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Research Article

PHYSIO-CHEMICAL AND MICROBIAL QUALITY OF BOILED MILK

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ABSTRACT
The microbiological quality and safety of boiled bovine milk obtained from urban consumers and open market areas of Jabalpur were examined. Milk samples (n=1800) were aseptically collected from the consumers and evaluated in the laboratory. Samples were analyzed for several Physico-chemical and microbial quality including fat **p**ercentage, solid not fat, corrected lactometer reading, titratable acidity, Methylene blue reduction test, total plate count and total coliforms. Furthermore, the presence of selected pathogens such as *Bacillus spp.*, *Enterobacter sp.*, *Escherichia coli*, *Proteus sp.*, *Pseudomonas spp.*, *Salmonella spp.*, *Shigella sp.*, *Vibrio sp., and Staphylococcus spp.*, were detected. The mean ± standard deviation of total plate count and the coliform count recorded 1108.90±217.145 cfu/ml and 3.32±0.64 cfu/ml respectively. The Wilcoxon Signed-Rank test indicates that there is no seasonal variation in physio-chemical and microbial parameters of sampled boiled milk during the study periods. The results of the present study serve to indicate the continued good condition of boiled milk sampled in terms of physio-chemical parameters, however; during the present study 20%, samples failed to achieve the standard microbiological norms and the presence of potential pathogenic microorganism is suggestive of unhygienic practices in some house kitchen.

Key Words: *Boiled Milk, Seasonal Variation, Bacteria, Quality*

INTRODUCTION
Milk is the lacteal secretion of mammals, which are the complete foods on Earth. In India, the term 'milk', refers to cow or buffalo milk or a combination of the two (De, 2004), widely accepted as a national drink due to the benefit associated with it. Chemically, milk is a complex mixture of fat, protein, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet (Haug *et al.*, 2007). Milk considered as an attractive source of energy, proteins, and calcium for infants, young children and elderly people who have few alternative sources available for nutrients; even it is a part of daily diets of most of the people.

The rural families produced the major parts of the unprocessed milk marketed to the urban consumers. The rural milk producers were not acting as retailers instead some intermediary act as retailers, these urban retailers were delivered milk door- to- door without cooling containers, by their own vehicles or sell it in milk booth. Door-to-door raw milk deliveries in the urban areas commonly practiced with virtually no quality control at all levels. Poor hygiene and mal practices of the intermediary of these products were responsible for poor quality of milk distribution in the community (Siva and Sannabhati, 1994).

In India, milk subjected to boiling before consumption by the end users. Boiling of milk at or above the 100.5°C make milk free from most of the pathogenic microorganism (Mattick *et al.*, 1931) and increases its palatability. Milk contains many substances like lipid and proteins in colloidal forms, these substances have a protective effect on micro-organisms against heat treatments, thus there is a possibility of their survivability in milks even after boiling as they have receiving un-certified milks from retailers. Keeping in the mind of above facts, the aim of this work was to evaluate the quality of boiled milk utilized by the urban consumers. The samples of boiled milk were further evaluated to determine the bacteriological quality with relation to the seasonal variation.

MATERIALS AND METHODS

Jabalpur (Latitude: 23.2, Longitude: 79.95, Altitude: 391) also known as *Sanskaardhaani* is the third largest urban agglomeration in the state of Madhya Pradesh, India. The climate of Jabalpur is divided into four main season's viz., the summer season (from middle of March to middle of June), the monsoon season (from middle of June to the end of September), the post monsoon season (October to middle of November) and the winter season (from middle of November to middle of March).

Source of Milk Samples

Boiled milk (Temperature≥100°C and holding time ≥2 min.) samples in triplicate from five different community areas at random were obtained from domestic and market area fortnightly for a period of one year in order to cover all major seasons; that made 1800 samples in a year. Each batch was collected and after recording initial temperature in terms of °Celsius, kept in an ice-box and transported to the laboratory for physio-chemical analysis and microbiological examination.

Physic-Chemical Analysis

Fat percentage, solid not fat (S.N.F.), corrected lactometer reading (C.L.R.), titratable acidity (T.A) and Methylene blue reduction test (M.B.R.T.) for microbial activity was determined according to the modified method of AOAC.

Microbiological Examination

After receiving the sampled milk in the laboratory, the milk was kept at room temperature for 15 minutes to bring milk at room temperature level. The samples further subjected to the serial dilution before inoculation by transferring one ml milk serially in sterile buffered peptone saline (0.5% w/v; peptone; 0.85% w/v; NaCl) to make dilution of 10^{-1} to 10^{-8}. 0.1 ml and 1ml of the milk from each diluted samples transferred to surface of Plate Count Agar medium for standard plate count (SPC) and Violet Red Bile Agar (VRB) plates for the detection and enumeration of coliform organisms in milk. In addition to these media, Hektoen Enteric Agar, Thiosulfate Citrate Bile Salts Sucrose Agar, Bacillus Cereus Agar (Polymyxin – B Sulphate 100 units per ml.), Eosin Methylene Blue Agar, Blood Agar and Hi Crome ECO157:H7 Agar (all, Hi Media, Mumbai, India) media were used for selective isolation and cultivation of pathogenic bacteria. The sample on the plate was spread evenly using sterile L-spreader. After incubation of each plate at 37±2°C for 24-48 hours, the colonies were counted in terms of colony forming units (cfu), from the plate having 20-200 colonies [(Collee *et al*., 1999), (Dworkin *et al*., 2006), (Forbes *et al*., 2002), (Jones and Sackin, 1980) and (Krieg and Holt, 1984)]. The numbers of bacteria per ml in milk samples calculated by taking average of three plates and the colonies were counted in terms of cfu per ml of milk by the methods as described previously by Khan *et al*.,(2008).

Statistical Analysis

Data of the experiments were analyzed for the affect of various physio-chemical and meteorological factors on the prevalence of bacterial population in milk statistically by using Pearson's correlation coefficient. The Wilcoxon Signed-Rank test was used to detect the differences in the distributions of various physio-chemical and microbiological variables. Small significance values (<.05) show that the two variables differ in distribution. The cfu of standard plate count (SPC) and coliform counts compared by the paired Student's t-test by using the SPSS Win 12.0 program (SPSS Inc, Chicago, U.S.A.).

RESULTS

Physio-chemical and microbiological qualities of consumable boiled milk were monitored at the urban community area for a period of one year to cover a full cycle of the season. The study revealed that a mean ± standard deviation (SD) 1108.90±217.145 cfu/ml and 3.32±0.64 cfu/ml total plate count and the coliform count respectively of bacterial count in the sampled boiled milk collected from community area of jabalpur; ranges from 894-1596 cfu/ml. of the total plate count and the coliform count was 2.3-4.8 cfu/ml. of the sampled milk. Range, mean and standard deviation of other variables were given in Table 1. In summer season total 150 boiled milk samples were examined in which 1,300 pathogenic bacteria were isolated out of which 11 reported pathogenic bacteria were *Bacillus cereus* (25.38%), *Bacillus*

subtilis (17.69%), *Enterobacter aerogenes* (6.92%), *Escherichia coli* (18.46%), *Proteus vulgaris* (0.77%), *Pseudomonas aeruginosa* (16.15%), *Salmonella enteritidis* (5.38%), *Salmonella typhimurium* (1.54%), *Shigella flexnerii* (3.08%), *Staphylococcus aureus* (3.08%) and *Vibrio cholerae* (1.54%).
In monsoon season total 200 boiled milk samples were examined in which 980 pathogenic bacteria were isolated out of which 11 reported pathogenic bacteria were *Bacillus cereus* (29.59%), *Bacillus subtilis* (10.20%), *Enterobacter aerogenes* (15.31%), *Escherichia coli* (18.37%), *Proteus vulgaris* (1.02%), *Pseudomonas aeruginosa* (11.22%), *Salmonella enteritidis* (1.02%), *Salmonella typhimurium* (3.06%), *Shigella flexnerii* (2.04%), *Staphylococcus aureus* (6.12%) and *Vibrio cholerae* (2.04%).

Table 1: Descriptive statistics of the boiled milk samples

Parameters	Minimum	Maximum	Mean	Std. Deviation
Initial Temperature (ºC)	12.48	20.28	15.9417	2.04645
MBRT (min.)	292	350	330.85	18.149
Acidity	0.12348	0.13788	0.1288350	0.00306816
Fat%	5.092	5.916	5.53733	0.224120
CLR	25.7	26.2	25.976	0.1605
SNF	8.207	8.445	8.31825	0.065126
Coliform/ml	2.28	4.80	3.3183	0.63907
Total count/ml	894	1596	1108.90	217.145

In post monsoon season total 100 boiled milk samples were examined in which 510 pathogenic bacteria were isolated out of which 11 reported pathogenic bacteria were *Bacillus cereus* (23.53%), *Bacillus subtilis* (21.57%), *Enterobacter aerogenes* (5.88%), *Escherichia coli* (17.65%), *Proteus vulgaris* (3.92%), *Pseudomonas aeruginosa* (3.92%), *Salmonella enteritidis* (1.96%), *Salmonella typhimurium* (7.84%), *Shigella flexnerii* (1.96%), *Staphylococcus aureus* (7.84%) and *Vibrio cholerae* (3.92%).
In winter season total 150 boiled milk samples were examined in which 620 pathogenic isolates were obtained out of which eight reported pathogenic bacteria were *Bacillus cereus* (24.19%), *Bacillus subtilis* (14.52%), *Enterobacter aerogenes* (8.06%), *Escherichia coli* (20.97%), *Pseudomonas aeruginosa* (12.90%), *Salmonella typhimurium* (6.45%), *Shigella flexnerii* (4.84%) and *Staphylococcus aureus* (8.06%).

Table 2: Mean of the physio-chemical and microbiological parameters boiled milk samples based on seasons

Parameters	Winter	Post Monsoon	Monsoon	Summer
Initial Temperature (ºC)	16.08667	16.05	16.12	15.48667
MBRT (min.)	333.4	343.2	315.75	340.2
Acidity	0.12882	0.12807	0.131117	0.12894
Fat%	5.614667	5.56	5.399	5.629333
CLR	26.01333	25.92	25.9875	25.95933
SNF	8.347	8.31	8.286625	8.337167
Coliform/ml	3.366667	3.86	3.27	2.973333
Total count/ml	1058.667	949	1306.5	1002.267

Mean of the physio-chemical and microbiological parameters boiled milk samples based on season was also analyzed (Table 2). There was very little variation in these parameters recorded during the study period in the milk sampled from the households. The Wilcoxon Signed-Rank test was done to detect the differences in the distributions of the physio-chemical and microbiological parameters of boiled milk samples analyzed during the study period. The result of Wilcoxon Signed-Rank test as the significance

value is more than 0.05 for all the paired tested indicated that there was no difference in these parameters based on seasonality (Table 3).

In boiled or Ultra high treated (UHT) milk samples; there was no correlation established among the initial temperature and coliform count of boiled milk with any other variables studied; means that contaminations from extraneous sources were responsible for coliform count in milk. MBRT (min.) was significantly (p=0.05) positively correlated with fat% and SNF, and significantly negatively correlated with acidity and total plate count. Total plate count was significantly positively correlated with fat%, and significantly negatively correlated with acidity and MBRT (min.); means that adulteration augmented the bacterial bio load of milk which further deteriorate the quality of milk (Table 4).

Table 3: Wilcoxon Signed-Rank test statistics

	Post Monsoon - Winter	Monsoon - Winter	Summer - Winter	Monsoon - Post Monsoon	Summer - Post Monsoon	Summer - Monsoon
Z	-.700(a)	-.840(a)	-.980(a)	-.280(a)	.000(b)	-.560(a)
Asymp. Sig. (2-tailed)	0.484	0.401	0.327	0.779	1.000	0.575

a*-Based on positive rank and.****b****-The sum of negative ranks equals the sum of positive ranks.*

DISCUSSION

Boiling of milk is a common practice in developing countries that not only increases the self-life of milk but also increases the flavour of the milk. Previous study has reported that there is a marked reduction of total bacterial count in milk when raw milk was subjected to 15s of boiling (Metwally *et al.,* 2011). The results of the present study serve to indicate the continued good condition of boiled milk sampled. Mean value of all physio-chemical and microbiological parameters revealed that during the summer the coliform counts and during post-monsoon season the total plate count was lowest, average initial temperature of the sampled milk was lowest during the summer is due to habit of the people, that is; keeping milk in low temperature immediately after the boiling.

In India, Bhopal Sahakari Dugdha Sangh (BSDS) has set up the standard plate count for pasteurized milk and below 35,000/ml is permissible. For coliform count, there should be no coliform 1:10 dilution in pasteurized milk based on lactose fermentation test; acidity of milk varies between 0.10-0.17 percent as lactic acid. Fat percentage of double toned milk, standardized milk and full cream milk is 1.5%, 4.5% and 6.0% respectively. Solid-not-fat (SNF) percentage vary among double toned milk, standardized milk and full cream milk is 9.09%, 8.51% and 9.06% respectively. According to the PFA, total solids content of unadulterated cow milk should be around 13.7 % while that for buffalo milk should be 17.2 %. Present study was reported higher Fat percentage, high MBRT (min.), SNF and CLR value indicative of good quality of milk probably due to boiling of milk after receiving from any sources.

During the present study 20%, samples were failed to achieve the standard norms. *Bacillus spp.* were found dominants (38%) in all the sample collected, that is probably because these spore formers require a temperature above 135°c to be completely eliminated from the milk and this temperature range could hardly be achieved in non industrial boiling procedures, therefore; some *Bacillus* spores survived in milk even after heat treatment (Janstova and Lukasova, 2001).

Based on standards of UHT milk, the coliform bacteria count in each ml. of it must not be above 10 (based on the US regulations) and *Escherichia coli* must be negative (Hillerton and Berry, 2004; Potter and Hotchkiss, 1995; Lues *et al.,* 2003). During present study, coliforms counts (30%) was recorded next to the *Bacillus spp.*, in which *Escherichia coli* accounted 47%, despite of the fact that no *Escherichia coli* were recovered from the sampled milk during the winter, probably due to the low ambient temperature.

According to Patil *et al.*, the satisfactory level for *Staphylococcus aureus* is 10/gm., *Salmonella* is nil/25gm., *Escherichia coli* is nil/gm., *Listeria monocytogenes* is nil/gm., coliform should be less than

10/gm., and total plate count is 5×10^4/gm., and for UHT milk total plate count should be nil (Patil *et al.*, 2008). Presence of *Listeria monocytogenes* was not tested during this study period, however; *Pseudomanas sp.* (13%), *Salmonella spp* (8%), *Shigella sp.* (3%), *Styphylococcus/Micrococcus spp.*(6%) and spp. of *Vibrio*(2%) were reported during the present works indicative of presence unhygienic practices both in sellers and consumers.

Table 4: Correlation coefficient: physio-chemical and microbiological variables

Variables		Initial Temperature (ºC)	MBRT (min.)	Acidity	FAT%	CLR	SNF	Coliform / ml	Total Count /ml
Initial Temperature (ºC)	**Sig. (2-tailed)**	.	0.379	0.305	0.828	0.434	0.503	0.462	0.328
	D		NS	NS	NS	NS	NS	NS	NS
MBRT (min.)	**Sig. (2-tailed)**	0.379	.	0.011	0.008	0.948	0.028	0.505	0.000
	D	NS	.	S	S	NS	S	NS	S
Acidity	**Sig. (2-tailed)**	0.305	0.011	.	0.944	0.344	0.522	0.272	0.021
	D	NS	S	.	NS	NS	NS	NS	S
FAT%	**Sig. (2-tailed)**	0.828	0.008	0.944	.	0.599	0.000	0.885	0.011
	D	NS	S	NS	.	NS	S	NS	S
CLR	**Sig. (2-tailed)**	0.434	0.948	0.344	0.599	.	0.009	0.971	0.782
	D	NS	NS	NS	NS	.	S	NS	NS
SNF	**Sig. (2-tailed)**	0.503	0.028	0.522	0.000	0.009	.	0.919	0.052
	D	NS	S	NS	S	S	.	NS	NS
Coliform /ml	**Sig. (2-tailed)**	0.462	0.505	0.272	0.885	0.971	0.919	.	0.467
	D	NS	NS	NS	NS	NS	NS	.	NS
Total count/ ml	**Sig. (2-tailed)**	0.328	0.000	0.021	0.011	0.782	0.052	0.467	.
	D	NS	S	S	S	NS	NS	NS	.

D., decision (α=0.05); S., significant; NS., Non-significant.

In conclusion, boiling of milk for periods more than 2min on direct flame prevents consumer from economic and health loss by removing unwanted micro organism and increasing self-life of milk subjected to further maintenance of cold chain. Presence of some pathogenic micro organism in sampled milk is a major threat to the consumer as milk by itself act as enriched medium which is able to promote the growth of potentially pathogenic micro organism in case of mishandling, however; till date no outbreak of disease were recorded out of milk borne pathogens at study area.

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