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Keeping quality of pasteurized milk at different temperature

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ABSTRACT

The experiment was undertaken to study the keeping quality of pasteurized milk at different temperatures (4°C, 7°C and 15°C. One hundred eight pasteurized milk samples were obtained from three different renowned milk company, out of which thirty six samples were collected from each of the three different renowned milk company as replication. All the samples were subjected to physical and microbiological examination. Total viable count and coliform count were done for the microbiological quality at different holding periods at day 0, 2, 5, 6, 8 and day 10 storage at 4°C, 7°C and 15°C. The results revealed that the average highest total viable count in the pasteurized milk was log 8.8325 at 15°C for 10 day storage and lowest value was log 4.0828 at 4°C for 0 day. Average highest coliform was log 2.8808 at 15°C for day 10 storage and lowest value was 0 at after 2nd days of storage at 4°C. It was concluded that pasteurized milk should be held at 4°C to achieve maximum shelf life. Further quality and shelf life of pasteurized milk depends on the raw milk quality, hygienic production of milk and improvement of storage facilities.

Introduction

Pasteurization derives its name from the eminent French scientist, Louis Pasteur, who during his studies in the year 1860 to 1870 found that heating certain liquids, especially wines, to a high temperature improved their keeping qualities. This process is widely employed in all branches of the dairy industry. Pasteurization came into use on a commercial scale in dairy establishments shortly after 1880 in Germany and Denmark.

Postpasteurization of bacterial contamination provides a serious obstacle to maintaining and extending fluid milk product shelf life. Two major sources contribute to postpasteurization contamination: equipment milk residues and aerosols. Ineffective cleaning procedures of the interior of processing equipment create milk residues which can allow bacteria to multiply and contaminate subsequent milk flow. Filler nozzles, carton-forming mandrels, and pasteurizers have all been pinpointed as sources of postpasteurization contamination (Gruetzmacher and Bradley, 1999). Bacterial biofilms, which are difficult to remove the clean-in-place (CIP) procedures, can also form within processing equipment and provide a constant source of contamination for both raw and pasteurized milk (Austin and Bergeron, 1995).

Unenclosed milk contact surfaces provide a route for microbial aerosols to contaminate pasteurized milk (Kang and Frank. 1989). During cleaning or operation, airborne yeasts, molds, bacteria, and spores can land on a milk contact surface and thus enter the milk flow. An unenclosed filling unit (e.g., a federal-style filler) can allow exposure of the pasteurized milk to airborne bacteria, which can result in levels of postpasteurization contamination higher than those of milk packaged in a self-enclosed system (Doughlas et al., 2000).

Most microorganisms present in raw milk are destroyed by exposure to time and temperature combinations currently used in milk pasteurization. Minimizing the time between production and pasteurization and maintaining low storage temperatures will help control enzymatic degradation of raw milk through growth of heat-sensitive organisms. However, some spores and thermoduric organisms can survive pasteurization and affect the quality of fluid milk and other processed dairy products. Thermoduric organisms, such as some species of *Streptococcus* and *Lactobacillus*, and spore-forming rganisms, such as *Bacillus*, can multiply within pasteurized milk products resulting in off-flavors and protein and lipid degradation. Psychrotrophic spore formers present particularly difficult challenge, as they can survive pasteurization, germinate, and multiply in refrigerated conditions, under which milk is stored (Boor et al., 1998; Douglas, 2000).

Pasteurization typically uses temperatures below boiling since at temperatures above the boiling point for milk casein micelles will irreversibly aggregate (or "curdle"). There are two main types of pasteurization used today: high temperature/short time (HTST) and ultra-high temperature (UHT, Also known as Ultra-heat treated). In the HTST process, milk is forced between metal plates or through pipes heated on the outside by hot water, and is heated to 71.7°C (161°F) for 15-20 seconds. UHT processing holds the milk at a temperature of 138°C (250) for a fraction of a second. Milk simply labeled "pasteurized" is usually treated with the HTST method, whereas milk labeled "ultra-pasteurized" or simply "UHT" must be treated with the UHT method. (Rosenau, M.J., 1913).

It is however unfortunate that in Bangladesh, the practice of pasteurization is very recent methods of production, handling, distribution and storage of milk, takes place under much disorganized conditions. The importance of hygienic quality milk is not given due importance. Supply of milk from widely scattered sources, non-availability of cooling of milk before and during transportation, careless handling and distribution under most unhygienic condition and willful adulteration by unscrupulous milkman with polluted water from ponds, ditches, rivers, unwholesome milk powder and treacle to add flavour are all in sanitary conditions under which milk is produced, handled and marketed. In addition the transportation and distribution of market milk obtained from villagers to urban market centers of towns and cities and to domestic homesteads present enormous problems.

The fresh milk processing trade in Bangladesh has grown from practically nothing since independence in 1971 to very limited small scale dairy plants producing about 30,000 metric tons (approximately) of pasteurized milk annually at the present time. The governmental agency has established one semiautomatic plant at Savar in Dhaka and few other private enterprises and cooperative union have taken interest to build processing plants e.g. Milk Vita, Arong` Milk, Tulip, Aftab Dairy etc. These plants are not essentially producers of fresh milk; rather they receive unprocessed raw milk from different dairy farms and remote areas of villages. The raw milk is just pasteurized in plants, packaged and distributed to retail stores through ambulatory outlets. The standard and regulations governing microbiological quality, public health safety and hygienic condition and labelling of the products are not practically enforced.

At present, keeping this angle in view, government dairy farm, extensions of dairy farming in private sector are playing an important role to the lives of people and also contribute a lot on the agricultural economy of the country. In all developed countries the establishment of dairy industry improves the health of people and economic well being of the nation. But in Bangladesh, the dairy establishments still have little satisfactory food control service to ensure safety food supplies and protection of the health of people. Most of the private farms collect milk form villagers and some small dairy farmers of different areas of Bangladesh. The collected milk pasteurized in plants are packed, cooled and later distributed to the retail stores. The consumers obtain the pasteurized milk from retail storage. In the retail store the pasteurized milk is kept in refrigerator storage. Although the microbial quality of pasteurized milk is maintained limitedly before supply to the retail store but the pasteurization plant establishment authorities, having no accountability for direct microbiological quality measure or storage conditions at different refrigeration temperature and shelf-life of heat processed milk as it leaves the plant, the effective control of food quality is not ensured.

In view of above fact and its relationship to the public health safety assessment of sanitary quality of pasteurized milk, it is of prime concern to find out whether the sanitary, safety and organoleptic qualities of the products stored at different refrigeration temperatures meet the recommended microbiological limit that would assure that the milk food product safe, wholesome and of good quality. Keeping the end in view, the research work was undertaken with the aim to determine the keeping quality of pasteurized milk at different temperature by observing the pattern of microbial growth in pasteurized milk during different storage period and temperature.

MATERIALS AND METHODS

Collection and processing of samples

Pasteurized milk from three companies (Arong, Milk vita and Aftab) from KR market, Bangladesh Agricultural University, Mymensigh, were collected. The packaged samples were transferred to a wide mouthed thermo flask, kept cool in ice and brought to the bacteriology Laboratory, Department of Microbiology and Hygiene of the said university. All samples were brought in about 30 minutes to one hour for subsequent studies to determine the hygienic quality of milk before delivery to consumers. All the samples (108) were opened aseptically and processed for physical test (color, flavor, consistency, taste, COB tests) and microbiological tests (Total Viable Count, Coliform Count).

Physical Tests (Sensory Evaluation)

The milk samples of three farms were judged by a panel of experts at different holding periods to evaluate organoleptic quality in which color, flavor, taste, consistency characters and COB tests were determined. A legend of taste panel scores was taken as a standard to evaluate the organoleptic quality of samples. For example 5 marks were given for excellent quality of milk, 4 marks were given for good quality, 3 marks were given for moderately good, 2 marks for fair quality and 1 mark for bad quality and 0 for spoiled. The physical change of milk is COB. If milk clotted on boiling it was indicated as (+)ve and if not clotted it was indicated as (-)ve test. The microbiological tests including total viable count and coliform count were performed as per recommendation of APHA (1960).

Data analysis

The data on the total viable counts and coliform count per ml of milk and taste panel scores of different farms for different holding periods were analysed statistically to find out the level of significant correlationship. The analyses of variance were studied by F test. The mean differences were evaluated at 0.1% level of significance by Duncan's New Multiple Range Test (DMRT). Linear correlation co-efficient and regression equation were calculated with the standard statistical method.

RESULTS AND DISCUSSION

Total viable count

Results reveal that there were wide variations in average total viable count for milk at different temperature. Average highest total bacteria count/g was found $2.84x10^4$ (log 4.4533), 3.8 X 10^4 (log 4.5795) and $6,8x10^8$ (log 8.8325) for milk samples at 10 days of storage, while that was lowest 1.21 X 10^4 (log 4.0828), 1.24 X 10^4 (log 4.093) and 1.25x 10^4 (log 4.0969) at 0 days of storage at 4°C, 7°C and 15°C respectively (Table 1 and 5). There were significant differences (P<0.001) among those milk samples for total viable count at different temperature.

Ashenafi and Beyene (1994) reported similar finding, where the microbial load, microflora and keeping quality of raw and pasteurized milk from a dairy farm. The initial total count of pasteurized milk was 1.5 X $10^4$and this increased by 2 log units in 12 h. when stored at room temperature.

Table 1. Total viable count at 4°C, 7°C and 15° C temperatures.

Days	4°C		7°C		15° C	
	Average	Log average	Average	Log average	Average	Log average
0	121	4.0828± 0.0305	124	4.093± 0.027	125	4.0969± 0.0542
2	137	4.1367 ± 0.0425	161	4.2065± 0.0148	640	4.8062± 0.048
4	163	4.2122 ± 0.04536	241	4.382± 0.0233	2370	5.3747± 0.0156
6	203	4.3075± 0.02722	316	4.4995± 0.019	16300	6.2122± 0.0491
8	243	4.3856 ± 0.03797	337	4.527± 0.0141	580000	7.7634± 0.0758
10	284	4.4533± 0.02595	380	4.5795± 0.0304	6800000	8.8325± 0.0817
LSD		**		**		**

LSD= Level of significant difference
** = Significant at 0.1% level (P<0.001)

Table 2. Coliform count at 4°C, 7°C and 15° C temperatures.

Days	4°C		7°C		15° C	
	Average	Log average	Average	Log average	Average	Log average
0	107	2.0394± 0.075	110	2.0398± 0.0506	115	2.0607± 0.0499
2	44	1.6435± 0.2374	178	2.2498± 0.0279	250	2.3979± 0.0435
4	0	0	251	2.3995± 0.0148	370	2.5682± 0.1245
6	0	0	293	2.4666± 0.021	510	2.7076± 0.0646
8	0	0	359	2.555± 0.007	650	2.8129± 0.0426
10	0	0	405	2.6074± 0.016	760	2.8808± 0.0327
LSD		**		**		**

LSD= Level of significant difference
** = Significant at 0.1% level (P<0.001)

Coliform count

Results demonstrate that there were wide variations in average coliform counts per gram of samples for milk at different days and at different temperature. There were significant differences (P<0.001) among those milk samples for coliform count at different temperature. Average highest 107 (log2.0607) coliform count/g was found for milk samples at 0 days of storage, while that was lowest 0 at after 2nd days of storage at 4°C. Average highest 405 (log2.6074) and 760 (log2.8808) coliform count/g was found for milk samples at 10 days of storage, while that was lowest 110 (log2.0398) and at 0 days of storage at 7 °c and 15° c respectively (Table 2 and 6).

Coliforms possessed the ability to grow in pasteurized milk kept at low temperature. Inoculating into deoxycholate agar incubated at 4°C resulted into isolation of 120 coliform strains from pasteurized milk. (Barbaro et al., 1983). Exponential bacterial growth began after a lag of at least 3 days at 0-4°C. (Tirard-Collet et al., 1991).

The result of microbiological status of pasteurized milk are supported by the findings of Siva et al, (1993) where it was found that plate count of 10 samples of pasteurized milk varied from 10,000 to 62,000 with an average of 3.7 + 0.54 x 10,000 CFU/ml. The Coliform count in 10 sample varied from <10 to 80 with an average of 22 + 8.13 CFU/ml. The authors further confirmed that the

Coliform counts were significantly correlated with total plate count in pasteurized milk (r=0.754)

It was observed that day by day increase of TVC and TCC was similar to Jung et al, (2001) where the bacterial counts for the LTLT and HTST pasteurized milk samples were about $4.0x10^3$ and $1.5x10^1$ CFU/ml at the production day, respectively. The bacterial counts rapidly increased to $>10^7$ CFU/ml at room temperature (12-30° C) for 3) days and were kept $<2×10^4$ at refrigerator temperature (10° C) for 7 days of storage.

Physical and organoleptic changes

Clot on boiling (COB)

If milk is clotted on boiling (COB), it was indicated as +ve and if milk not clotted it was indicated as –ve test. In case of COB, all samples were found negative at 0 days and 2nd days of holding period. At 4th days, 4°C and 7° C temperature found negative but 15° C both negative and positive results were found. At 6 day of storage in 4°C and 7° C temp found negative, in case of 15° C all are positive. At 8th day 4° C temperature all positive. 7° C both positive and negative and 15° C all positive were found. At day 10th 4°C both positive and negative but at 7°C and 15° C all positive were found (Table 3).

Color

The color of all milk samples at 0 day holding was freshly normal and attractive (golden yellowish white). 5 marks were given for normal color of milk, 4 marks were given in case of white color, 3 marks were given for bluish white color and so on. Total number of 6 samples thus 30 marks maximum. In of 4°C temperature, at day`0 score was 30 marks and at day 2, 4, 6. 8 and 10 the score were 30, 29, 28, 24 and 19 respectively. In case of 7° C temperature. 0 day obtain 30 marks at 2nd day 29, at 4th day 29, at 6th day 23, at 8th day 21 and 10th day 15, scored respectively. Lastly, in case of 15° C temperature, 0 day obtain 30 marks, at 2nd day 27, at 4th day 20, at 6th day 16, at 8th day 14 and 10th day 12 scored respectively (Table 4).

Flavor

The Flavor was scored on the basis the sweet aroma of milk 5 marks were given for flavored milk, 4 marks for good, 3 marks were given for moderately good, 2 marks were given for fair 1 marks were given for bad and 0 marks were given for spoiled. Total number of 6 samples thus 30 marks maximum. At 0 day 4° C temperature obtain 30 marks, at 2nd day 30, at 4th day 29, at 6th day 27, at 8th day 23 and 10th day 17 scored respectively In case of 7° C temperature, 0 day obtain 30 marks, at 2nd day 28, at 4th day 25, at 6th 18, at 8th day 10 and day 6 scored respectively. Lastly, in case of 15° C temperature, 0 day obtain 30 marks, at 2nd day 25, at 4th day 15, at 6th day 8, at 8th day 4 and 10th day 1 scored respectively. (Table 4).

Table 3. COB test for pasteurized milk at different temperature.

Temperature Days	4°C	7°C	15°C
0	-	-	-
2	-	-	-
4	-	-	±
6	-	-	+
8	-	±	+
10	±	+	+

- = Negative, no clotting.
+ = Positive

Table 4. Changes in physical properties of pasteurized milk at different temperature.

Physical tests	Days Temperature	0	2	4	6	8	10
Color change	4°C	30	30	29	28	24	19
	7°C	30	29	29	23	21	15
	15°C	30	27	20	16	14	12
Flavor change	4°C	30	30	29	27	23	17
	7°C	30	28	25	18	10	6
	15°C	30	25	15	8	4	1
Consistency change	4°C	30	30	28	26	21	16
	7°C	30	28	25	18	10	6
	15°C	30	25	15	8	4	1
Taste change	4°C	30	30	30	27	25	18
	7°C	30	26	24	20	14	10
	15°C	30	24	16	10	3	1

5=Excellent; 4=Good; 3= Moderate good ; 2= Fair ; 1= Bad; 0= Spoiled

Consistency

Total number of 6 samples thus 30 marks maximum. At 0 day 4° C temperature obtain 30 marks, at 2nd day 30, at 4th day 28, at 6th day 26, at 8th day 21 and 10th day 16 scored respectively. In case of 7° C temperature, 0 day obtain 30 marks, at 2nd day 27, at 4th day 25, at 6th day 19, at 8th day 12 and 10th day 7 scored respectively. Lastly, in case of 15° C temperature, 0 day obtain 30 marks, at 2nd day 26, at 4th day 17, at 6th day 11, at 8th day 5 and 10th day 3 scored respectively (Table 4).

Taste

Total number of 6 samples thus 30 marks maximum. At 0 day 4° C temperature obtain 30 marks, at 2nd day 30, at 4th day 30, at 6th day 27, at

8th day 25 and 10th day 18 scored respectively. In case of 7° C temperature, 0 day obtain 30 marks, at 2nd day 26, at 4th day 24, at 6th day 20, at 8th day 14 and 10th day 10 scored respectively. Lastly, in case of 15° C temperature, 0 day obtain 30 marks, at 2nd day 24, at 4th day 16, at 6th day 10, at 8th day 3 and 10th day 1 scored respectively (Table 4).

Table 5. Comparison of Total viable count (Log average) at 4°, 7° and 15°C temperature

Tem	Micro. Count	Days of storage					
		0th	2nd	4th	6th	8th	10th
4°C	TVC	4.0828	4.1367	4.2122	4.3075	4.3856	4.4533
7°C	TVC	4.093	4.2065	4.382	4.4995	4.527	4.5795
15°C	TVC	4.0969	4.8062	5.3747	6.2122	7.7634	8.8325
LSD		NS	**	**	**	**	**

NS= Not significant; **= Significant at 0.1% level (P<0.001); LSD= Level of significant different

Table 6. Comparison of Total coliform count (Log average) at 4°, 7° and 15°C temperature.

Temp.	Micro. Count	Days of storage					
		0th	2nd	4th	6th	8th	10th
4°C	TCC	2.0394	1.6435	0	0	0	0
7°C	TCC	2.0398	2.2498	2.3995	2.4666	2.555	2.6074
15°C	TCC	2.0607	2.3979	2.5682	2.7076	2.8129	2.8808
LSD		NS	****	**	**	**	**

NS= Not significant; **= Significant at 0.1% level (P<0.001); LSD= Level of significant different

The investigation on the assessment of keeping quality of pasteurized milk at different temperature demonstrated that pasteurized milk at 4°C was acceptable upto 10 days but better upto 8 days of storage, at 7°C upto 8 days of storage and acceptable upto 4 days at 15°C storage temperature.

Grade A pasteurized milk and their products should never contain over 10 coliforms/ml and for certified pasteurized milk it should not contain over 1 coliform/ml (James, 1978). The result also revealed storage life of pasteurized milk which is also similar to the finding of Plaksanguansri, (1991) the storage life of processed and packaged milk from good quality raw milk was found to be >21 days. The storage life reduced to 4 days due to contamination after pasteurization by a rapid increase in the number of microorganisms. Deterioration of pasteurized milk was dependent on the process of pasteurization, not on seasonal changes.

However, the best keeping quality resulted from milk pasteurized on the third and fourth days; even milk pasteurized on the seventh and ninth days had superior keeping quality to that pasteurized on the first day. (Ravanis and Lewis, 1995). The keeping quality of pasteurization milk was found to be wholly determined by those thermoduric bacteria capable of multiplying and affecting the milk quality during storage.

The post-pasteurization contamination level was the most critical factor determining keeping quality at 6 or 10°C. The results also showed that the sample volume and the samples storage container influence the plate count of cold-stored samples. (Stepaniak and Abrahamsen, 1995). The organoleptical quality and acidity of milk samples gradually changed in proportion to bacterial counts. However, the specific gravity did not significantly change after 30 days. No significant correlation was found in the keeping quality of milk between dark and light exposure at room temperature for 30 days. The compositions of fat, solids not fat, protein, and lactose in milk did not significantly change according to the storage conditions for 30 days. (Jung et al, 2001). The flavour was affected mainly by acid development and proteolytic activity. The thermal stability test was found to be a possible alternative to organoleptic evaluation for determining the shelf life of pasteurized milk (Reinheimer et al. 1993).

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