2cfu/ml and at 35°C were 3.8 x 10^2cfu/ml. On 75 days TPC of S2 were calculated and it was 2 x 10^2cfu/ml at 4°C, no changes in TPC were observed at 25°C and 1 x 10^3cfu/ml were found at 35°C, which showed an increase in TPC as compared to 60 days count at this temperature. On 90 days TPC of S2 were decreases at 4°C and 25°C were 1 x 10^2cfu/ml and 10^1cfu/ml respectively, but at 35°C TPC were increases 3 x 10^3cfu/ml as compared to the previous storage days. It was observed that S1 were containing more microbial load during storage at these temperature condition as compared to S2.

TPC of S3 on 45 days at 4°C, 25°C and 35°C were 3 x 10^1cfu/ml, 6 x 10^3cfu/ml and 5 x 10^2cfu/ml respectively, on 60 days TPC were increases at 4°C, 25°C and 35°C were 2 x 10^2cfu/ml, 10x 10^4cfu/ml and 1 x 10^3cfu/ml respectively. TPC were decreases at 75 days and calculated 1 x 10^2cfu/ml, 6 x 10^2cfu/ml and 1 x 10^2cfu/ml at 4°C, 25°C and 35°C of storage condition. At 90 days the TPC of S3 at 4°C, 25°C and 35°C were 9 x 10^1cfu/ml, 4 x 10^2cfu/ml and 8 x 10^1cfu/ml respectively, it showed more decrease of TPC during storage time.

The brand milk sample S4 TPC were 2 x 10^1cfu/ml, 4 x 10^2cfu/ml and 3 x 10^2cfu/ml at 4°C, 25°C and 35°C respectively after 45 days storage time, TPC were increases at 60 days period time and showed that at 4°C, 25°C and 35°C were 3x 10^1cfu/ml, 6 x 10^2cfu/ml and 5 x 10^3cfu/ml respectively after 75 days storage time, after 90 days storage time at 4°C TPC were 4 x10^2cfu/ml, which showed an increased during storage time as compared to 75 days storage time. But at 25°C and 35°C TPC were 1 x 10^3cfu/ml and 2 x 10^1cfu/ml respectively, which showed that it was decreases at time.

Conclusion: Our results indicate that raw milk samples sold in Peshawar City are contaminated with pathogenic and indicator bacteria, such as *E. coli*, coliforms, and Salmonella, indicate that to the growth of these organisms may lead to a hazard against public health. Milk has been considered safe because of pasteurization, all the branded milk are free from any microbial load and pathogens, during storage of branded milk show that TPC were observed. So it is recommended that food safety programs should be designed to ensure that adequate pasteurization treatments are achieved and that the microorganism is absent from postpasteurization processes.

It is imperative that stricter quality control measures be imposed in Pakistan to assure that consumers are provided with truly wholesome milk. Chillers at the collection centres and the transportation of milk in an insulated containers and vehicles to achieved a reduction of milk temperature and possibly in microbial multiplication. Finally, it is also recommended that the members of the public should always boil raw milk before consumption because of their microbial content. Therefore, it is highly recommended that hygienic practices and regulations, such as on-site pasteurization and implementation of HACCP following established standards, should be introduced to facilitate the production of raw milk of high quality and safety.

REFERENCES

Adams, M.R. and M.O. Moss, Food Microbiology. 3rd Edition, The Royal Society of Chemistry.

Allen, J.C. and G. Joseph, 1985. Deterioration of pasteurized milk on storage, J. Dairy Res., 52: 469-478.

Andrews, 1992. Manual of Food Quality Control 4. Rev. 1. Microbiological analysis, Published by Food and Agriculture Org. of the U. N.

Anjum, M. S., K. Lodhi and A.A. Raza, 1989. Pak Dairy, Issues and policy alternatives Special report series No.14.Pak. Econom. Analysis network project, Islamabad.

Barbara, P.M., M.F. Elisabeta, D. Maura, C. Arianna and C. Sofia, 2006. Microbiological and chemical characterization of Fiore Sardo, a traditional Sardinian cheese made from ewe's milk. Int. J. Dairy Tech., Vol. 59, No 3 August.

Bei-Zhong. H., M. Yun, Li. Min, Y. Ying-Xiao, R. Fa-Zheng, Z. Qing-Kun and N.M.J. Robert, 2007. A survey on the microbiological and chemical composition of buffalo milk in China. Food Control, 18: 742-746.

Bleem, L.A., 1994. *Escherichia coli* O157:H7 in raw milk, a review. In animal health insight fort callins, co: Centers for epidemiology and animal health.

Bramley, A.J. and C.H. Mckinnon, 1990. In: Dairy Microbiology, Volume 1, Elsevier Applied Science Publishers, London.

Bryan, F.L., 1988. Risks associated with vehicles of food borne pathogens and toxins. J. Food Prot., 51: 498-508.

Bryan, F.L., 1983. Epidemiology of milk born diseases. J. Food Prot., 146: 637-649.

Deman, J.C., M. Rogosa and M.E. Sharp, 1960. A medium for the cultivation of lactobacilli., J. Appl. Bacteriol., 23: 130-135.

Desmasures, N., F. Bazin and M. Gueguen, 1997. Microbiological composition of raw milk from selected farms in the Camembert region of Normandy, J. Appl. Microbiol., 83: 53-58.

Fenlon, D.R., D.N. Logue, J. Gunn and J.A. Wilson, 1995. A study of mastitis bacteria and herd management practices to identify their relationship to high somatic cell counts in bulk tank milk, Br. Vet. J., 151: 17-23.

Fook, Y.C., A. Aminah and K.A. Mohd, 2004. Bacteriological quality and safety of raw milk in Malaysia, Food Microbiol., 21: 535-541.

Godden, S.M., J.T. Jansent, K.E. Leslie, N.L. Smart and D.F. Kelton, 2002. The effect of sampling time and sample handling on the detection of Staphylococcus aureus in milk from quarters with subclinical mastitis, Can. Vet. J., 43: 38-42.

Hancock, D.D., T.E. Besser, M.L Kinsel, P.I. Tarr, D.H Rice and M.G Paros, 1994. The prevalence of *E.Coli* O157:H7 in dairy and beef cattle in Washington State, Epidemiology and Infection., 113: 199-207.

Jervis, D.I., 1988. Behavior of pathogens in dairy products. Dairy Ind. Int., 53: 15-19.

Kleter, G., 1975. The bacterial flora in aseptically draw milk neth. Milk dairy J., 28: 220-237.

Lewis, M.J., 1994. Heat Treatment of Milk. In: Modern Dairy Tech., Vol.1 Advances in Milk Processing) Ed. R.K. Robinson, Chapman and Hall.

Maxcy, R.B., 1967. Nature and growth response of the microflora of pasteurized, packaged milk, J. Milk Food Tech., 30: 213-220.

Muir, D.D., 1996. The shelf-life of dairy products: Raw milk and fresh products. J. Society of Dairy Tech., 49: 44-50.

Padhye, N.V. and M.P. Doyle, 1991. Rapid procedure for detecting enterohemorrhagic *E.Coli* O157:H7 in food. Appl. Environ. Microbiol., 57: 2693-2698.

Pesic, M.D., L. Stojanovic and L. Jovanovic, 2005. Moulds associated with milk depending on macroclimate and geographical location, Appl. Ecology and Environ. Res., 3: 61-65.

Riadh AL-Tahiri, 2005. A Comparison on Microbial Conditions between Traditional Dairy products Sold in Karak and Same Products Produced by Modern Dairies, Pak. J. Nutr., 4: 345-348.

Srikandakumar, A., E.H. Johnson, H. Nsanzi and K.S Al-Abri, 2004. Microbes and Anti-microbial Substances in Pasteurized Milk Sold in Oman. Int. J. Food Prop., 7: 615-627.

Upton, P. and J. Coia, 1994. Outbreak of *E.Coli* O157:H7 infection associated with pasteurized milk supply. Lancet., 344: 1015-1022.

Villanueva, M.R., J.W. Tyler and M.C. Thurmond, 1991. Recovery of Streptococcus agalactiae and Staphylococcus aureus from fresh and frozen bovine milk, J. Am. Vet. Med. Assoc., 198: 1398-1400.

Zehner, M.M., R.J. Farnsworth, R.D. Appleman,K. Larntz and J.A. Springer, 1986. Growth of environmental mastitis pathogens in various bedding materials, Dairy Sci., 69: 1932-1940.