NUTRITIONAL AND MICROBIOLOGICAL QUALITY ASSESSMENT OF PASTEURIZED MILK IN AZH AGRO INDUSTRIES PVT. LTD., GAZIPUR, BANGLADESH

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Abstract: Milk is one of the most valuable and regularly consumed natural food which is rich in nutritional value. A study was carried out with raw liquid milk collected from Shahzadpur and pasteurized, packed in AHZ Agro Industries Pvt. Ltd, Gazipur, Bangladesh. One hundred and forty five pasteurized milk samples collected from August, 2011 to February 2012 were analyzed for the nutritional and chemical composition such as acidity, fat, solid non fat, protein, lactose, specific gravity; and microbiological characteristics. Platform tests (alcohol and clot-on-boiling) were found negative. From the study it was found that the nutrient composition of the pasteurized milk samples were within the standard limits with average specific gravity of milk was 1.029, fat 3.575%, protein 3.43% and lactose 5.02 %. Acidity% refers the quality of milk. The 7 months average highest acidity was 0.15% and the lowest acidity was 0.146%. Protein percentage was found to be 3.36% in August (Rainy season), 3.45% in winter and 3.42% in spring. All the samples had solid non fat (SNF) with standard limits of 8.17 to 8.38 percent. It was found that total bacterial count in milk varies with seasons. In rainy season, autumn season and winter the average total viable count was 1715, 1744.3 and 1481.6 cfu/ml respectively. From 145 pasteurized milk samples, 121 samples showed excellent quality and 24 samples showed good quality in methylene blue reductase test (MBRT). No coliform and Salmonella/Shigella were observed. SNF and protein percentage varied with season, been highest in winter while less in spring. The overall result found was ensuring good quality of pasteurized milk. All the nutritional composition and microbiological count complies with the BSTI standard.

Keywords: Pasteurized Milk, Nutritional composition, Chemical tests, Total Bacterial Count, Coliform, Methylene Blue Reductase Test, Seasonal Effect.

Introduction

Milk is one of the most important food of human beings. It is defined as the whole, fresh, clean, lacteal secretion obtained by complete milking of one or more healthy animals. The main animal sources of milk are cows, buffaloes, goat and sheep. Milk proteins supply those amino acids that help in the growth of adults and infants[1]. Cow milk contains 85.5-89.5% water, 2.5-6% fat, 2.9-5% protein, 3.6-5.5% lactose, 10.5-14.5% total solids and 0.6-0.9% minerals[2]. The constituents may vary with breed, types of food given, stage of lactation, season and age of the cow[3]. Quality of milk in the aspects of microbiology depends on the presence of coliform and other pathogenic bacteria. Milk

from healthy cows contains relatively few bacteria 10^2-10^3cfu per ml and the health risk from drinking raw milk would be minimal[4]. The safety of dairy products with respect to food born diseases is of great concern around the world. In developing countries milk and other milk products produces in unsanitary conditions and poor production practices[5]. Quality milk refers to harmful toxic substances and sediment free and should contain good flavor with normal composition[6]. Bangladesh is a tropical country with high humidity and heavy rainfall and hence this environment is favorable for the growth and multiplication of bacteria, virus, fungus and parasites[7]. In Bangladesh, milk is produced mostly non-organized way and usually it is supplied to the consumers from the urban and rural areas by milkmen. Contamination may occur from different sources such as udder and body of cows, dust from the air, litter, floor, flies, insects and rodents, water supply, hands and clothes of the milker, utensils, bottles and atmosphere etc.[8]. The presence of *E. coli* is the indicator of faecal contamination as well as it indicates the presence of toxigenic or enteropathogenic bacteria which are major public health hazard. Salmonellosis which is a disease of cattle and calves is caused by different species of *Salmonella. Shigella* species is a major cause of food borne gastrointestinal illness. It is mostly found in the intestinal tract of humans and other primates[9]. *Shigella* usually transmitted from human to human through poor hygiene, although food and water have been associated with outbreaks of shigellosis[10]. Pasteurization remains an essential stage impossible to reduce the microbiological risk of food and to prolong the preservability[11]. This is a thermal process widely used in the food and dairy industry with the objective of minimizing health hazards from pathogenic microorganisms and to prolong product shelf life. Pasteurization does not impair the nutritional quality of milk fat, calcium and phosphorus. It does not affect fat soluble vitamins (A, D and E), as well as the B complex vitamins such as riboflavin, pantothenic acid, biotin and niacin[4]. For Bangladesh the Standard Testing Institute (BSTI) given requirements for pasteurized milk[12]are Fat, percent by mass, min 3.5, Solid Non Fat (SNF) percent by mass, min 8.0, Density g per ml 1028-1032, Acidity as Lactic acid 0.15% max, Lactose percent by mass, min 4.4, Protein percent by mass, min 3.3, Total Bacterial Count per ml, max 20,000 cfu, Coliform Count per ml, max ,<10 cfu[7].

Due to spread consumption by infants, children and adults, milk has been marked as the most important food for which chemical and microbiological quality control should be attained[13]. There is no such findings regarding in this field that demonstrate any exact picture of the parameters to confirm the quality of milk. This study was carried out to investigate the Total Bacterial Count, Total Coliform Count, Total *Salmonella-Shigella* Count; To do analysis of the Chemical and Microbiological quality of the pasteurized milk and also to study the Nutritional and Microbiological properties temporal seasonal variations in the pasteurized milk.

Materials and Method

Study Population and Samples : This study was conducted at Microbiological and Chemical Laboratory under Quality Control Department in AHZ Agro Industries Pvt. Ltd., Hotapara, Gazipur with 145 samples during August 2011 to February 2012. This is a Food and Beverage industry which processes full cream pasteurized milk.

Physicochemical Tests

Alcohol test and Clot on Boiling (COB) test : 5 (five) ml of milk and 5 ml of 68% alcohol (ethanol) were placed in a test tube. The test tube was inverted several times with the thumb held tightly over the open end of the tube. Then the tube was examined for formulation of curd particles[5]. COB test is performed by heating 5 (five) ml of milk in a test tube over a flame for four minutes. Heating will precipitate the proteins in the milk if it is sour. So milk what clots should be rejected. If no coagulation occurs the milk is good in primary testing[14].

Chemical and Nutritional Tests by Lactoscan

Fat, Protein, SNF and Lactose test : The milk constituents (fat, protein, lactose, and SNF) of the milk samples were determined by milk analyzer, Lactoscan. Milk samples were mixed gently 4-5 times to avoid any air enclosure in the milk. Then 25 ml samples were taken in the sample-tube and put in the sample- holder one at a time with the analyzer in the recess position. Then when the starting button activated, the analyzer sucks the milk, makes the measurements, returns the milk in the sample-tube and the digital indicator (LED display) shows the specified results[14].

Chemical and Nutritional Tests by Manual Methods

Titratable acidity test : 10 (ten) ml of milk was pipetted into a beaker and then 3-5 drops of 0.5% phenolphthalein indicator was added into the milk. Then the sample was titrated with 0.1 N NaOH solution until definite pink color persists. Percent lactic acid is then calculated[5].

Specific Gravity by Pycnometer : Specific gravity of milk was determined by using pycnometer. The density of milk was measured against the density of standard (water). Firstly pre weighed pycnometer was filled with standard reference liquid (water) to some predetermined level at 20^0C and weight was noted. Then milk sample was filled in a similar pycnometer to similar level and temperature and weighed. Specific gravity of milk was calculated by the following formula: Specific gravity: Weight of milk sample/ Weight of distilled water[15].

Microbiological Tests

Total Plate Count : The total bacterial count was made by adding 1 ml of milk sample into sterile test tube having 9 ml distilled water. After thoroughly mixing, the sample was serially diluted up to 1:10. Then 1 ml sample taken in a sterile Petri dish and 15-20 ml Plate Count Agar media were poured. After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 37^0C for 24 hours. Finally colony counts were made using colony counter[5].

Coliform Count : The coliform count was made by adding 1 ml of milk sample into sterile test tube having 9 ml distilled water. After thoroughly mixing, the sample was serially diluted up to 1:10. Then 1 ml sample taken in a sterile Petri dish and 15-20 ml

Mac Conkey Agar media were poured. After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 37⁰C for 24 hours. Finally colony counts were made using colony counter[5].

Salmonella and Shigella Count : The *Salmonella* and *Shigella* count was made by adding 1 ml of milk sample into sterile test tube having 9 ml distilled water. After thoroughly mixing, the sample was serially diluted up to 1:10. Then 1 ml sample taken in a sterile Petri dish and 15-20 ml SS Agar (SS: Oxoid, UK) media were poured. After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 37⁰C for 24 hours. Finally colony counts were made using colony counter[16].

Methylene Blue Reductase Test (MBRT) : MBRT is probably the most extensively used bacteriological test for milk. 10 ml milk was taken in a test tube and 1 ml methylene blue dye was added. The tube was slowly inverted twice for mixing so that the whole column of contained air rises above the level of the milk. Then the tube placed in a water bath at 37⁰C temperature[17].

Results

In this study 145 pasteurized milk sample individuals were tested for Microbiological qualitative analysis and Nutritional status of rural area of Shahzadpur, Bangladesh. The studies carried out for 7 months (August 2011 to February 2012). The Alcohol test and Clot on Boiling (COB) test is the most important test. These tests were performed with the 145 pasteurized milk samples immediately and the results of these tests refer the good quality of the milk.

Physicochemical and Microbiological tests of 7 months

The Average Nutritional and Microbiological data of pasteurized milk of 7 months were summarized (Table 1).

Table 1: Average Microbiological and Nutritional Analysis Result of 7 Months of Pasteurized Milk.

Month	Chemical & Nutritional Analysis						Microbio logical Analysis
	Specific Gravity	Fat %	SNF* %	Acidity (%)	Lactose%	Protein%	TPC* Cfu/ml
August, 2011	1.028	3.72	8.26	0.148	4.18	3.36	1715
September, 2011	1.029	3.51	8.17	0.149	4.31	3.40	1889
October, 2011	1.028	3.68	8.17	0.148	4.25	3.47	1722
November, 2011	1.029	3.53	8.27	0.150	4.31	3.40	1622
December, 2011	1.029	3.51	8.32	0.149	4.47	3.47	1488
January, 2012	1.030	3.52	8.32	0.150	4.64	3.41	1457
February, 2012	1.030	3.56	8.38	0.146	4.58	3.47	1500

SNF: Solid Non Fat, TPC: Total Plate Count.

Physicochemical Tests

Average Specific Gravity: Average specific gravity of 7 months ranged from 1.028 to 1.030. It increases with season. In winter the Specific Gravity found higher than spring and rainy season.

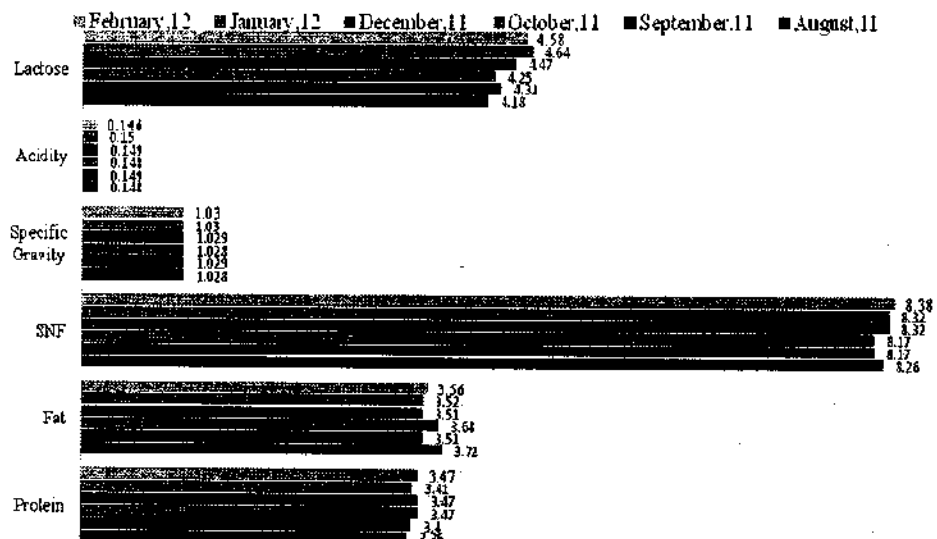**Average Fat%:** The average fat content of the 7 months ranged from 3.51% to 3.72%.

Average Protein%: Protein content in 7 months. In August the protein% is 3.36 but gradually it increases and ranged from 3.40 to 3.47%. In Spring and Winter season protein% found higher than Rainy season.

Average SNF%: In Rainy season (August) the SNF% is 8.26%. In Spring season (September-November) the SNF % were 8.17%, 8.17% and 8.27%. The highest SNF% found in winter season (December-February). The SNF % in winter season was 8.32%, 8.32% and 8.38%.

Average Acidity% (as lactic acid): The average Acidity% of 7 months ranged from 0.146 to 0.150%. As per BSTI standard acidity 0.15% (max) and it is found that the acidity% was in range.

Average Lactose%: Lactose% ranged from 4.18 to 4.59%. It was less in Rainy season but increases in Spring and Winter. The average Lactose% of 7 months was 4.59%.

Nutritional & Chemical Analysis of Pasteurized Milk of 7 months

Figure 1: Nutritional and Chemical Analysis of Pasteurized Milk from August11-February12

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Physicochemical Tests

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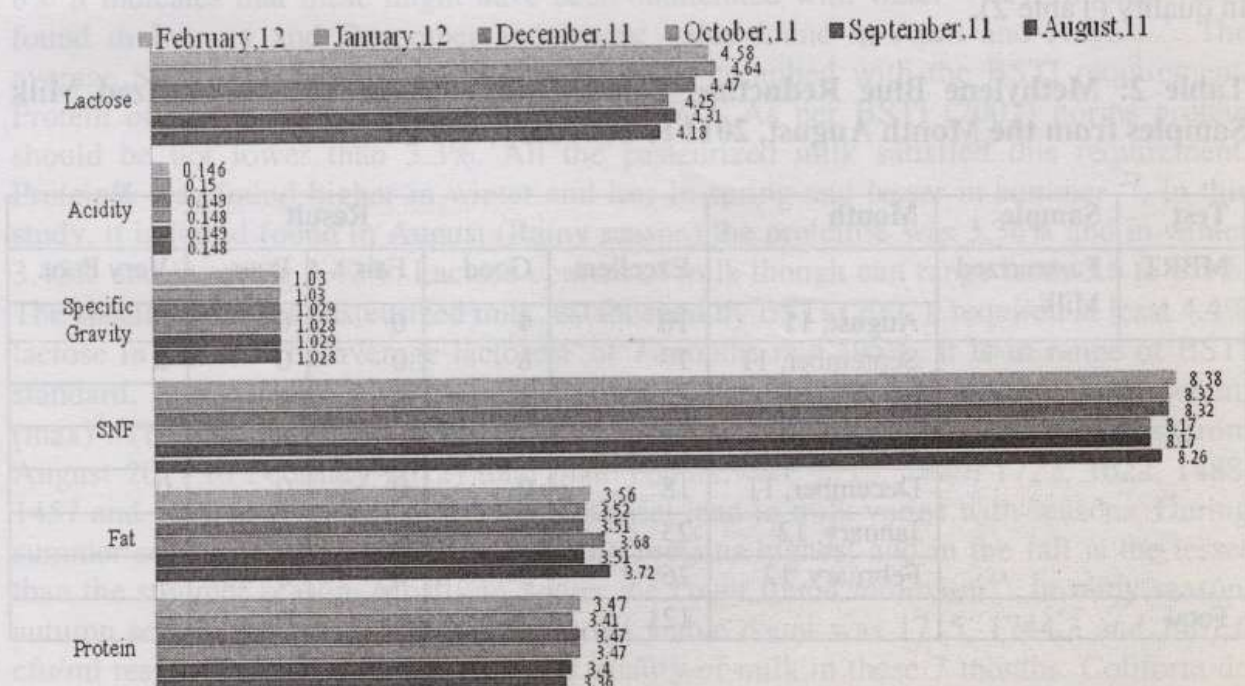### Nutritional & Chemical Analysis of Pasteurized Milk of 7 months

Figure 1: Nutritional and Chemical Analysis of Pasteurized Milk from August11-February12

Microbiological Tests

Average Total Plate Count (TPC): In Rainy season (August) the count was high, 1715 cfu/ml. In spring season (September-November) the counts were 1889, 1722 and1622 cfu/ml. In winter season (December-February) the counts were 1488, 1457 and 1500 cfu/ml. In winter season the count was less than spring (Figure 2).
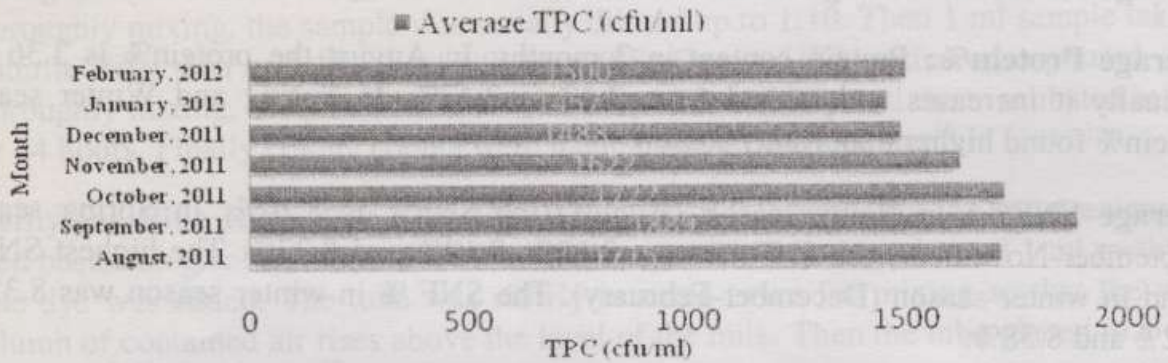

Figure 2 : Average Total Plate Count (cfu/ml) of 7 months.

Average Coliform Count: In 145 samples of 7 months, no Coliform was found.

Average _Salmonella/Shigella_ Count: In 145 samples of 7 months, no _Salmonella_ or _Shigella_ was found.

Average Methylene Blue Reductase Test: There were 145 pasteurized milk samples was tested. 121 samples are found Excellent in quality and 24 samples were found Good in quality (Table 2).

Table 2: Methylene Blue Reductase Test (MBRT) of the 145 Pasteurized Milk Samples from the Month August, 2011 to February, 2012.

Test	Sample	Month	Result				
MBRT	Pasteurized Milk		Excellent	Good	Fair	Poor	Very Poor
		August, 11	10	4	0	0	0
		September, 11	7	8	0	0	0
		October, 11	23	5	0	0	0
		November, 11	14	4	0	0	0
		December, 11	18	0	0	0	0
		January, 12	23	1	0	0	0
		February, 12	26	2	0	0	0
Total			121	24	0	0	0

Discussion

Milk is one of the most common food sources in the human diet and is also a product that is directly available for consumption. 145 pasteurized milk samples were taken to determine the temporal seasonal effect on microbiological and nutritive quality analysis of the milk. The alcohol and colt on boiling tests are prime importance in milk to detect milk which has a tendency to curdle during processing, sterilization or pasteurization. Clotting of milk on the side of tube or at the bottom indicates poor keeping quality. For good quality of milk alcohol and COB test should be negative. The Acidity of the Pasteurized milk ranged from 0.144-0.162% where BSTI allows max 0.15% for the pasteurized milks. Titrable acidity is a measure of freshness and bacterial activity in milk. High quality milk has to have <0.14% acidity[8]. The 7 months average highest acidity of 145 samples was 0.15% and the lowest acidity was 0.146%. Normal average milk has a specific gravity of 1.027 to 1.035[17]. The average specific gravity of 7 months of 145 samples was 1.029. From the specific gravity it can be assured that no water has been added in these milks. It is found that in winter the Specific Gravity found higher than spring and rainy season. Commercially, the fat of milk is unquestionably the most valuable constituent of milk. The FDA (Food and Drug Administration) requires not less than 3.25% milk fat for fluid whole milk. The BSTI requirement for fat content of pasteurized milk is a minimum of 3.5%[18] which is fulfilled in 7 months average fat% and it was 3.575%. As per BSTI requirement (SNF) content should be 8.0% (min). The requirements are fulfilled in the whole study. The highest SNF was found in winter season and that is from December-February (8.32, 8.32 and 8.38%). In Spring SNF was found 8.17%. Generally SNF varied from 6.39 to 8.88%[20]. When the SNF% goes below 8% it indicates that these might have been adulterated with water[21]. SNF highest value found in January and December and lowest value found in April and August [1]. The average SNF of 7 months was 8.27% which is complied with the BSTI requirement. Protein of cow's milk ranged from 2.98-3.87%[18]. As per BSTI (2002) norms protein should be not lower than 3.3%. All the pasteurized milk satisfied this requirement. Protein% was found higher in winter and less in spring and lesser in summer [22]. In this study, it is found found in August (Rainy season) the protein% was 3.36% and in winter 3.45% and in spring 3.42%. Lactose content of milk though can range from 3.6 to 5.5%. The specification for pasteurized milk, established by BSTI (2002), required at least 4.4% lactose in milk[21]. The average lactose% of 7 months is 4.395%. It is in range of BSTI standard. The standard for total viable count of pasteurized milk is 20,000 cfu per ml (max)[8]. The total viable count was in range of BSTI (2002) standard. The 7 months (from August 2011 to February 2012) total plate counts were 1715, 1889, 1722, 1622, 1488, 1457 and 1500 cfu/ml. It is found that bacterial load in milk varies with seasons. During summer season the bacterial load generally remains highest and in the fall is the lesser than the summer season. Mostly in winter the count found minimum[23]. In rainy season, autumn season and winter the average total viable count was 1715, 1744.3 and 1481.6 cfu/ml respectively. It refers to the good quality of milk in these 7 months. Coliform do not survive in pasteurization. If their presence found in pasteurized milks it indicates recontamination after pasteurization[8]. In this study 145 pasteurized milk samples were tested and found no coliform. So it indicates good quality of milk and proper

pasteurization process. Methylene Blue Reductase Test (MBRT) is a marker to understand the quality of milk[24]. From 145 pasteurized milk samples 121 samples showed decolorization after 4.5-5 hours that holds excellent quality and 24 samples showed decolorization between 3-4 hours and holds good quality. Microbial standards for milk (FAO WHO, codex standard 2000) of Pasteurized milk for EU and ES stated that *Salmonella* should be Nil[19]. Mostly Pasteurized milk didn't contain *Salmonella* and *Shigella*[21]. In this study there were no *Salmonella* and *Shigella* found. So it proves that the sanitary and hygienic quality conditions of all those pasteurized milks are good for consumption.

Conclusion

We conclude in the present study that, the nutritional composition, chemical and microbiological quality of the pasteurized milk has passed the quality parameters. SNF% and Protein% also varies with season. SNF% and Protein% found highest in winter, less in Spring. Fat% was minimum 3.5%. The pasteurized milk contains a good range of Lactose%. No coliform indicates good sanitation practice. Methylene Blue Reductase Test results showed maximum samples were excellent in quality. Total Viable Count varies with season. Highest count observed in rainy and spring season. Mostly lower count found in winter season. *Salmonella* and *Shigella* found Nil that proves no post pasteurization process contamination and good hygiene practice.

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