Chemical Composition And Microbial Quality Of The Milk Samples Collected From Nandini Diary, Vijayapura, Karnataka

Sana R. Kolhar*

*Department of Zoology, Anjuman Art's, Science and Commerce College, Vijayapura-586101 (Karnataka), India; E-Mail: sanakolhar.sk@gmail.com; Mobile: 9686781894

Article History	ABSTRACT
Received: Revised: Accepted:	*Milk is an essential commodity in daily life. It is not only a source of good quality protein, but also of calcium and riboflavin besides other nutrients. However, in local products to increase the yield certain adulterants are added which may affect the nutritional quality of milk. Raw milk contains many adulterants, such as water, urea etc when compared to the packaged milk. Packaged milk do not have any adulterants, because they are well treated and sterilised. Qualitative analysis of the raw milk sample can detect the adulterants present in the milk samples and can easily be found out. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces. The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health. It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk.*

INTRODUCTION

Milk may be defined as the whole, clean, fresh lacteal secretion obtained by the complete milking of healthy milch animals. Milk is a considerable resource of products whose composition varies [1]. There are four components which are dominant in qualitative terms, such as, water, fat, protein and lactose, while the minor components are minerals, enzyme, vitamins, uric acid. Milk has a short life; however, products such as milk powders have allowed a global industry to be developed. Milk is a widely consumed beverage that is essential to the diet of several millions of people world wide because it provides important macro and micro nutrients [2-5]. Milk proteins have a high biological value but, unlike egg proteins, they lack sulphur containing amino acids. The proteins in the cow's milk have balanced amino acid profiles and good digestibility.

Milk and milk products also contain fat. Cow's milk contains fat that is in the form of glycerides. The fat in the cow's milk is a poor source of essential fatty acids. The main carbohydrate in the milk is a disaccharide called lactose. It is made up of two simple sugars, glucose and galactose. Lactose support the absorption of calcium and phosphorous and synthesis of some B complex vitamins in the small intestine [6-10].

Milk and milk products are an excellent source of vitamins and minerals, particularly calcium. Milk has a significant amounts of vitamin A and B such thiamine, riboflavin and nicotinic acid. Milk contains many natural enzymes and other enzymes are produced in the milk as a result of bacterial growth [11-15]. Enzyme

action in milk systems is extremely important for its effect on the flavour and body of different milk products. Lipase, oxidases, proteases and amylases are among the more important enzymes that occur naturally in milk. These classes of enzymes are also produced in milk by microbiological action [16-19].

TABLE 1: COMPOSITION OF RAW MILK

CONSTITUENTS	COW MILK	BUFFALO MILK
Water	86.50	83.18
Fat	4.39	6.71
Protein	3.30	4.52
Lactose	4.44	4.45

TABLE 2: BREED AVERAGES FOR PERCENTAGES OF MILK FAT AND PROTEIN

BREED	TOTAL FAT	TOTAL PROTEIN
Guernsey	4.46	3.47
Holstein	3.64	3.16
Jersey	4.64	3.73

Another study in 1700 healthy people found that drinking raw milk in the first year of life was associated with a 54% reduction in allergies and 49% reduction in asthma. Milk is high in calcium and phosphorus, which are both needed for healthy bones, cell function, muscle health and metabolism. These minerals are heat stable [17,18].

About 80% of the milk protein is CASEIN, while the remaining 20% is WHEY. These may help in muscle growth, improve insulin resistance and lower heart disease risk.

Composition vary by breed, animal and point in the lactation period. Seasonal variation in milk fat percentages are well recognised, with summer months averaging 0.4 % units less than winter months. The higher temperature during summer also effect the milky fatty acid composition.

The fat percentage of milk increases continuously during milking process with the lowest fat milk drawn first and the fat milk drawn last. The increase in the fat percentage throughout the milking process is due to the clustering of fat globules trapped in the alveoli. Thus, if cows are not milked out completely fat percentage will be lower than normal but at the next milking, fat content will be higher than normal.

PROCESSING OF RAW MILK

1. Milk arrives at the milk dairy processing plant over the weighbridge and the weight is automatically recorded [19]. The temperature of the milk should be at 4-6 degree Celsius. Milk samples using sterile containers are collected automatically from each supplier at source and are delivered to a laboratory technician for detailed analysis. Milk that deviates in composition, taste and smell from natural milk receives a lower quality rating. The technician also takes a composite sample from each bottle and are tested for acidity, antibiotics, added water, fat, protein urea content (Gwandu, 1988).

There are some organoleptic tests that are done to check the quality of the raw milk sample, the tests are as follows,

ORGANOLEPTIC TESTS

PROCEDURE: The can is opened at the milk collection point and the milk is smelled and if any is odour is found in the milk, it is rejected. Even the milk is tasted and rejected if any bad taste occurs [20].

Abnormal smell and taste may be due to the following reasons,

1) atmospheric smell (methane or cowy odour).
2) advanced acidification (pH greater than 6.4).

CLOT ON BOILING TEST

PROCEDURE: 5 ml of sample is taken in the test tube and it is holded with the test tube holder and heated using bunsen burner for 3-4 minutes. The sample may clot or coagulate on boiling [21].

RESULT: The coagulation or clotting results in the poor quality and is rejected.

This clotting is appeared due to the following reasons,

1) may be due to the bacterial contamination in the milk.
2) may be due to low acidity of the sample.

TEST FOR FAT PRESENT IN THE RAW MILK BY MILKOSCREEN
PROCEDURE: As soon as the raw milk is taken from the vehicle, they are proceeded for the quality check in the lab. The milk sample of each bottle is tested [22].
This is done by the following tests,
First, the sample of each bottle is taken in a measuring cylinder, and the temperature of the milk is recorded. The same is done for all the samples and their temperature is recorded.
Then the lactometer is introduced into the sample. First the lactometer sinks in the milk and later it floats and then the reading of the lactometer is recorded for each sample. Next, small amount of sample is taken in cylinder and is placed in the milkoscreen machine, which automatically displays the percentage of fat, SNF and urea present in the sample.

Fig.1.Milkoscreen

RESULT: If the reading is less in the lactometer, than we can come to conclusion that more amount of water is being added to the milk. If the reading is high, than we can say that milk is having low amount of water in it. By this we can also come to know about the adulteration of milk.

METHYLENE BLUE DYE REDUCTION TEST
PROCEDURE: The test has to be done under sterile conditions. Take 10 ml of milk sample in sterile test tube. Add 1 ml methylene blue dye solution (0.005%). Stopper the tubes with sterilized rubber stopper and carefully place them in a test tube stand dipped in serological water bath maintained at 37-38 degree Celsius.
The sooner the decolourization, more inferior is the bacteriological quality of milk assumed to be. This is widely used at the dairy reception dock, processing units and milk chilling centres where it is followed as acceptance / rejection criteria for the raw and processed milk.
RESULT: Based on the time of decolourization, the quality of milk is decided.

TABLE 3: BASED ON THE TIME OF DECOLOURIZATION, THE QUALITY OF MILK IS DECIDED

TIME	QUALITY
5 hours and above	very good
3 – 4 hours	Good
1 – 2 hours	Fair
Less than ½ hour	Poor

1. **DETERMINATION OF FAT BY GERBER METHOD**

Fig.2.Gerber centrifuger

PROCEDURE: First the calibrated butyrometer is washed properly, later 90% of Concentrated Sulphuric acid of 10 ml is poured in the butyrometer with the help of pipette, from the sides of the test tube by rotating the butyrometer. Then add 1 ml of Amyl alcohol to it. Then stop the butyrometer by stopper and it is inverted and shaked [23,24].

After this, the butyrometer is inserted in the Gerber centrifuge, invertedly in the tubes. Run the centrifuge for 10 minutes, the separation of the fat layer from the milk takes place in this process. This is because of the reaction of Concentrated Sulfuric acid and Amyl alcohol, that leads in the separation of fat content from the milk proteins. After 10 minutes the butyrometer is removed out and there we can see the separation of fat layer from the milk, which is quite clear in the appearance compared to the bottom solution.

Reading of fat is taken and recorded.

RESULT: The fat content is read directly on the butyrometer and an average value of the all the sample is recorded.

MICROBIOLOGY TEST FOR THE GROWTH OF BACTERIA

PROCEDURE: All the apparatus mentioned above were sterilized, with alcohol. The Laminar Air Flow was also sterilized with alcohol with the help of cotton.

Laminar air flow is the chamber which is used to prevent the contamination of biological samples in which the cultures are kept in it [14, 25-30].

The conical flask is poured with 2.25 gm of NaCl, and to which 250 ml of distilled water is added and shaked well. This conical flask is then fitted with cotton plug and heated around 90 degree celsius for 10 minutes and cooled.

In the laminar air flow, air will be moving at same speed and in the same direction, it provide aseptic or sterile conditions for the tissue culture. The prepared solution is poured in the test tubes with the sterilized pipette.

9ml of the solution is poured in all the test tubes and covered with cotton plug. The three different samples of milk are taken for the experiment.

Toned milk, Shubham and Samruddhi were taken for the test.

1ml of toned milk is poured in the first test tube and next sample in the fourth test tube and another sample in seventh test tube. Serial dilution is done. The 1ml of sample from the first test tube is poured in the second test tube and 1ml of sample from the second test tube is poured into third test tube, the same is done for the two samples and closed with the cotton plug.

The petrifilms were taken and the media prepared was poured on it. The petrifilms were incubated for 24-48 hours at 32 degree Celsius. After the incubation period the petrifilms were taken out and the bacterial colonies were counted by using bacterial colony counter.
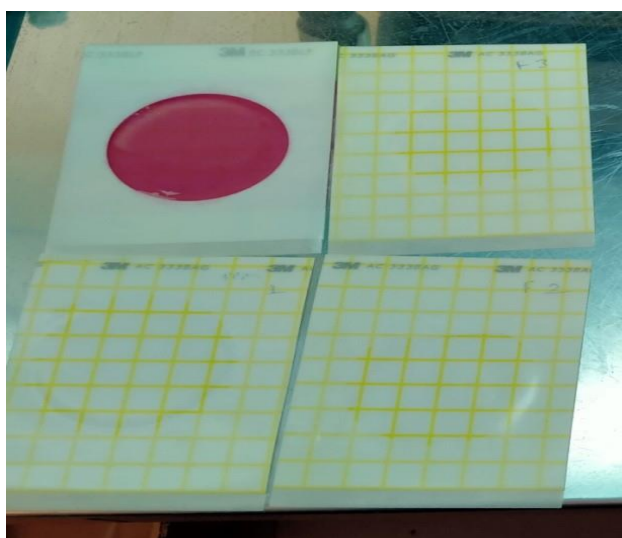
Fig.3. Petrifilms

RESULT: If the sample is containing more than 300 colonies than it will be having more amount of microbial growth.

BACTERIAL ANALYSIS OF RAW AND PACKED MILK.
MATERIAL AND METHODS
SAMPLE COLLECTION: Ten raw milk samples and ten pasteurized milk samples were collected in sterile containers. After collection, the samples were transported to the laboratory and processed for MBRT and total coliform count and total viable count within one day hours [15, 31-33].

MICROBIOLOGY ANALYSIS
In the methylene blue reduction test 1 ml of methylene blue is added to 10 ml of milk. The tube is sealed with rubber stopper and slowly inverted three times to mix. It is placed in water bath at 35 degree Celsius and examined at intervals upto 6 hours. The time taken for the methylene blue to become colourless is the mythelene blue reduction time. The grading of milk sample's on the basis of methylene blue reduction test in different milk samples are presented in table.

The methylene blue reduction test depends upon the ability of bacteria in milk to grow and to consume the dissolved oxygen, which reduces the oxidation reduction potentials in the medium.

ENUMERATION OF MICROORGANISMS DETERMINATION OF TOTAL VIABLE COUNT
Different dilution of milk sample ranging from 10^1 to 10^6 was prepared by using sterile peptone water. For the determination of Total Viable Count 0.1 ml of each dilution was inoculated on Nutrient Agar using a sterile pipette for each dilution.

The diluted sample was sprayed as quickly as possible on the surface of the plate with sterile glass spreaders. 1 sterile spreader was used for each plate, the plates were then kept in an incubator at 35 degree Celsius for 24- 48 hours.

After incubation, plates showing 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by dilution factor to obtain the TVC.

ENUMERATION OF TOTAL COLIFORM COUNT (TCC)
For the enumeration of Total Coliform, TVC method was employed for TCC method. MacConkey Agar plates were used as above. The milk samples were inoculated on MacConkey Agar and incubated aerobically at 37 degree Celsius for 24 hours. The plates were observed for growth of Escherichia Coli. Single isolated circular pink colony was picked and subcultures on MacConkey Agar for purification of the isolate simultaneously another single colony showing similar characters was picked for the Gram Staining, morphological characters of the isolates using bright field microscope [16, 34-39].

All the milk samples were subsequently sub-cultured on to Eosin Methylene Blue Agar, for primary screening of E. coli and incubated aerobically at 37 degree Celsius for 24 hours.

STATISTICAL ANALYSIS
All the microbial counts were converted to the base – 10 logarithms of the number of colony forming units per ml of milk samples.

TABLE 4: MICRO FLORA OF RAW MILK

Milk sample	TVC/ml	TCC/ml
1	1.08×10^7	0.43×10^1
2	1.42×10^7	1.65×10^1
3	1.48×10^7	1.07×10^1
4	1.39×10^7	0.45×10^1
5	1.46×10^7	1.06×10^1
6	1.32×10^7	0.26×10^1
7	1.19×10^7	0.31×10^1
8	1.45×10^7	0.19×10^1
9	1.31×10^7	0.21×10^1
10	1.28×10^7	0.95×10^1

TABLE 5: MICRO FLORA OF PASTEURIZED MILK

Milk sample	TVC/ml	TCC/ml
1	2.7×10^7	Absent
2	2.9×10^7	Absent
3	3.2×10^7	Absent

4	2.6×10^7	Absent
5	3.7×10^7	Absent
6	2.5×10^7	Absent
7	3.8×10^7	Absent
8	2.1×10^7	Absent
9	2.9×10^7	Absent
10	3.1×10^7	Absent

RESULTS

There was no significant difference observed with respect to the average counts of E. coli. Raw milk contained an average TVC of 1.338×10^7 cfu/ml and TCC of 0.647×10^1 cfu/ml. Pasteurized milk contained an average TVC of 2095×10^4 cfu/ml and TCC of absent.

TABLE 6: MICROBIOLOGICAL QUALITY OF RAW MILK

Milk sample	MBR time (hours)	Quality of milk
1	1.30	Fair
2	1.15	Fair
3	1.00	Fair
4	1.20	Fair
5	1.10	Fair
6	1.25	Fair
7	1.15	Fair
8	1.20	Fair
9	1.30	Fair
10	1.00	Fair

TABLE 7: MICROBIOLOGICAL QUALITY OF PASTEURISED MILK

Milk sample	MBR time (hours)	Quality of milk
1	6.30	Good
2	7.00	Good
3	6.00	Good
4	6.15	Good
5	6.45	Good
6	7.15	Good
7	6.45	Good
8	6.30	Good
9	7.00	Good
10	6.30	Good

DISCUSSION

The literature reviewed in the present study shown evidence that *Escherichia coli* are frequently occurring organisms in the milk. The methods of production, transportation, handling and sale of milk are entirely unhygienic, the raw milk poses a great hazard to public health without adapting hygienic measures because of the possibilities of contamination with E. coli,the result of milk sample shows that all sample were contaminated with Escherichia coli.

The unclean hands of workers, poor quality of milk, unhygienic conditions of manufacturing units, inferior quality of material used and water supplied for washing the utensils could be the source of accelerating the bacterial contamination.

It is recommended that the milk collection should be done with utmost hygienic measure and that milk should be pasteurized immediately after collection to reduce the load of bacteria especially the pathogenic ones. For this, consciousness and hygiene is required from the point of generation to the point of consumption of these widely consumed milk products.

CONCLUSION

It is recommended that the milk collection should be done with utmost hygienic measure and that milk should be pasteurized immediately after collection to reduce the load of bacteria especially the pathogenic ones. For this, consciousness and hygiene is required from the point of generation to the point of consumption of these widely consumed milk products.

CONFLICT OF INTEREST
None

FUNDING SUPPORT
Nil

REFERENCES
1. Aditya sood, Abhijit kumar, Microbial quality analysis of milk, Chandigarh, 2015.
2. Guetouache, Mourad Guessas, Bettache, Algeria, 2014.
3. Kandpal SD, Srivastava SK, Negi KS, Estimation of quality of raw milk, 2012.
4. Abebe Tessema, Markos Tibbo, Milk quality control, 2009.
5. Oliver S.P, Jayarao B.M and Almeida R.A, Foodborne pathogens in milk and the dairy farm environment, 2005.
6. Rehman ZU, Salaria AM , Effect of storage conditions on the nutritional quality of UHT processed milk, 2005.
7. Sanjeev K Anand, Quality assurance and public health safety of milk and milk Product, 2004.
8. Tatini, S.R. and Kauppi, K.L, Encyclopedia of Dairy Science, 2003.
9. Deeth.H, Aseptic packaging of sterilized milk, 2001.
10. Lewis, M. And Heppell. N, UHT sterilization, 2000.
11. Murphy, S.C. and Boor.K.J, Trouble shooting sources and causes of high Bacteria counts   in raw milk, 2000.
12. E. Koldeweij, U. Emanuelson, L. Janson, Relation of milk production loss to milk Somatic cell count, 1999.
13. Y.T.Grohn, C.E.Mcculloch, C.L.Guard, Effects of clinical mastitis on milk yield in Dairy cows, 1999.
14. Auldist, M.J, Hubble, I.B, Diary technology, 1998.
15. N. Gengler, Persistency of lactation yields, 1996.
16. Bramely, A.J./ McKinnon, Microbiology of Raw milk, New York, 1990.
17. SH. Gwandu, Assessment of Raw Cow Milk Quality,1988.
18. Walstrs and Jenness , Diary Chemistry , 1984.
19. P. Hortet, H. Seegers, loss in milk yield and related composition changes Resulting from clinical mastitis in dairy cows, 1978.
20. Larson, B.L. and Smith, Nutrition and Biochemistry of Milk, New York, 1974.
21. Webb BH, Johnson AH, Alford JA, Fundamental of Dairy Chemistry, 1974.
22. Linzell.J, Peaker, Mechanisms of milk secretion, 1971.
23. www.diary.com, Iowa State University, Ames, 1968.
24. J.C. Hartley, G.W. Reinbold and E.R.Vedamuthu, Bacteriological methods for Evaluation of raw milk quality, Ames, 1968.
25. Smith.V.R, Physiology of lactation in the udder, 1964.
26. Rolleri, G.D., Larson, B.L. and Touchberry, Individual variations in the specific Protei constituents of milk, 1956.
27. Judkins HF, Mack MJ, The Principles of Dairying, 1955.
28. Keservani, R. K., Sharma, A. K., & Kesharwani, R. K. (2017). An overview and therapeutic applications of nutraceutical and functional foods. Recent advances in drug delivery technology, 160-201.
29. Keservani, R. K., Kesharwani, R. K., Vyas, N., Jain, S., Raghuvanshi, R., & Sharma, A. K. (2010). Nutraceutical and functional food as future food: a review. Der Pharmacia Lettre, 2(1), 106-116.
30. Sharma, A. K., Keservani, R. K., & Kesharwani, R. K. (Eds.). (2018). Nanobiomaterials: applications in drug delivery. CRC Press.
31. Vyas, N., Keservani, R. K., Nayak, A., Jain, S., &Singhal, M. (2010). Effect of Tamarindus Indica and Curcuma Longa on stress induced Alopecia. Pharmacologyonline, 1, 377-384.
32. Jarouliya, U., Keservani, Raj K. (2019a). "Pathways Leading to Child Obesity", Global Perspectives on

Childhood Obesity: Current Status, Consequences and Prevention, Second Edition, Edited by Debasis Bagchi, Academic Press, Elsevier, chapter 12, pages 137-146. ISBN: 9780128128404.

33. Jarouliya, U., Keservani, Raj K. (2019b). "Protein function as cell surface and nuclear receptor in human diseases". Medicinal Chemistry with Pharmaceutical Product Development, Edited by Sanjay Bharti, Debarshi Kar Mahapatra, Apple Academic Press, CRC Press, Taylor & Francis Group. Chapter 1, Page 1-32. ISBN: 9781771887106.

34. Singh, P., Kesharwani, R. K., & Keservani, R. K. (2017a). Antioxidants and Vitamins: Roles in Cellular Function and Metabolism. In Sustained energy for enhanced human functions and activity (pp. 385-407). Academic Press. ISBN:9780128054130.

35. Singh, P., Kesharwani, R. K., & Keservani, R. K. (2017b). Protein, carbohydrates, and fats: Energy metabolism. In Sustained Energy for Enhanced Human Functions and Activity (pp. 103-115). Academic Press. ISBN:9780128054130.

36. Kesharwani, R. K., Singh, P., & Keservani, R. K. (2016). "Green Coffee Bean Extract and Chlorogenic Acids: Chemistry and Novel Antioxidant Benefits" In: Green Coffee Bean Extract in Human Health, Edited by D. Bagchi, H. Moriyama, A. Swaroop, Boca Raton, Taylor and Francis, USA. Chapter 1, 1-17. ISBN: 9781498716376.

37. Rane, Bhushan R., Bharath, Mounika S., Patil, Rutuja R., Keservani, Raj K., Jain, Ashish S. (2020b). Novel Approaches in Nutraceuticals, In: Enhancing the Therapeutic Efficacy of Herbal Formulations through Novel Drug Delivery Systems Edited by Rajesh Kumar Kesharwani, Raj K. Keservani, Anil K. Sharma, IGI Global International Publisher, Pennsylvania, USA, Chapter 11, 241-266. ISBN13: 9781799844532.

38. Rane, Bhushan R., Patil, Aishwarya S., Keservani, Raj K., Jain, Ashish S. (2020a). Novel Approaches in Herbal Formulation, In: Enhancing the Therapeutic Efficacy of Herbal Formulations through Novel Drug Delivery Systems Edited by Rajesh Kumar Kesharwani, Raj K. Keservani, Anil K. Sharma, IGI Global International Publisher, Pennsylvania, USA, Chapter 2, 43-68. ISBN13: 9781799844532.

39. Rane, Bhushan R., Tadavi, Sandip A., Keservani, Raj K. (2020c). "Naturopathy" In: Herbal Product Development, Edited By, Anil K. Sharma, Raj K. Keservani, Surya Prakash Gautam, Apple Academic Press, CRC Press, Taylor & Francis Group, Chapter 12, 321-347. ISBN: 9781771888776.