A comparative analysis of quality of milk from different breeds of cattle

Mini V S[1]* and Chaithanya M R

Abstract: Milk can be graded by physical, chemical and microscopical examinations. The Methylene blue reduction test (MBRT) is a chemical test for counting the germs in milk. The methodology employed the enzymatic reduction of methylene blue by a metabolically active organism turning the Methylene Blue colorless. The rate of decoloration by the metabolically active cells can be correlated to the number of viable cells. The principle of methylene blue reduction test depends on the fact that the color imparted to the milk by adding a dye such as methylene blue will disappear more or less quickly, which depends on the quality of the milk sample to be examined. Methylene blue is a redox indicator, that lose its color under the absence of oxygen and is thought to be reduced. The depletion of oxygen in the milk is due to the production of reducing substances in the milk due to the enhanced rate of bacterial metabolism. The dye reduction time refers to the microbial load in the milk and the total metabolic reactions of the microorganism.

Key words: MBRT, Exotic, Indigenous breeds, Decolourisation, Incubation time

Correspondent author: Dr. Mini V S, Assistant Professor (FDP) in Zoology, St. John's College, Anchal Contact No: 7907298991

Introduction

Milk is a good medium for the growth of microorganisms. A variety of microorganism can be found in both raw milk and pasteurized milk. These actively growing microorganisms reduce the oxidation reduction potential of the milk medium due to the exhausted oxygen by the microorganism.

Normally the milk is contaminated with microorganisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeroginosa*, *Enterobacter spp*., *Bacillus spp*., *Paenibacillus spp*., etc. Contaminated milk is one of the important sources for transmission of diseases from animals to humans. The main reason for this contamination is the un-proper handling of milk. Normally milk is contaminated during the milking process by the microorganisms present in the exterior surface of the animals, pipelines such as udder and adjacent areas. Unsterilized dairy utensils such as milking machines, milk cans are also a good source of contamination by the microorganism (Cappuccino and Natalie, 2002).

The milk contains energy sources such as lactose (sugar), nitrogenous compounds such as proteins, amino acids, ammonia, urea etc. for the growth of microorganism. Acid fermentation by the bacteria is common under ordinary conditions. Souring of milk indicates the milk is spoiled. Acid formation in the milk is indicated by the sour flavor, coagulation of milk to give a jelly like curd appearance or clear whey nature. Lactic acid fermentation is common in the raw milk at the room temperature. At temperature from 10 to 37 ^{o}C souring is mainly due to *Streptococcus lactis*, *Enterococci*, *Lactobacilli* and other coliform bacteria. At temperatures from 37 to 50 ^{o} C the most common contaminants of milk are *S. faecalis* and *S. thermophilus*. Thermophilic bacteria such as *L. thermophilus* can grow in the milk at higher temperatures (Nandy and Venkatesh, 2010).

Milk can be graded by physical, chemical and microscopical examinations. The Methylene blue reduction test (MBRT) is a chemical test for counting the germs in milk. It is based on the fact that the coloring matter, methylene blue is blue in the presence of oxygen. When oxygen is removed from milk to which the dye is added, the dye immediately loses its colour (Chacko *et al*., 1988, 1992). The bacteria that are ordinarily found in milk, use oxygen in their growth and multiplication. Many germs will quickly use up all the oxygen, while a small number will require a much greater length of time. Fresh milk has a considerable amount of oxygen dissolved in it. If the dye methylene blue was added to fresh milk, it will turns to a blue colour. This blue colour will remain until all the oxygen is used up, then the milk will almost immediately change blue to white again. The larger the number of bacteria in milk, the sooner the colour change takesplace. Hence the use of methylene blue becomes a valuable test for determining the relative number of bacteria present in a given number of milk samples. The formation of Methylene blue reductase is thus becoming a popular tool for determining the quality of the milk (Hatch, 1927).

Methylene Blue Dye Reduction Test for Assessing the Raw Milk Quality

Methylene Blue Dye Reduction Test, commonly known as MBRT test is used as a quick method to assess the microbiological quality of raw and pasteurized milk. This test is based on the fact that the blue colour of the dye solution added to the milk get decolourized when the oxygen present in the milk get exhausted due to microbial activity. The sooner the decolourization, more inferior is the bacteriological quality of milk assumed to be. This test 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.

Grading of raw milk based on MBRT:

MBRT test may be utilized for grading of milk which may be useful for the milk processor to take a decision on further processing of milk. As per BIS 1479 (Part 3): 1977 criterion for grading of raw milk based on MBRT is as below:

Incubation Time	Quality
5 hrs and above	Very good
3 to 4 hrs	Good
1 to 2 hrs	Fair
Less than ½ hrs	Poor

In India, the rural population depends heavily on the livestock for their livelihood. Among the livestock, dairy cattle play a pivotal role in the economy of rural poor (Nair, 1973). Dairy farming provides a livelihood for a large section of people across our country. In the selection of suitable dairy animals criteria such as breed, pedigree, production records and physical appearance are to be considered (Kunzi, 1984., May *et al*., 2003; Menzy *et al*., 1982). Apart from a large number of non-descript breeds, there are 26 well defined breeds of cattle and 6 breeds of buffaloes in our country (Patel, 1976; Sinha., 1951). Some of the important breeds are Sindhi, Sahiwal, Gir, Jaffarabadi, Jersey, HF, Sunandini, Swiss brown, Red Dane etc.

Dairy breeds or milch breeds are high milk yielding cows and we selected five breeds such as Holstein-Friesian (HF), Jersey, Red Dane, Sahiwal, Swiss Brown and two cross breeds such as JerseyXHF and SunandiniXHF from Aiswarya Dairy farm, Anchal for qualitative analysis of milk samples. Among these Holstein-Friesian (HF), Jersey and Swiss Brown are popular exotic breeds reared in India.

Farm visit: Aiswarya Dairy Farm, Thumbodu, Bharathipuram

Aiswarya dairy farm is situated in Thumbodu near Bharathipuram,Yeroor. The farm gives all the facilities for the neat and hygienic maintenance of dairy animals. The farm consists of 20 cows. Most of them are indigenous and some exotic breeds are also there. The indigenous breeds include Gir (Gujarat), Sahiwal (Hyderabad), Vechur (Kerala) and Kasaragod Kullan (Kerala). Among these the milk of Vechur has high medicinal quality and economic value. The exotic breeds found in that farm include Jersey, HolsteinFriesian, BrownSwiss, Red Dane etc. All these breeds have their own identification features. Cross breeds like Sunandini X HF and Jersey X HF were also found.

Sunandini is a composite breed of cattle developed in India by crossing non descript cattle with exotic breeds like Brown Swiss, Jersey, HF etc. We collected milk samples for our experiments from pure breeds such as HF, Jersey, Red Dane, Brown Swiss and Sahiwal. Among these HF, Jersey, Red Dane and Brown Swiss were exotic breeds and Sahiwal is an indigenous breed. The crossbreed samples we collected include Jersey X HF and Sunandini X HF.

Objectives

(1) To check the quality of non-refrigerated and refrigerated milk samples from different breeds of cattle,

(2) To compare the breed wise quality of milk samples from indigenous and exotic breeds of cattle.

Materials Required:

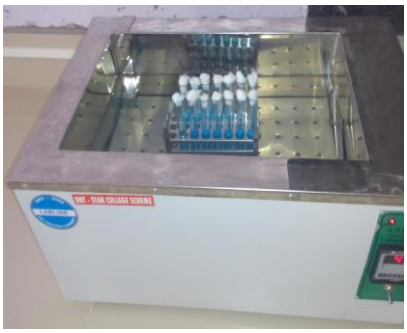
Milk samples to be analyzed, Methylene blue reductase test (MBRT) dye solution (dye concentration 0.005%), Test tubes, Test tube racks, Measuring cylinders (10 ml), Droppers, Water bath (42 ± 1^0C), Cotton, Bunsen burner.

Procedure:

The test has to be done under sterile conditions. Take 10 ml milk sample in sterile MBRT test tube. Add 1 drop of redox indicator MBRT dye solution (dye concentration 0.005%) to each test tube containing milk sample. Tighten the test tube mouth with cotton swab. Gently shake the tubes at about four or five times to ensure proper mixing of the methylene blue solution. Keep the tubes in the water bath at 42 ± 1^0C. This time is recorded as the beginning of the incubation period. Note the incubation time. Incubation time is the time elapsed for the colour to turn whitish appearance (Decolourization is considered complete when only a faint blue ring (about 5mm) persists at the top. Stabilize the tubes for 5 minutes.

Image 1, 2 and 3: Incubation and decolourisation of samples inside the water bath (42±1^{0}C)





Results obtained

(1) Starting time of incubation at 10 am (Without Refrigeration)

Name of breed	Time of decolourization	Total incubation time	Quality
Holstein-Friesian	10.40 AM	40 Minutes	Poor
Jersey	10.40 AM	40 Minutes	Poor
Red Dane	10.40 AM	40 Minutes	Poor
Sahiwal	10.40 AM	40 Minutes	Poor
Swiss Brown	11.30 AM	1 Hour 30 Minutes	Fair
Jersey X HF	11.05 AM	1 Hour 5 Minutes	Fair
Sunandini X HF	10.50 M	50 Minutes	Poor

(2) Starting time of incubation at 11 am (After refrigeration for 1 Hour)

Name of breed	Time of decolourization	Total incubation time	Quality
Holstein-Friesian	11.50 AM	50 Minutes	Poor
Jersey	11.40 AM	40 Minutes	Poor
Red Dane	11.30 AM	30 Minutes	Poor
Sahiwal	11.20 AM	20 Minutes	Very Poor
Swiss Brown	01.15 PM	2 Hours 15 Minutes	Good
Jersey X HF	11.55 AM	55 Minutes	Poor
Sunandini X HF	11.40 AM	40 Minutes	Poor

Discussion

Methylene Blue Dye Reduction Test (MBRT) has uesd in evaluating cell viability in a very short time (Nandy et al., 2007). In the current study, MBRT was adopted to develop a protocol for determining the quality of milk samples from different breeds of cattle. The modified protocol was extended to demonstrate the qualitative comparison of milk samples from indigenous, exotic and cross breeds. The methodology employed the enzymatic reduction of methylene blue by a metabolically active organism turning the Methylene Blue colorless. The rate of decolouration by the metabolically active cells can be correlated to the number of viable cells. For this purpose, the slope of the MB decolouration rate was calibrated with respect to colony forming units (CFU) obtained through plating. This method was successfully employed to characterize the viability of *E. coli* and *B.subtilis* (Bapat et al., 2006).

Studies revealed that MBRT can be successfully employed to quantify viable cell count in a very short time (less than 4min). Methylene Blue (MB) dye has been employed to check for the overall microbial load and quality control of milk and other liquid foods (Impert et al., 2002). Because of its size and positive charge, it does not enter into the cells appreciably. It gets reduced to 'leuko' or colourless form of MB at the cell surface via reductase enzymes present in the cell membrane. This colourless form of methylene blue (MBH) is uncharged, lipophilic, and enters cells by diffusion across the plasma membrane where it is re-oxidized and thus sequestered within the cells (May et al., 2003). If oxygen is available, reduced MB can be oxidized by the mitochondrial electron transport system. This will result in the reappearance of the blue color. Up to now, the exact mechanism of dye reduction is not known, but some reports available suggest that MB is reduced by transmembrane reductases (Bongard et al., 1995; Merker et al., 1997). This mechanism is applied to evaluate the microbial load in a liquid medium. The shorter time required for the disappearance of the blue colour is indicative of a higher microbial load. It is assumed that greater the number of microorganisms, more the oxygen demand and lesser the oxygen concentration in the medium resulting in the faster disappearance of the colour. This fact has been used as a broad indicative test of a microbial load representing microbial quality of milk.

There are so many factors plays significant role in the reduction of methylene blue in milk. Microbs such as bacteria may play but an insignificant part in the reduction of methylene blue in milk, though their de-oxygenating effect may be of influence in the commercial application of the test (Jepras *et al*., 1997). Milk as it exists in the udder, or milk drawn anaerobically, reduces methylene blue almost instantaneously, whereas the same milk exposed to oxygen will usually take more than ten hours to reduce. The oxidation-reduction potential of anaerobically drawn milk is much lower than the same milk exposed to oxygen, and in accordance with its behaviour toward methylene blue (Bapat *et al*., 2006). Evidence is given for the presence of a redox system, present in low concentration, as responsible for the reduction of methylene blue. Although the addition of small amounts of cysteine or glutathione to milk leads to the reduction of methylene blue, their absence from milk excludes them as possible factors in the normal reduction. The possibility that lactoflavin may furnish the redox system is suggested (Bongard *et al*., 1995). The reduction of methylene blue in milk is also catalysed by light in the visible spectrum. The presence of light fastens the reduction rate; hence the test tube under observation should be tightened properly (Cappuccino and Natalie, 2002). Uniform concentration of methylene blue dye should use in all test samples since addition of more methylene blue dye will result in more reduction time. Increased incubation time reduces the reduction time since the activity of some organism

increases with increased incubation temperature. The test tubes, periodically invert at regular intervals during incubation time to improve the accuracy of the test result. Otherwise microorganisms may not be evenly distributed in the milk sample leading to wrong result interpretations (William *et al*., 1998).

Summary and Conclusion

In the first experiment we placed the non-refrigerated, fresh milk samples in the water at 10 AM. The reduction was observed simultaneously after 40 minutes in the first four samples taken from the breeds, Holstein Friesian, Jersey, Red Dane and Sahiwal. The second colour change was observed after 50 minutes in the samples taken from crossbreed, Sunandini X HF. Among the samples we took, fair quality was observed in the sample of exotic breed, Swiss Brown and cross breed Jersey X HF. In the second experiment, the milk was refrigerated for one hour and placed in the water bath. The decolourisation was first observed in the sample of Sahiwal, the indigenous species after 20 minutes. It indicates that the milk has very poor quality. Immediately after this, decolourisation was found in the following breeds; Red Dane, Jersey, crossbreed- Sahiwal X HF, HF and crossbreed- Jersey X HF within one hour. It indicates the poor quality of milk. Samples of Red Dane showed high quality by its decolourization after 2 hours and 15 minutes.

We expected that the milk quality of indigenous breeds were greater than that of exotic breeds. But our results showed that the milk quality of the indigenous breed, Sahiwal was very poor when compared to other exotic and crossbreeds taken. It may be due to some factors such as the contamination of microorganisms, light exposure during milk transferring etc. So from our results we conclude that the MBRT is very valuable and efficient test for determining the quality of milk samples. The advantage of the test is that the test can be easily made by anyone of ordinary skill and intelligence.

Acknowledgement

We would like to thank Department of Zoology, St. John's College, Anchal, DBT-STAR College Scheme and DST-INSPIRE Fellowship scheme for providing all the necessary facilities for the successful completion of our work.

References

Acharya,R.M. 1970.Crossbreeding of zebu cattle with exotic breeds for milk production.*Indian J.Anim.Sci.*,40(20):110-119.

Bhat ,P.N.,Taneja,V.K. and Garg,R.C. 1978. Effect of crossbreeding on reproduction and productive traits..*Indian J.Anim.Sci.*,48(2):7-78.

Bapat P, Nandy SK, Wangikar P and Venkatesh KV (2006). Quantification of metabolically active biomass using Methylene Blue dye Reduction test (MBRT): Measurement of CFU in about 200 seconds *J.Microbiol. Methods*. 65: 107-116.

Bongard RD, Merker MP, Shundo R, Okamoto Y, Roerig DL, Linehan H, Dawson CA (1995). Reduction of thiazine dyes by bovine pulmonary arterial endothelial cells in culture. *Am. J. Physiol*. 269: 78–84.

Chacko,C.T. and Jose,T.K 1988. Crossbreeding for milk production enhancement in Kerala.*Indian Dairyman*,40:697-709.

Chacko,C.T.,Unnithan,N.R. and Schneider, F.1988 .Progent testing in a large scale crossbreeding programme in Kerala:scope and limitations.*proc.VI World conf.Anim.Prod.*,Helsinki,Finland.

Chacko,C.T.,Angehrn,B. and Gopakumar,C.P. 1992. Consequences of MOET in crossbreeding programme: modelling with the Kerala programme.*Proc. Nat.Sem.Progeny Testing of Bulls in Tropics*,Trivandrum,Kerala,India.

Hatch KL (1927). The use of the methylene blue test in the grading of milk samples for cheese factories. Twenty eighth annual convention Southern Wisconsin Cheese makers ssociation, December9, 1927.

Impert O, Katafias A, Kita P, Mills A, Pietkiewicz-Graczyk A and Wrzeszcz G (2002). Kinetics and mechanism of a fast leuco-Methylene Blue oxidation by copper (II)–halide species in acidic aqueous media. Dalton Trans, pp. 348-353.

Jepras RI, Paul FE, Pearson SC and Wilkinson MJ (1997). Rapid Assessment of Antibiotic Effects on *Escherichia coli* by *bis*-(1, 3-Dibutylbarbituric Acid) Trimethine Oxonol and Flow Cytometry. Antimicrob. Agents Chemother 41: 2001-2005.

Kerala Livestock Department Board. *Annual report 1985-86,p*.43.Trivandrum,Kerala,India,KLDB.

Kunzi,N. 1984. *Consultancy report of KLD Board* .Trivandrum,Kerala,India.

May JM, Qu Z and Whitesell RR (2003). Generation of oxidant stress in cultured endothelial cells by methylene blue: protective effects of glucose and ascorbic acid. *Biochem. Pharmacol*. 66: 777–784.

Menzi,M., Kropf,W. and Von Siebenthal,W. 1982. An exotic cattle population under a tropical environment.Wld *Anim.Rev*.,44: 24-29.

Merker MP, Bongard RD, Linehan JH, Okamoto Y, Vyprachticky D, Brantmeier BM, Roerig DL, Dawson CA (1997). Pulmonary endothelial thiazine uptake: separation of cell surface reduction from intracellular reoxidation. *Am. J. Physiol*. 272: 673–80.

Nair,P.N.R. 1973. Evolutionary crossbreeding as a basis for cattle development in Kerala state(India).P.112. Faculty of Veterinary medicine, University of Zurich,Switzerland (Thesis).

Nandy SK, Bapat P and Venkatesh KV (2007). Sporulating Bacteria prefers predation to cannibalism in mixed cultures. FEBS Lett. 581: 151-156.

Nandy SK and Venkatesh KV (2010). Application of methylene blue dye reduction test (MBRT) to determine growth and death rates of microorganisms *Afr. J.Microbiol. Res*. 4(1): 061-070.