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Studies on the Microbiological and Chemical Qualities of Milk

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A B S T R A C T

The milk samples (Raw and Boiled) were collected from various vendors of Hoshiarpur and Ludhiana district. Three different samples of Pasteurized milk samples were taken from the market. These samples were evaluated for their quality by determining Standard Plate Count (SPC) and Coliform count. The bacterial colonies ranged from 0.7×10^4cfu/ml to 6×10^6cfu/ml in boiled milk. Methylene Blue reduction test (MBRT) had also been performed to check microbial quality of the samples and Sample 1 (both raw and boiled) was reported to be poor. All the three samples (Pasteurized) were reported to be found adulterated with borates and carbonates while no samples were found to contain detergent.

Introduction

Milk contains all the essential nutrients especially calcium and phosphorus required for growth and development of body (Thorning *et al.*, 2016). Thus milk is a crucial diet of vast population on earth. People of all age groups generally consume milk as a dietary and high-grade food. It is exceptionally important for the growing children. Milk is a major part of human food and plays a prominent role in the diet. The milk fat content varies within the same dairy products and between different dairy products. Raw farm milk and full fat milk have their own percentage of fat (Kala *et al.*, 2018). Approximately 50% of the milk produced is used as fresh or boiled, one sixth as yoghurt or curd and remaining is consumed for manufacturing indigenous variety of milk products such as butter, khoa, paneer, rubri, kheer etc.

The simplicity and rapidity with which milk can be adulterated has always tempted unscrupulous milk vendors to indulge in fraudulent practices and adulterate the milk (Ali Ahmed, 2009). The ever increasing greed

has given way to a new type of adulterated milk called synthetic milk which exactly looks like the natural milk, has same specific gravity, fat and Solid Not Fat (SNF) value and is prepared by mixing water, pulverized detergent or soap, sodium hydroxide, vegetable oil, salt and urea. It is very dangerous from health point of view too. The use of synthetic milk has been found to have harmful effects on human beings. Urea and caustic soda are very deleterious to heart, liver and kidneys. Urea is an additional burden for kidneys as they have to do more work to remove urea from the body.

Milk gets contaminated very easily leading to its early spoilage when it is handled carelessly (Oliver *et al.,* 2005). Milk is an efficient vehicle for transmission of diseases to humans. Approximately 90% of dairy related diseases in human being arise from unhygienic milk products.

Thus, the present study was undertaken with the objective to determine the microbial and chemical qualities of raw, boiled and pasteurized milk.

Materials and Methods

Raw and boiled milk samples were collected from various vendors, pasteurized milk samples of three different brands were collected and analysed for microbial and chemical qualities. The samples were collected in the sterile bottles, sealed properly, brought to the laboratory, kept at below 4^0C and tested within three to four hours after collection. All the samples were first of all evaluated for the microbial quality by standard plate count (SPC) then presumptive test were performed to observe the presence of lactose and non-lactose fermenting bacteria by using MacConkey agar. The samples were inoculated on MacConkey Agar and incubated aerobically at 37^oC for 24 hours. Then non-lactose fermenting colonies were sub cultured again on MacConkey agar for purification of the isolate. Gram staining was performed to ensure the purity of the organism. *E.coli, Micrococcus, Streptococcus, Klebsiella* were identified by standard methods.

Simultaneously all the milk samples (raw, boiled and pasteurized) were evaluated for their biochemical characteristics like phosphatase activity, presence of adulterants like carbonates and bicarbonates, borates, formaldehyde, urea, detergent and carbohydrates etc.

Results and Discussion

The quality of raw milk, boiled milk and pasteurized milk had been studied by using both chemical as well as microbiological methods. Milk is considered as a complete diet so is an important food for people of all age group. Pathogenic bacteria in milk have been a major factor for public health concern since the early days of dairy industry. Many diseases are transmitted through milk. Raw milk is a major source of pathogenic infections (Vasavada, 1988). The health of dairy herd and milking conditions determine the quality of raw milk. Other sources of contamination are unclean teats, the use of unclean milking and transport equipments (Bonfoh *et al.,* 2003). To check the microbial quality of milk the standard plate count (SPC) was done. It is an important measure of milk quality and can assist in evaluating mastitis outbreaks, farm sanitation efficacy and proper milk handling practices. It gives the total microbial load of the samples including the presence of pathogens and non-pathogens. It gives an indication of the sanitary quality of milk and most grading of milk is done on the basis of some methods for estimating number (Collin *et al.,* 1995). The total viable count of raw milk ranged from 2.1×10^4cfu/mL to

9.6×10^6cfu/mL. In Boiled milk samples, the total microbial count range between 0.7 $\times10^4$cfu/mL to 6×10^6cfu/mL. This figure can be regarded as a high count as mentioned by Bramley and Mckinnon (1990) that counts of greater than 10^5cfu/ml for raw milk are indicative of serious faults in production hygiene.

The Milk may get contaminated with different micro-organisms due to direct or indirect contact with any source of external contamination during the steps of milking, collection and transport. Direct physical contact of milk with unclean surfaces such as milking utensils, udders and teats and hands of handlers besides environmental factors such as design and cleanliness of building and installations, the adequacy of water supply, the manner in which dung and other wastes are disposed off and the amount of dust in the immediate surroundings are important in so far they may contribute to microbial contamination of surfaces with which milk comes in to contact. Pasteurized milk of different brands showed different values of standard plate count. It ranged from 1.7×10^4cfu/mL to 1.5×10^5cfu/mL. Post-pasteurization contamination may occur due to improper handling of milk.

According to Pasteurized Milk Ordinance (PMO) standards, SPC of Grade A milk for an individual producer should not exceed 100,000 cfu/mL. In the present study one sample out of three showed $>10^5$cfu/mL. Elevated numbers of bacteria in milk generally arise from at least one of 4 common sources: dirty teats, soiled equipment, mastitis infections and poor refrigeration (Murphy and Boor, 2000) (Table 1). From the results of present study it was found that most of the samples were contaminated with coliform bacteria shows the average number of coliforms, the minimum and maximum number of coliforms in each sample. These were then categorized as lactose fermentor and non-lactose fermentor. Table too shows the average, the maximum and the minimum number each of lactose fermentor and non-lactose fermentor. Coliforms were found both in raw, boiled and pasteurized milk but raw milk had higher contamination (90%) than pasteurized milk (60%). Raw milk showed an average lactose fermenting coliform count of 18600/mL whereas boiled showed count of 6000/mL and in pasteurized milk showed count of 2300/mL (Table 2).

Methylene blue reduction test (MBRT) performed for raw milk revealed that out of three samples one was poor, one was fair and one was good. Out of three samples of pasteurized milk two were good and one was excellent. In three boiled milk samples, only one was found to be excellent. MBRT is a fast method to check microbial quality of the samples (Table 3). But the results obtained with MBRT are not in cognizance with those obtained with standard plate count. It is not a very reliable method for detection of contamination in dairy products (Igumbor *et al.*, 2002). This method should be used in combination with other methods to assess bacterial quality.

Owing to high demand of milk unscrupulous elements often adulterate the milk. These days the practice of making synthetic milk is rampant. In the present study milk samples were examined for the presence of adulterants. One sample of raw milk and boiled milk was found to be positive for starch. Also the same raw and boiled milk sample showed the presence of carbonates. None of the pasteurized milk samples showed the presence of any adulterants except Borates and Carbonates (Table 4). Absence of major adulterant in the pasteurized milk indicates the strict laboratory testing standard followed by the companies. None of the sample was found to contain formaldehyde.

Table.1 Enumeration of micro-organisms in milk samples by standard plate count method (SPC)

Sample Number	Cfu/ml
Raw Milk	
1	9.6×10^{6}
2	1.2×10^{4}
3	1.0×10^{4}
Boiled Milk	
1	6×10^{6}
2	0.9×10^{4}
3	0.7×10^{4}
Pasteurized Milk	
1	1.5×10^{5}
2	2.3×10^{4}
3	1.7×10^{4}

Table.2 Number of lactose and non-lactose fermenting bacteria (Presumptive test)

Particular of sample	No. of sample	Lactose Fermenting (per ml per g)	Non-lactose Fermenting (per ml per g)
Raw Milk	3	18600	2500
Boiled Milk	3	6000	3900
Pasteurized Milk	3	2300	400

Table.3 Decolorizing time and grading of milk samples by MBRT test

Sample Type	Sample Number	Decolorization Time	Grade
Raw Milk	1	1.5h	Poor
	2	2.3h	Fair
	3	6.4h	Good
Boiled Milk	1	1.35h	Poor
	2	6.20h	Good
	3	>8h	Excellent
Pasteurized Milk	1	>8h	Excellent
	2	6.25h	Good
	3	7.15h	Good

Table.4 Chemical adulterants in milk

S.No	Type of Sample	Starch	Sugar	Borates	Carbonates	Formaldehyde	Urea	Detergent	Phosphatase Activity
1	Raw Milk	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve
2		-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3		-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
1	Boiled Milk	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve
2		-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
3		-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
1	Pasteurized Milk	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
2		-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
3		-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve

To protect public health against milk borne infections, there are regulations that require proper hygienic handling of milk. These regulations should be followed strictly to safe guard the human health.

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