

Microbial and Physico-Chemical Quality Assessment of the Raw and Pasteurized Milk Supplied In the Locality of Twin City of Pakistan

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Abstract

The present study was undertaken with the aim of investigating the physico-chemical and microbiological quality of raw and pasteurized milk consumed in the twin city of Pakistan. For this purpose ninety samples of buffalo raw milk and sixty samples of pasteurized milk were collected from different locally producer. The physicochemical examination included pH, fat, total solids and moisture to assess consumer's acceptance and the bacteriological analysis comprised enumeration of total viable count, coliform count, fecal count, yeast & mold E.coli, Enterobacteriaceae, Salmonella and Staphylococcus for the determination of sanitary. The chemical result showed that range of pH, fat, total solids and moisture were within the permissible limits in both raw and pasteurized samples while microbiological quality showed that raw milk samples were contaminated with total plate count, Coliform, fecal Coliform, E.Coli, Salmonella and yeast & molds and few samples of pasteurized milk were contaminated with total viable count (TVC).

Key words: Buffalo, Microbiological, Pasteurized, Physical, Milk, Contamination.

Introduction

Pakistan is an agricultural based country and Livestock plays a pivotal role in its economy by providing essential items of human diet in the form of milk, meat etc. The role of livestock in rural economy has assessed by the fact that 30 to 35 million of the total rural population are engaged in livestock related activities, having household holdings. Mostly they have 2 to 3 cattle, buffalo and 5 to 6 sheep and goats per family, deriving 30 to 40 per cent of income from it (Bilal and Ahmad 2004). These animals produced 29.472 million tones of milk during 2004-05. Out of the total milk produced, 68 per cent is contributed by buffaloes, 27 per cent by cows and the remaining 5 per cent by sheep, goats and camels. Keeping in mind such a big contribution in the country's milk production, buffalo becomes the most important animal of the country. (Economic Survey of Pakistan 2004-05). Due to its high nutritional value it is important part of food for vast population on earth. To serve its purpose it is a food that contains all the nutrients for the newly born baby to old age.

In Pakistan buffalo is the major dairy animal contributing maximum in total milk production followed by cattle and sheep/goat, respectively. India followed by Pakistan is the

top producer and consumer of buffalo milk. Buffalo milk is much richer than cow's milk due to its average butter fat content of over 7% and the SNF content is around 9-10.5 % and is generally slight higher than that of cow's milk. The milk is very popular throughout the country and sells at a higher price than cow's milk due to its high fat and solid contents. The milk provides the basis for dairy produce such as ghee, dahi, butter, milk powder and baby food. (Banerjee, 1983). Raw milk in its natural state is one of the most important sources of healthy and functional food ingredients. It is medium for the growth of large variety of bacteria such as Bacillus, Enterobacter, Micrococcus, Pseudomonas, and Salmonella that cause undulant fever, dysentery, Salmonellosis, and tuberculosis. The presence of food-borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and excretion from the udder of an infected animal, (El Zubeir et al. 2006).

The major problem with the raw milk supply system in Pakistan, from the consumer's point of view is that it is produced and supply by untrained person like Goalas, and, it has been observed that these supplier adulterated the milk by adding dirty water and additives such as low quality flour to bring the total solid to a level that is acceptable to consumer. The composition and amount of microflora in the raw material has a deceive effect on the quality and safety of dairy product. The objective of this study was to evaluate

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the hygienic quality of raw and pasteurized milk with special reference to food borne pathogens available in twin cities of Pakistan. The results obtained serve as baseline data and will be useful in future studies dealing with pathways of contamination and in the development of HACCP systems for hygienic processing of buffalo milk.

Material and Methods

Sample collection and Preservation. Total one hundred and eighty samples were collected from different locally producer which included ninety raw and ninety pasteurized milk sample. All samples were purchased randomly from supermarkets and transported in icebox maintaining cold state to the laboratory, and analyzed immediately.

Microbiological Methods. Traditional microbiological methods and media were used for the isolation and enumeration of Total viable counts(TVC), coliform and fecal coliform, Yeast & Mold, Pseudomonas, Enterobacteriaceae, Salmonella and Staphylococcus as described by Andrews (1992) and result were reported as the count of colony forming unit (cfu/ml).According to the colony morphology and gram staining bacteria and fungal were identified for the further conformation and bacteria organism were subcultures to differential growth media as shown in Table 1 and were classified according to Bergey, manual(Bergey,1984).

Physiochemical Methods .The milk samples were chemically analyzed for pH, 1 solid non fat(SNF), total fat content, and moisture. The pH of the milk samples was measured using digital pH meter, and it was firstly calibrated using buffer of pH 7.0 and 4.0(HANNA model 210). Solid non-fat, Fat content and Moisture were determined according to the method of Association of Analytical Chemist (AOAC, 2000) and the results were expressed as percentage.

Statistical Analysis. Statistical analysis including mean, standard deviation was calculated according to Caulcutt and Boddy1983.

Results

Results of physiochemical composition of buffalo's raw and pasteurized milk samples which were collected from local shops and market in Pakistan were shown in Table 2 and in Table3. Fat, Solid non fat, and Moisture content in buffalo's milk were (3.58,8.25, and 91.78% respectively) than those found in pasteurized milk (4.1, 11.33 and 89.16%).. The pH informs precisely about the freshness state of the milk. Fresh milk is neutral or with slightly acidic tendency. According to our result pH in pasteurized milk samples were lies between the ranges of 6.45-7.03 and in raw milk was found between the range of 6.35-6.98 .This study showed similarities with some recent studies by Ibtisam *et al.*, 2009; Agged *et al.*, 2010. Milk pH gives an indication of milk hygienic and it should not be ≤ 6.6 or ≥ 6.8 when milk temperature is 20°C, because cooling of milk reduces the risk of growth of bacteria while in high milk temperature must be considered as favorable to the growth of bacteria in milk (Walstra *et al.*, 2006).

In present study the solid non-fat content, Fat and moisture were recorded in raw and pasteurized milk sample. These results revealed lower values of fat and TS than that found by the Enb *et al.*, 2009 and Abou-Donia *et al.*,2010 who had recorded fat, TSN (4.90 5.70 13.40%) respectively. The water content as moisture were also observed in both samples which showed that it was higher in raw milk samples as compare to the pasteurized milk samples. The higher water content in raw milk were due to the adulteration of raw milk either by adding ice to maintain its temperature or directly addition of dirty water to increases the sale.. Siegentholer and Shulthess (1977) said that addition of water not only disturbed the mineral balance but it also change its natural taste and as well as it leads to increase the risk of microbial growth because water added may be grossly contaminated. The major reason in developing countries like Pakistan, India and Bangladesh mostly people used raw milk because locally supplier sale raw milk with low prices as compared to the pasteurized milk.

Table 1. The media used for the microbiological analysis and incubation condition.

Parameters	Media	Incubation Temperature °C	Incubation time
Total viable count	Plate count agar (,Oxoid Ltd., UK)	35	24-48 h
Total Coliform	MacConkey-agar (Merck, Germany)	37	24-48 h
Total Fecal Coliform	EC broth (Oxoid Ltd., UK)	44	24-48 h
Yeast & molds	Potato dextrose agar (Oxoid Ltd., UK)	25	4-5 days
	Chloramphenicol(Oxoid Ltd., UK)		
Enterobacteriaceae	Violet Red Bile Dextrose Agar(Oxoid Ltd., UK)	37	24-48 h
Enterococci	Slanetz-Bartley Medium(Oxoid Ltd., UK)	37	24-48 h
E.Coli	Eosin Methelyene blue agar(Oxoid Ltd., UK)	37	24-48 h
Staphylococcus	Baired-Parker agar base(Oxoid Ltd., UK)	37	24-48 h
	Egg yolk tellurite emulsion(Sigma, Germany)		
	Xylose lysine Desoxycholate agar(Merck, Germany)		
Salmonella	Hektoen enteric agar(Merck, Germany)	37	24-48 h
	Selenite Cystine Broth(Merck, Germany)		

Table 2. Physicochemical Properties of Raw milk.

Physiochemical composition	Sample number (N)	Min.	Max.	Mean ± SD
pH	90	6.35	6.98	6.71±0.19
Solid non fat (SNF %)	90	7.88	9.34	8.25±0.39
Moisture (%)	90	90.66	92.78	91.78±0.43
Fat dry matter (TF %)	90	3.01	3.95	3.58±0.23

Microbial studies were carried out for the detection of total viable count (TVC), Coliforms, fecal Coliform, yeast & mold. According to the results of pasteurized milk, only TVC were present with average count of $\log_{10}1.38$ cfu/ml. On the other side all raw milk samples were contaminated with TVC, Coliform, Fecal coliform, and yeast&molds with the mean count of ($\log_{10}4.52, 3.59, 1.83, 2.33$, cfu/ml) as shown in Figure 1 and Table. Different bacterial species were also recovered from the raw milk samples in which *Enterobacteriaceae* were present with higher frequency 30% with mean count of $\log_{10}3.93$ cfu/ml compared to the other bacterial species as *Staphylococcus spp* was with 26% with average number of $\log_{10}3.45$ cfu/ml Enterococci was with 24% with average number of $\log_{10}3.15$ cfu/ml *E.coli*, *Pseudomonas spp* were with 7% with mean count of $\log_{10}0.98$ and 0.92 cfu/ml. However *Salmonella spp* were detected with lowest frequency 6% with average count of $\log_{10}0.76$ cfu/ml as shown Figure 2.

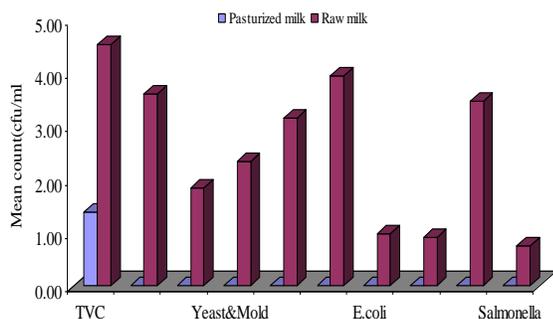


Figure 1. Microbial contamination of raw and pasteurized milk.

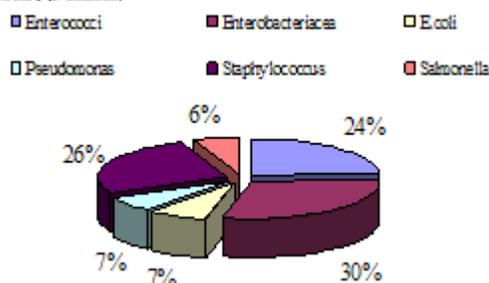


Figure 2. Frequency isolation of different bacterial species from Raw Buffalo milk.

Table 3. Physicochemical properties of Pasteurized milk.

Physiochemical composition	Sample number (N)	Min.	Max.	Mean ± SD
pH	90	6.45	7.3	6.78 ± 0.20
Solid non fat (SNF %)	90	10.31	13.5	11.33 ± 0.81
Moisture (%)	90	86.5	90.45	89.16 ± 0.99
Fat dry matter (TF %)	90	3.49	4.1	3.67±0.16

Discussion

The microbiological analysis of milk samples showed a significant deficiency in hygienic and sanitary quality conditions as shown in Figure, 1. Buffalo milk is considered as having unacceptable hygienic quality when bacteria count exceeded 2.0×10^4 cfu/ml according to the European Regulation as reported in NMC annual meeting 2004. In the present study the average TVC was $\log_{10}4.52$ cfu/ml which is higher than the limits recommended by either of these agencies. The finding in this study were consistence with the results of Ibtisam et al.,2009 and Wira et al., 2009 whereas it were lower than reported by Hamida et al., 2009 and higher than Franciosi et al., 2009) .Possible reasons for the high bacterial count in raw milk was due to increases holding time of milk during transportation or it may be unhygienic milking procedure. In pasteurized milk only TVC were observed with the average count of $\log_{10}1.38$ cfu/ml which is under the permissible range as presented in Table 4.

Table 4. Microbiological Standards for Milk (FAO WHO, Codex Standard (2000).

Raw milk	EU	US
Bacteria (TVC)	<100,000	<100,000/<300,000
Coliform	10	None detectable
Yeast & molds	<5	<5
E.Coli	Negative	Negative
Salmonella	Negative	Negative
Pasteurized milk		
Bacteria (TVC)	5 000/50,000	< 20,000
Enterobacteriaceae	5	
Coliform	5	Nil
Yeast & molds	-	<5
E.Coli	Negative	Negative
Salmonella	Negative	Negative

Our result showed similar result as some previous studies which were done by Ibtisam et al., 2009; Agged et al., 2009 and Ammra et al., 2009 they had observed the total viable count contamination in pasteurized milk while On the other hand Hamida et al., 2009, did not observe single

colony of bacteria in pasteurized samples. Microbial contamination of pasteurized milk can occur from different sources, like dirty milking equipment, inefficient pasteurization, contamination from the environments, and poor packaging, unsatisfactory sanitation and unsuitable storage temperature or a combination of these. (Fulya .T ,2011)

The Coliform group (*Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebisellia*, *Salmonella*, *Shigella*) belong to a family of *Enterobacteriaceae* that were isolated from animal intestine. The presences of *Enterobacteriaceae* family are considered important in food as indicator of spoilage or potential pathogenic organism. According to our finding the average count of coliforms in raw milk were \log_{10} 3.59cfu/ml, and average count of fecal coliforms were \log_{10} 1.83 cfu/ml. and. As some previous studies carried out by, Hamida *et al.*, 2009; Franciosi *et al.*, 2009 ;Wira *et al.*, 2009 and Fulya ,2011 determine that the Coliform count in raw milk sample were (\log_{10} , 2.12, 1.39 ,2.83-3.17 and 4.30cfu/ml, respectively. The detection rate of coliforms in our results were higher as compared to the study of ,Franciosi *et al.* ,;Hamida *et al.*, 2009 and lower than the Fulya ,2011. The presence of Coliform bacteria may not be the direct indicator of fecal contamination but it is precise indicator of poor sanitary practices during milking and handling process. In our findings *Enterobacteriaceae* were observed with mean count of \log_{10} 3.93cfu/ml 30% *Enterococci* with \log_{10} 3.15 cfu/ml and *E.coli* was few in number with lowest frequency 7%, which were lower than the reported by Fulya , 2011 while *E.coli* was not isolated by Ekici *et al.*, 2004 in milk samples. According to the Regulation of EU the *E.coli* should not present in milk samples. The presence of *E.coli* is the indicator of fecal contamination as well as it indicates the presence of toxigenic or enteropathogenic bacteria which are major public health hazard especially causes illness in young children, the elderly and immunocompromised persons (Hillerton *et al.*, 2004).

The average count of *Staphylococcus aureus* in current study were observed with average number of \log_{10} 3.45 cfu/ml with frequency of 24% which was lower than the previous reported by Fulya ,2011 and higher than the Aggad *et al.*, 2010. *Staphylococcus aureus* is typical pathogen that is mostly present in the mucus membrane and skin of warm blooded animals including human beings and its most lethal agent that causes chronic infection in mammary gland. The main sources of transmission of *S.aureus* usually occur during milking. In milk the minimum number of *S.aureus* that causes food poisoning estimated to be \log_{10} 5.0 cfu/ml but if its count increases from \log_{10} 7.0 cfu/ml resulted production of enterotoxin, that causes the gastroenteritis syndrome and it cannot be destroyed by heating, drying or freezing .(Hillerton *et al.*, 2004).

According to the current studies *Pseudomonas* were present in 7% in raw milk with average number of \log_{10} 0.92 cfu/ml which were higher than the previously observed by

Srikandakumar *et al.*, 2004. *Pseudomonas* is an environmental contaminant commonly originating from water sources. Excessive environmental bacteria have adverse effect on milk flavor and shelf life .The presence of *Pseudomonas* indicate that the water pipes lines were contaminated with this organism and use of water from these pipes lines to clean the milking equipment may lead to high *Pseudomonas* count.(Srikandakumar *et al.*,2004). Isolation of *Salmonella* from raw milk of buffalo is of public health significance because *Salmonella* is a zoonotic pathogen and its causes Salmonellosis, which is a disease of cattle and calves is caused by different species of *Salmonella* especially *Salmonella enteric* subspecies enteric serovars, *Typhimutium* and *Dublin* (Veling *et al.*, 2001).we had recorded 6% *Salmonella* in raw milk with average count of \log_{10} 0.76 cfu/ml while Ekici *et al.*, 2004 did not observed single colony of salmonella in milk and milk product. Environment play important role in spread of *Salmonella* e.g. contamination of animal occur through fodder water, wildlife or pasture. Worldwide *Salmonella* prevail between 2.6 and 25.3% from bulk tank milk, meat and fecal samples of cull dairy animals.

Yeast and mould are common contaminate in foods. While yeast does not result in food poisoning, it does cause food to spoil .Different types of molds produce toxic substances that were designated as mycotoxins. Some are mutagenic and carcinogenic, some display specific organ toxicity and some are toxic by other mechanism (Deak, 2008).The mean value of yeast & molds were found in this study were \log_{10} 2.33 cfu/ml respectively which are higher than reported by Godic-Torkar, 2008. Many researchers have been reported that the higher concentration of mycotoxins found in ensiled feed which was used mostly in winter seasons and due to the feeds of animals molds were transferred to milk. The big difference in microbial conditions between the pasteurized milk and the raw milk and this completely agreed with previous studies which show 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. (Lewis, 1994).

However milk is a natural food that has no protection from external contamination and it easily contaminated when it is separated from Buffalo which result in infection and threat to consumer, s health .Raw milk normally has varied microflora arising from several sources, such as the exterior surfaces of the animals and the surfaces of milk handling equipment such as milking machines, pipelines and containers. Therefore raw milk is susceptible to contamination by many pathogenic microorganisms, which result in infection and threat to consumer, s health According to the microbiological and chemical analysis result, it has been concluded that the raw milk samples sold in twin city were inappropriate for human consumption, because it had contaminated with pathogenic bacteria such as *E.coli*, and *Salmonella*, *Pseudomonas*, *Staphylococcus*. Milk has been considered safe because of pasteurization,

according to our study all the pasteurized milk are free from any microbial load and pathogens as compared to the raw milk. Therefore, it is recommended that food safety programs should be designed and training should be given to farmers, milk seller and collector on the hygienic and the physical aspects of raw milk. It is imperative that stricter quality control measures should be imposed in Pakistan to assure that consumers are provided with truly wholesome milk. Chillers at the collection centers 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 suggested that the awareness in public should created that they 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 introduce to facilitate the production of raw milk of high quality and safety

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