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Study on Physico-Chemical and Microbial Quality of Raw Milk Collected From Different Places of Assi Region in Varanasi City, Varanasi

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ABSTRACT

The present study was conducted to evaluate the physico-chemical characteristics including Adulteration and Microbiological quality of cow raw milk collected from four different places of *ASSI* region in Varanasi. Samples were analysed to know the chemical composition, the results showed that the statistically average percentage of Moisture (87.46), Fat (3.87), Protein (3.15), Lactose (4.42), Ash (.712), pH (6.43) and acidity (0.147). The keeping quality of milk was evaluated by Methylene Blue Reduction Test (MBRT). This phenomenon testified that milk sample 1 is fair quality and remained sample were found good and excellent. The microbiological conclusion confirmed the presence (less or more) of microbial load in all the raw milk samples. The highest level of microbial quality in standard plate count (SPC) was 19.1×10^6 cfu/ml. in sample 1 and in logarithm value is 7.28 cfu/ml at the same time, the highest coliform bacteria 2.3×10^2 in logarithm value is 2.36 was found in the sample 2. The adulterations in raw milk were checked by the standard procedure. In cow's raw milk the different mixed adulterant were found in two samples contaminated with detergent and pulverized soap. Besides different hazardous chemical adulterant, raw milk from sample 1 was detected with presence of hydrogen peroxide and sample 2 was contaminated formalin whereas urea was present in sample 2 and 4. Milk adulteration is a global concern and social problem. Increased demand, growth in competition in dairy industry and financial gain makes some producers to adulterate the milk thereby decreasing milk quality.

Key words: Adulterated milk, Coliform, MBRT, Microbial quality, Raw milk, SPC.

INTRODUCTION

Milk is an essential component of the diet of more than 7 billion peoples. The world production of milk reaches 730 million tons/year (Hemme *et al.,* 2010 and FAO, 2012). India is the largest milk producing country in the world since last two decade. In India, milk production recorded in 2016-17 was 165.40 Mt and per capita availability of milk is about 355grm/day (Source: NDDB). Milk is a complex combination of proteins, carbohydrates, fats, minerals, vitamins and other constituents. This makes milk essential for human consumption as a complete food supplement in various parts of the world which is obtained from different animal species such as goats, cows, buffaloes and camels.

However, cow's milk remains the most preferred type of milk. Generally, all types of milk are composed of the same kind of constituents, but in different concentrations. The overall concentration of major elements in goat and sheep milk is relatively higher in relation to cow milk (except for sodium) and several times higher in relation to human milk. All essential mineral elements are found in milk because by its definition it contains the nutritional requirements for growth of the young (Bates *et al*.,1996). Minerals are the inorganic components and make up only a relatively small portion of the animal diet but are vital to the animal (Yadav *et al*., 2018). Also, milk and milk products are main constituents of the daily diet, especially for vulnerable groups such as infant school age children and old age (Davies *et al*., 1986). Even though mammals produce milk to feed their offspring, in many areas of the world humans continue to consume milk throughout their life. However, it must be emphasized that lactose intolerance is widespread throughout the world and that a large proportion of the world's population would not benefit from the putative benefits of milk.

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms because of the specific production. It is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining

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its quality. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass. The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed, animal health etc. Milk is an important source of nutrients to human and animals, but due to its high-water activity and nutritional value it serves as an excellent medium for growth of much kind of microorganisms under suitable conditions. The number and kinds of microorganisms fluctuate with the circumstances associated with the production of a particular lot of milk and the degree and source of contamination. Milk adulteration encourages the deterioration of raw milk that apt to favourable condition for microbes to grow, particularly pathogenic bacteria which are liable to cause various types of diseases. Milk is highly vulnerable to bacterial contamination and hence is easily perishable (Kim *et al.,* 1983, Steele *et al.,* 1997). Depending upon the number of microbes present in raw milk it's criteria has been distinguished. The criteria of raw milk monitoring is presented in Table 1.

Milk as well as dairy products are important sources of food borne pathogens and numerous epidemiological reports have implicated inadequate heat-treated milk and raw-milk products are the major factors for illnesses caused by food-borne pathogens (Harrington *et al.,* 2002). Milk borne epidemics of human diseases spread through consumption of contaminated milk (Parekh and Subhash., 2008).

Quality control tests for milk are very important to assure adulterant free milk for consumption. Adulteration of milk reduces the quality of milk and can even make it hazardous.

Adulterants like soap, acid, starch, table sugar and chemicals like formalin may be added to the milk. Most of chemicals used as adulterants are poisonous and cause health hazards. Adulterants are mainly added to increase the shelf life of milk. Some of the preservatives like acid and formalin are added to the milk as adulterants, thereby increasing the storage period of milk. Generally, water is added to the milk to increase the volume content of the milk. Unfortunately, milk is being very easily adulterated throughout the world. Possible reasons behind it may include- demand and supply gap, perishable nature of milk, low purchasing capability of customer and lack of suitable detection tests (Kamthania *et al.,* 2014).

MATERIALS AND METHODS

Raw milk samples were collected randomly from different places of Assi region (Lanka road, Ravi Das Gate near BHU, Godoulia Road and near Hanuman temple on *Assi* to Ravi das road) of Varanasi. Thus, collected samples were divided into 4 treatment with 4 replications of each treatment.

Table 1: Interpretive Criteria for Raw Milk Monitoring.

Parameter	Low	Medium	High
Standard Plate count	<2,00,000	1 million-5 million	>5million
Coliform count (cfu/ml.)	<50	50-100	>100
Yeast and Mould count	<8	8-80	>80

Altogether a total of 16 raw milk samples were analysed. All samples were collected in a sterilized labelled screw capped jars kept in icebox and immediately carried to the laboratory of Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences; Banaras Hindu University, Varanasi U. P. in 2019. Approximately 250 ml of milk was used for analysis. All milk samples collected from containers containing milk during that day was either to be consumed at household level or sold to the public or both. For easy identification all samples were coded with random numbers.

Parameters of Physico-chemical composition of milk samples tested for analysis

The milk samples were analyzed for determining moisture, fat, protein, lactose, ash, pH and acidity compositional ingredients as per AOAC (2005).

Moisture percentage:

The moisture content was calculated by the following formula:

Moisture% by weight =

$$\frac{(W_1\text{-}W_2)}{(W_1\text{-}W_3)} \times 100$$

Where,

W_1 = Weight of dish + sample (gm) before drying

W_2 = Weight of dish + dried sample after drying

W_3 = Weight of empty dish

Fat content:

Fat percentage of the milk sample was determined by using Gerber method. 10 ml Sulphuric acid (Gerber acid) was taken into milk butyrometer (range 0–10%) using automatic measure, then the sample of milk was mixed well and then 10.75 ml of milk was drew with the help of milk pipette, after that transfer of milk slowly from the wall side of butyrometer. 1.0 ml of amyl alcohol was added to differentiate fat particles. Stopper key was used to lock the neck of butyrometer and the contents were shaken slowly for uniform mixing. Then after the butyrometer was placed in Gerber centrifuge and the contents were centrifuged for 3-4 min at 1100 rpm. The butyrometer was then placed in water bath at 65°C for 5 min and then fat percentage was read on the butyrometer stem.

Total ash content:

Ash percentage was calculated by using following formulae

Ash (%) =

$$\frac{W_1\text{-}W_2}{W} \times 100$$

Where,

W = Weight of sample

W_1 = Weight of silica dish

W_2 = Weight of silica + ash

Test of milk pH:

The pH of the milk samples was determined in the laboratory using a digital pH-meter (EUTECH, Serial No. 1366514,

Model P/N: 54×002606; made in Malaysia) (AOAC, 2005). The pH meter was first calibrated using buffers of pH 7.0 and 4.0 each time before the pH of milk sample was measured.

Titratable acidity

The Titratable Acidity of the milk is measured by using known volume and concentration of Sodium hydroxide solution using phenolphthalein as an indicator. The calculation is done by using following formula.

$$\text{Lactic acid} = \frac{\text{No of ml. of 0.1 N NaOH solutions required for neutralization x 0.009}}{\text{Weight of sample (Weight of sample = Volume of milk x specific gravity)}} \times 100$$

Lactose content

25 ml of milk was taken into the 500 ml conical flask and diluted with 200 ml. of distilled water and 3.75 ml of 10% acetic acid solution was added and boiled then left for cooling the contents and transferred to 250 ml volumetric flask and volume was made to increase with distilled water and filtered through No. 42 filter paper. The filtrate was collected in a clean dry beaker and then the filtrate was filled in burette with the help of funnel. This contains lactose and was determined by using Fehling A and B reagent along with titration.

Protein content

10 gm milk was taken in a clean and dry Kjeldahl flask. 25 ml of concentrated sulphuric acid, 0.2 gm of copper sulphate and 10 gm of potassium sulphate added. Then after the flask was placed on digestion heater under fume chamber and heated gently to boil until contents were clear and then for another 2 hours the liquid was then allowed to cool and diluted with 200 ml of distilled water. The flask with micro-Kjeldahl distillation unit was then fitted and added with 75-80 ml of 50% NaOH down the neck of the flask to form a layer under the acid. 50 ml of N/10 sulphuric acid in a 250 ml conical flask was then taken and it was placed under the condenser so that the tip of the condenser is dipped in the liquid. After sometime heating was started and the contents were distilled until all ammonia has passed over as indicated by collection of 150 ml distillate. After distillate was obtained the heating was stopped and the conical flask was removed. The tip of condenser was washed to remove all traces of condensate. Finally, the contents were titrated against N/10 NaOH using mixed indicator.

Microbiological parameters tested for analysis

Methylene Blue Reduction Test (MBRT)

1ml of methylene blue (1:2500) was added to 10 ml of milk. The tubes were sealed with rubber stopper and slowly inverted 3 times in order to mix. Tubes were placed in water bath at 35°C and examined at interval up to 6 hour and then the decolorizing time was recorded and the milk was graded as per guide for raw milk supplies as:

MBRT Time (in hours)	Grade
5 and above	Very good
3 and 4	Good
1 and 2	Fair
½	Poor

Total Bacterial Count (TBC)

In the dilution plate method, a sample from a liquid culture is inoculated and poured on a plate. Then mixed gently by shaking. The plate was incubated to allow bacterial growth and colonies were counted. Because every cell in the population will divide and produce a visible colony, the colonies on the plate represent the number of cells that were present in the sample taken from the population. More than 300 colonies are termed as "too numerous to count" (TNTC) and fewer than 30 is not statistically significant. In order to achieve a countable number from a high concentration culture, the serial dilution technique was employed: a series of dilutions of the original population was made and samples from each dilution were spread onto agar plates. The plate that had the appropriate number of colonies (30-300) were counted and the count was then multiplied by the dilution factor of the plate in order to determine the number of bacteria in the original population.

$$\text{No. of cell/ml} = \frac{\text{no. of colonies x Dilution Factor}}{\text{wt. of milk sample (ml.)}}$$

Standard Plate Count (SPC)

To determine the SPC, the plate count agar method was used. Plate Count Agar was formulated as described by Buchbinder *et al*., (1951) which is recommended by APHA and FDA. The samples were diluted and appropriate dilutions were added to Petri plates. Sterile molten agar was added to these plates and plates were rotated gently to ensure uniform mixing of the sample with agar. The poured plate count method was preferred to the surface inoculation method, since it gives higher results.

Coliform count

Violet Red Bile Agar (VRBA), a modification of Mac Conkeys original formulation was used for the enumeration of coli-aerogenes bacterial group. 10.38 grams of sample was suspended in 250 ml distilled water. Then the medium was heated to dissolve completely along with constant stirring. Autoclaving was not performed. The sample was then cooled to 45°C and poured into the petri plates containing the inoculum. If desired, the medium can be sterilized by autoclaving at 15 lbs. pressure at (121°C) for 15 minutes.

Detection of different mixed adulterants in milk

Milk samples were tested for presence of Detergent, Pulverized soap, Colouring materials.

To determine the presence of detergents in milk we took 5 ml. of milk in a test tube. Then we added 0.1 ml 0.5%

Bromocresol Purple (BCP) solution to the milk. After addition of BCP if Violet colour appears then it indicates presence of detergent in milk.

To determine the presence of soap we took 10 ml milk sample in a test tube and added equal quantity of hot water to it. Then 1-2 drops of phenolphthalein indicator were poured which shows presence of soap in milk only if pink colour appears.

10 ml milk sample was taken in a test tube and added with 10 ml diethyl ether and then mixed well. Then after it was allowed to stand, the presence of colouring matters was detected only if yellow colour was present in ethereal layer.

Detection of different hazardous chemical in milk

Milk samples were tested for presence of Hydrogen Peroxide (H_2O_2), Formalin, Urea, Borax/ Boric Acid.

Inorder to detect H_2O_2 in milk we took 1 ml. milk sample in a test tube and added 1 ml. of potassium iodide-starch reagent solution to it and mixed well. The appearance of blue colour in sample indicates the presence of hydrogen peroxide as adulterant.

To determine presence of formalin we took 10 ml milk sample in a test tube and then added 5 ml conc. Sulphuric acid with a little amount of Ferric chloride without shaking the sample. If violet or blue colour appeared at the junction of two liquid layers it indicates the presence of formalin.

To determine the presence of borax we took 5 ml. milk sample in a test tube and added 1 ml. conc. HCl to it. After that a turmeric paper was dipped into it and was allowed to dry in a watch glass at 100°C. If the turmeric paper turns red, it indicates the presence of borax or boric acid.

Statistical analysis

The complete randomized design (CRD) with one-way analysis of Variance followed by Duncan's multiple range test using standard statistical procedure was used for the statistical analysis of the data obtained from research. Analysis was done by Statistical Package for Social Sciences (SPSS) version 19 (V.19). Data was tested at P<0.005 to check its significance.

RESULTS AND DISCUSSION

Treatment details

The detail of treatment that was taken during investigation is presented in Table 2.

Moisture content

The moisture content of different raw milk samples is presented in the Table 3. It is evident from the Table 3 that maximum moisture content was recorded in cow milk in sample 2 was significantly different as compared to others sample. Moisture content of cow milk in sample 2 was significantly (P<0.05) higher i.e. 88.22% over milk sample 4 i.e. 86.61%. The sample 1 and 3 were both found to be statistically at par with each other having moisture percentage of 87.52% and 87.5% respectively. This finding is in accordance with (Banda, 2010), that milk consists of 86-87% water which means that milk is a bulky and heavy commodity. Also, these findings concur with findings of Mohammed *et al.* (2013), where the proximate chemical compositions of milk samples were analysed and reported that the moisture content for fresh milk was 86.8%.

Fat content

The fat content of milk in different sample is presented in the Table 4. It was revealed that the maximum fat content recorded in the sample no. 4 (4.26%) was significantly higher among others. The minimum fat content was observed in sample 3 (3.67%) which was statistically similar with the fat content of sample 1 (3.86%) and sample 2 (3.77%).

Protein content

The protein content of different sample is presented in Table 5. From above Table it is cleared that the maximum protein content was observed numerically in sample 3 (3.22%) followed by sample 4 and 1 i.e. (3.20%) and (3.19%) respectively, while minimum was found in sample 2 (2.99%).

Table 2: Details of treatment.

Sample or Treatments	Place collected from
1	Lanka road
2	Near to Ravi Das Gate
3	Godowlia road
4	Near Hanuman temple (Sangkat Mochan) on *Assi* to Ravi das road.

Table 3: Moisture Content in different samples.

Treatment	Mean	SEM	P value
1	87.520^{ab}	0.232	
2	88.220^{c}	0.211	0.048
3	87.500^{ab}	0.380	
4	86.615^{a}	0.270	

Mean bearing different superscript in a row differ significantly (P<0.05).

Table 4: Fat content in different samples.

Treatment	Mean	SEM	P value
1	3.868^{ab}	0.123	
2	3.770^{ab}	0.112	0.045
3	3.675^{a}	0.157	
4	4.260^{c}	0.145	

Mean bearing different superscript in a row differ significantly (P<0.05).

Table 5: Protein content of different samples.

Treatment	Mean	SEM	P value
1	3.190^{ab}	0.053	
2	2.993^{a}	0.031	0.038
3	3.225^{c}	0.048	
4	3.203^{ab}	0.081	

Mean bearing different superscript in a row differ significantly (P<0.05).

Samples 1, 2 and 4 were found to be statistically at par with each other. These findings are in accordance with the findings of (Dehinenet *et al*., 2013) whose research shows the protein content of milk to be 3.12% as well.

Lactose content

Data in Table 6 represents the lactose content present in different milk samples. In the present investigation among the milk samples the higher lactose content was found in sample 4 (4.59%) which was followed by sample no. 3 and 1 is each with 4.45% and 4.39% lactose respectively, whereas the minimum lactose content (4.27%) was found in the sample 2. The results showed that there was significant (P<0.05) difference among treatments in lactose content of milk. The highest lactose content in the present study (4.59%) was higher than what was reported by (Haftu and Degnet 2018) (4.24%). Another study, shows the lactose percentage found in the present investigation (4.59%) is similar to that reported by (Hertzler *et al*., 1996), i.e. (4.59%±0.12%) lactose.

Ash content

Ash content of different milk sample is represented in above Table 7. All the content of ash differs significantly among each sample. It is evident that the highest ash content (.0732%) was found in the sample 4 and was simultaneously followed by the sample 1 (.0730%) and 2 (.0715%) whereas the minimum ash content (.0685%) was found in the sample 3. The ash content found in the sample 4 was significantly higher (P<0.05) among the group samples. The current study of the ash content was statistically almost similar as reported by the (Teklemichael *et al.,* 2015) who found ash content (0.713 ± 0.043%) in their investigation. Also, our study concurred with the findings of (O'Connor, CB. (1995) who reported that the ash content of cow milk remains relatively constant from 0.7 to 0.8% during their analysis and also was found to be significantly influenced by breed, stage of lactation and feed of the animal.

pH

pH of different milk samples is presented in Table 8. All the values of pH differ significantly among each other. The maximum pH was found in the sample 4 (6.57), followed by the sample 1 and 3 that are (6.41) and (6.40) respectively, whereas the minimum pH (6.34) was found in the sample 2. The pH obtained from sample 1, 2 and 3 were found to be statistically similar with each other. The pH of the present investigation found to be lower than the reported by (FAO, 1999). The normal pH of fresh cow milk ranges from pH 6.6-6.8. When milk is left for some hour without disturbing the milk temperature gradually increases.

Acidity percentage

Acidity present in milk sample is presented Table 9. It is evident that the maximum acidity percentage (0.164 %) was found in the sample 3 and followed by the sample 4 and 2 that is (0.16%) and (0.148%) respectively, whereas the minimum acidity percentage (0.132%) was found in sample no. 1. Acidity of cow milk in sample number 3 was significantly (P<0.05%) higher among the group samples, every group samples differ from each other statistically. The present investigation of the acidity in cow milk was found to be similar with that reported by (Teklemichael *et al.,* 2015), who found the acidity percentage as (0.165%) in their study. (Hossain and Dev., 2013), reported that, to keep good quality for long time storage, acidity of milk should be less than 0.15% as BDS (Bangladesh Standard). Also, the present finding resembles with the Indian standard (FSSAI, 2011) where the acidity percentage in cow milk should vary between 0.12-0.14%.

Methylene blue reduction test

Table 10 represent the Methylene Blue Reduction Test of milk sample. Methylene Blue dye has been employed to check the overall microbial load and quality control of milk and other liquid foods (Impert *et al.,* 1994). Sample 4 retained

Table 6: Lactose content in different samples.

Treatment	Mean	SEM	P value
1	4.390^{ab}	0.099	
2	4.275^{a}	0.068	0.021
3	4.453^{ab}	0.062	
4	4.593^{c}	0.041	

Mean bearing different superscript in a row differ significantly (P<0.05).

Table 7: Ash content of different sample.

Treatment	Mean	SEM	P value
1	0.730^{bc}	0.011	
2	0.715^{b}	0.010	0.420
3	0.685^{a}	0.010	
4	0.733^{bc}	0.005	

Mean bearing different superscript in a row differ significantly (P<0.05).

Table 8: pH of different samples.

Treatment	Mean	SEM	P value
1	6.413^{ab}	0.073	
2	6.340^{a}	0.056	0.047
3	6.408^{ab}	0.031	
4	6.578^{c}	0.042	

Mean bearing different superscript in a row differ significantly (P<0.05).

Table 9: Acidity percentage in different samples.

Treatment	Mean	SEM	P value (0.05 %)
1	0.133^{a}	0.005	
2	0.148^{b}	0.002	0.041
3	0.163^{bc}	0.011	
4	0.160^{bc}	0.006	

Mean bearing different superscript in a row differ significantly (P<0.05).

maximum time period (>5 hrs) to change its colour from blue to white which showed that this milk sample comes under excellent quality and followed by sample 2 and 3 (nearly 3-4 hrs). This decolourization time showed grade of milk comes under good whereas the sample 1 was decolorized within 2 hours that means this milk was more reliable from above raw milk samples. (Muhammad *et al*., 2009), (Bongard *et al*., 1995) and (Merker *et al*., 1997), reported that blue color disappearance in short time indicates higher microbial load in the milk sample. (Bhattacharjee *et al*., 2006), performed methylene blue test for ten raw milk samples, out of ten samples, the five samples were poor, two samples were fair, two samples were good and only one sample was found to be excellent. Out of ten pasteurized samples, nine samples were of good quality and one was found to be excellent. In this study, most of cow's raw milk shows very short decolorization time of the dye. This may be due to poor milk handling practices during milking, poor animal health services and use of poor potable water.

Table 10: Methylene Blue Reduction Test.

Samples	Decolorization time (MBRT)	Grade /Quality
Sample 1	From 1-2	Fair
Sample 2	From 3-4	Good
Sample 3	From 3-4	Good
Sample 4	More than 5	Very Good

Table 11: cfu/ml of milk sample.

Treatment	Mean	SEM	P value
1	191.500^{c}	7.274	
2	124.750^{ab}	24.527	0.010
3	97.750	9.499	
4	92.500^{a}	5.172	

Mean bearing different superscript in a row differ significantly (P<0.05).

Table 12: Coliform Count in (cfu/ml.) different milk sample.

Treatment	Mean	SEM	P value
1	1.700	0.147	
2	2.325	0.239	0.017
3	1.925	0.229	
4	1.100	0.216	

Mean bearing different superscript in a row differ significantly (P<0.05).

Standard plate count

Table 11 is representation for Cfu/ml of milk sample collected from different regions of Varanasi. Total Viable Count (TVC) of bacteria was carried out on plate count agar media using pour plate techniques. The event of microbiological analysis showed that the highest growth of standard palate count bacteria in raw milk was in the sample 1 i.e. 19.1×10^{6} (log 7.28), followed by the varied to some extent in between the value of sample 2 and 3, that is 12. 4×10^{6} (log 7.09) and 9.7×10^{5} (log 5.98) respectively, whereas the minimum standard plate count bacterial loaded 9.2×10^{5} (log 5.96) was found in the sample 4. The sample no. 1 was significantly (P<0.05) higher in among the group samples which are statistically found different from each other. High bacterial contamination was found in raw milk collected from sample 1. The basic reason behind this is due to improper handling of milk. All the above detailed description of colony forming unit is presented in Fig 1 given below.

Coliform count

Table 12 represents the Coliform Count (cfu/ml.) in different milk sample. The measures of TPC (cfu/ml) of raw milk was found highest in the sample 2 (2.3×10^{2}) (log 2.36) which was followed by the sample 3 and 1 that is the value of 1.9×10^{2} (log 2.27) and 1.7×10^{2} (log 2.23) respectively whereas the minimum total coliform count was found 1.1×10^{2} (log 2.04) in the sample 4. The coliform count was significantly higher (P<0.05) among raw milk samples. Every sample were statistically different from each other. In the present investigation the minimum coliform bacterial load was found similar as reported by the (Md. *et al.,* 2015) which showed that the value of 9.4 $x10^{2}$ (log 2.97). The presence

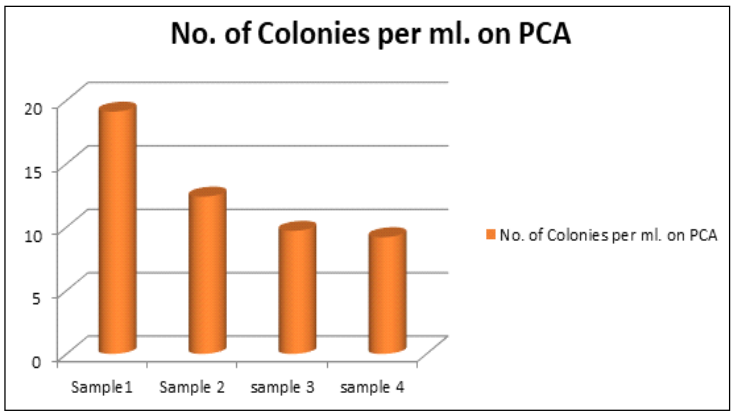

Fig 1: Average number of Colonies in different milk samples.

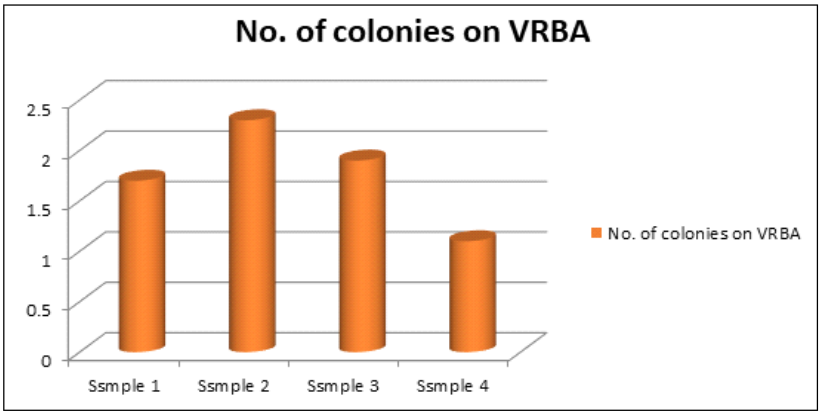

Fig 2: Average number of Colonies of different milk samples.

of these group bacteria in milk is considered as an indicator of the degree of unhygienic practices during production, processing or storage and is intended to measure general care taken in handling these products. Detailed description of coliform count described above is presented in Fig 2.

Rapid qualitative detection

Table 13 depicts the information on rapid qualitative detection of different mixed adulterant in raw milk. The different mixed adulterant was tested for cow raw milk in the laboratory; these are the adulterant such as: detergent, pulverized soap and colouring agent. During study the detergent was found to be positive (violet colour presence) in the sample 2. Detergents are added to emulsify and dissolve the oil in water giving a frothy solution, the characteristic white colour of milk (Singuluri and Sukumaran, 2014), it enhances the cosmetic nature of milk. Detergents cause gastro-intestinal complications that are most harmful for human being health. In addition to, colouring agent was also found to be positive (yellow colour) in the sample 3. Pulverized soap was not detected in any samples. Food colourant are added in the milk to improve the appearance and has hazard our effect on health. The analytical method called capillary electrophoresis was employed for the separation of food colourant.

Table 14 presents the rapid qualitative detection of different hazardous chemicals adulterant in raw milk. Hydrogen peroxide (H_2O_2) was found to be positive (blue colour) in the sample 1and 2. Hydrogen Peroxide is added to milk to prolong its freshness, but peroxides damage the gastro intestinal cells which can lead to gastritis and inflammation of the intestine. H_2O_2 disturbs the antioxidants in the body disturbing the natural immunity hence increasing ageing. Testing of the formalin in raw milk was found to be positive (Violet or Blue Colour) in the sample 2. In the raw milk, formalin is generally used as a preservative but they lead to the many harmful diseases in human being and also lead to cancer if used for long time. During the quality checking of raw milk every sample showed negative test to boric acid/Borax preservative. During the quality checking of raw milk in the laboratory, the Urea content was found to be positive (Yellow Colour indicate) in the sample 4.

Table 13: Rapid qualitative detection of different mixed adulterant in raw milk.

Adulterant	Samples			
	Sample 1	Sample 2	Sample 3	Sample 4
Detergent	-ve	+ve	-ve	-ve
Pulverized soap	-ve	-ve	-ve	-ve
Coloring agent	-ve	-ve	+ve	-ve

Table 14: Rapid qualitative detection of different hazardous chemicals adulterant in raw milk.

Adulterant	Samples			
	Sample 1	Sample 2	Sample 3	Sample 4
H_2O_2	+ve	+ve	-ve	-ve
Formalin	-ve	+ve	-ve	-ve
Borax/Boric acid	-ve	-ve	-ve	-ve
Urea	-ve	-ve	-ve	+ve

Commercial urea is added to milk to increase non-protein nitrogen content (Sharma *et al.*, 2012). Urea is added to milk to provide whiteness, increase the consistency of milk, increase non-protein nitrogen content and for leveling the contents of solid-not-fat (SNF) as are present in natural milk. Urea is also used to prepare synthetic milk. Health hazards associated are acidity, indigestion, ulcers and cancers. Urea is harmful to heart, liver especially for kidneys as the kidneys have to do more work to remove urea from the body (Kandpal *et al.*, 2012).

CONCLUSION

The effect of different treatment was found significant for each of the parameter tested i.e. either physico-chemical or microbiological. During the entire study of this research the results showed the statistically average percentage of Moisture (87.46), Fat (3.87), Protein (3.15), Lactose (4.42), Ash (.712), pH (6.43) and acidity (0.147) were observed from different samples collected. Sample 1showed fair grade while other samples showed good and excellent grade while testing with MBRT. All raw milk samples were confirmed with presence of more or less microbial load. Milk collected from

Lanka road was found with high level of microbial quality in SPC i.e. 19.1×10^6 cfu/ml. meanwhile highest coliform bacteria (2.3×10^2) were present in milk sample collected from Ravidas gate area. Presence of detergent and coloring agent was found in sample collected from Ravidas gate and Godoulia area respectively. Milk samples from Lanka road and Ravidas gate showed presence of hydrogen peroxide while other samples were free from this adulterant. Also sample from Ravidas gate was detected with presence of formalin. Urea was detected from sample collected from Hanuman temple (Sangkat Mochan) area while there was absence of boric acid in either of milk samples.

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