Determination of Microbiological Quality of Milk from Different Breeds of Cattle Using Methylene Blue Reduction Test

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Abstract ***Milk is very important due to its special nutritive value and important role for human and animal health. It has all the substances needed by organisms in its easiest assimilable form. The aim of the present study is to determine the microbiological quality of milk samples from different breeds of cattle using methylene blue reduction test (MBRT). It is a rapid and inexpensive test for analysing the microbiological quality of milk. Milk samples were collected from indigenous and exotic breeds of cattle such as Holstein-Friesian (HF), Jersey, Gyr, Chembra, and cross breeds such as Jersey cross, Red Sindhi, Gyr cross, Sindhi-Jersey cross and four different cross varieties of Jersey with HF. Production of maximum quantities of high quality milk is an important goal of every dairy operation. On the other hand, poor milk quality affects all segments of the dairy industry, ultimately resulting in milk with decreased manufacturing properties and dairy products with reduced shelf-life. The result showed that 5 samples found to be poor quality and refrigeration increased the quality of milk. However, the study shows the significance of proper handling and processing of milk for healthy consumption of milk.***

Key words: MBRT, Milk, Jersey, Holstein-Friesian, Red Sindhi, Gyr cross

I. INTRODUCTION

Milk, a highly nutritious food which contains proteins, fats, carbohydrates, vitamins, minerals and essential amino acids, all at a near neutral pH and at a high water activity, provides an ideal environment for the growth of many microorganisms (Quigley *et al*., 2013). It has been generally accepted that these microorganisms can also negatively impact on milk quality and shelf life. Previous reports suggested that during refrigeration, psychotolerant bacteria can proliferate and produce extracellular lipases and proteases, result in milk spoilage (Desmasures and Gueguen, 1997; Hantsis-Zacharov and Halpern, 2007). Similarly, microorganisms can bring about the fermentation of milk through the production of lactate and have a variety of different impacts on the sensory, texture, flavour and organoleptic properties of resultant products (Wouters *et al*., 2002). In contrast, it has been reported that the beneficial microorganisms present in raw milk can contribute to health by aiding digestion or by reducing the frequency of allergies, including asthma and atopic diseases, in individuals who consume raw milk during the early years of life (Deberry *et al*., 2007; Braun-Fahrlcander and Von Mutius, 2011). However, rapid, simple and inexpensive microbiological quality determination methods including Methylene Blue Reduction test (MBRT) could be commonly used as a quick method to assess the microbiological quality of raw and pasteurized milk.

Many microbial communities are complex and they are comprised of many different taxonomical groups of microorganisms (Quigley *et al*., 2013). Previous studies suggested that raw milk is an example of an environment that contains a diverse and complex microbial population (Quigley *et al*., 2011; Vacheyrou *et al*., 2011). Microbial contamination of raw milk can occur from various sources like air, milking equipment, feed, soil, grass etc. In appropriate conditions milk can act as a carrier of disease from milking animals to human *via* microorganisms (Muhammad Naseer Abbas, 2013). Harmful bacteria in raw milk causes various diseases like diarrhoea, stomach cramping, and vomiting etc. All cases of dairy illness continued to be of bacterial origin, pathogens that have involved in communicable diseases associated with the consumption of milk include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter*, *Yersinia*, Pathogenic *Escherichia coli* and *Clostridium botulinum* (Adesiyun *et al*., 1995). The detection of harmful microbial count in milk indicates the hygienic level exercised during milking includes cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal. Milk from healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the cow until it is used for further processing. Milk produced under hygienic conditions from healthy animals should not contain more than 5X105cfu/m (O'Connor *et al*., 1994).

In the present study, we selected 12 Dairy breeds or milch breeds such as Holstein-Friesian (HF), Jersey, Gyr, Chembra, and cross breeds such as Jersey cross, Red Sindhi, Gyr cross, Sindhi X Jersey cross and different cross varieties of Jersey with HF like Jersey X HF (equal), Jersey X HF cross (leg Variety), Jersey 75 X HF 25 cross and Jersey 25 X HF 75 cross from Gilbert dairy farm at Chaamavila, near Kattakada for qualitative analysis of milk samples. The farm gives all the facilities for the neat and hygienic maintenance of dairy animals. The farm consists of 22 cows. Among these, Holstein-Friesian (HF) and Jersey are popular exotic breeds reared in India. All these breeds have their own identification features.

Plate.1: Different breeds of cattle such as Holstein-Friesian (HF), Jersey 75 X HF 25, Jersey X HF cross breeds (leg variety), Jersey 25 X HF 75, Jersey X HF (equal) and Jersey breeds.

Holstein-Friesian (HF) breed was developed in the northern parts of Netherlands, especially in the province of Friesland. They are ruggedly built and they possess large udder. They are the largest dairy breed and mature cows weigh as much as 700kg.

Plate .2: Different breeds of cattle such as Jersey cross, Red Sindhi, Sindhi Jersey cross, Gyr, Chembra and Gyr cross.

They have typical marking of black and white that make them easily distinguishable. The average production of cow is 6000 to 7000 kg per lactation. However, the fat content in their milk is rather low. There are different cross breeding varieties of HF with jersey. In Jersey X HF (equal) cross breed, the body is small like Jersey but it yield highest milk production similar to HF. However, in Jersey 75 X HF 25 cross breeds, both the size and milk production is less. It is reverse in Jersey 25 X HF 75 cross breeds. Similarly, the HF percentage is more in Jersey X HF cross breeds (leg variety). However, the main objective of the present study was to determine the breed wise quality of raw milk and refrigerated milk (refrigeration for one hour) samples from indigenous and exotic breeds of cattle.

Gir breeds are originated in Gir forests of South Kathiawar in Gujarat. This breed produces the highest yield of milk amongst all breeds in India. It has been used extensively to make hybrid varieties in India and in other countries like Brazil. They are unicoloured. Horns are absent but short horns with suspended necks are seen in Gir cross. Jersey is the smallest of the dairy types of cattle developed on island of Jersey, U.K. In India, this breed has acclimatized well and is widely used in cross breeding

with indigenous cows. They are small sized. The typical colour of jersey cattle is reddish fawn. Compact and angular body with dished fore head are present. These are economical producers of milk with 5.3% fat content. Jersey crossbreeds are developed by crossing this breed with jersey in different regions. These are moderate sized breeds. However, the fat content in their milk is rather high. Red Sindhi is originated in Sindh, Pakistan. Now it is widespread. This breed mostly found in Karachi and Hyderabad district of Pakistan. They are smallest exotic cattle breeds. Colour is red with shades varying from dark red to light. It is widely used in crossbreeding programmes. Sindhi-Jersey cross is one among them. They are good milk producers but they possess small udders. Chembra is a local breed with average milk production of 12 L. Its milk has high protein content.

II. MATERIALS AND METHODS

Materials required

Milk samples, Methylene blue reductase test (MBRT) dye solution (dye concentration 0.005%), test tubes, test tube rack, measuring cylinders (10 ml), 1 ml Pipette, water bath (37±1°C), Cotton.

Procedure

All glassware and rubber stoppers sterilized either in an autoclave or in boiling water. Milk sample of 10ml was taken in a sterile test tube. Methylene blue solution (dye concentration 0.005%) of 1ml was added to each test tube containing 10 ml raw milk sample. The test tubes were stoppered with rubber bungs, which had been previously sterilized by immersing in boiling water for at least 10 minutes. Gently invert the tubes at about four or five times to ensure proper mixing of the methylene blue solution. Then tubes were kept in thermostatically controlled water bath previously set to 37 ± 1^0C. This time is recorded as the beginning of the incubation period. Observe every half hour for any decolourization in the tubes. 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. The total time taken for decolourization was recorded and the average of times in duplicates was taken to interpret the methylene blue reduction time of that sample.

Table.1: RESULT INTERPRETATION TABLE

Reduction time	Grade
Within 30 minutes	Very poor
Between 30minutes and 1 hour	Poor
Between 1 to 2 hours	Fair
Between 2 to 4 hours	Good
Between 4 to 5 hours	Very good
More than 5 hours	Excellent

III. RESULT

Milk collected from different breeds of cattle was refrigerated for one hour. Methylene blue reduction time and their quality of raw milk and refrigerated milk are shown in Table.1. By comparing the results with standard table for MBRT (see Result interpretation Table 1), the milk samples were classified as of different grades (Table 2). The results support the view that cold milk holds more oxygen than warm milk (Fig.1).

Table: 2. Methylene Blue Reduction time of different milk samples

Name of Breed	Reduction time (hrs) of raw milk samples	Quality	Reduction time (hrs) of refrigerated samples	Quality
Gyr	0.35	Poor	0.40	Good
Gyr Cross	0.45	Poor	4	Very Good
Pure Jersey	5	Very Good	6	Excellent
Jersey Cross	2.20	Good	3	Good
Red Sindhi	1	Fair	2	Fair
Sindhi Jersey Cross	3	Good	3	Good
Chembra	1	Fair	1.30	Fair
Holstein-Friesian	0.4	Poor	0.50	Poor
Jersey X HF (Equal)	0.45	Poor	2.35	Good
Jersey 75 X HF 25	3.5	Good	6	Excellent
Jersey 25 X HF 75	1.30	Fair	1.45	Fair
Jersey X HF (Leg Variety)	0.45	Poor	1	Fair

IV. DISCUSSION

Methylene Blue 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 assed to the milk get decolourized when the oxygen present in the milk get exhausted due to microbial activity (Chacko *et al*., 1988, 1992) and the rate of decoloration by the metabolically active cells can be correlated to the number of viable cells (Nandy *et al*., 2010). The present study demonstrates the microbiological quality of both raw and refrigerated milk samples from different breeds of cattle. 12 milk samples from indigenous and exotic breeds of cattle were tested and our results showed that the milk quality of gir varieties and some breeds of HF cross were poor when compared to other breeds. However refrigeration of milk samples for one hour improved the milk quality. Previous studies suggested that the bacteria that are ordinarily found in milk use oxygen in their growth and multiplication (Impert *et al*., 2002). The depletion of oxygen in milk is due to the production of reducing substances in the milk due to the enhanced rate of bacterial metabolism. Many germs will quickly use up all the oxygen, while a small number will require a much greater length of time. The larger the number of bacteria in milk, the sooner the decolourization and more inferior is the bacteriological quality of milk assumed to be. Similarly, this test is widely used at the dairy reception dock, processing units and milk chilling centers where it is followed as acceptance or rejection criteria for the raw and processes milk (Hatch, 1927).

Methylene blue dye has been employed to check for the microbial load and quality control of milk and other liquid foods (Impert *et al*., 2002). If oxygen is available in the sample, reduced MB can be oxidized by the mitochondrial electron transport system (Merker *et al*., 1997). Though the exact mechanism of dye reduction is not known, the previous studies suggested that MB is reduced by transmembrane reductase (Bongard *et al*., 1995; Merker *et al*., 1997) and this mechanism is applied to evaluate the microbial load in a liquid medium. Close relationship between oxidation reduction potential and methylene blue reduction test to determine the oxygen content of raw milk for grading has been suggested (Jenitta *et al*., 2014). The study reported a parallel relationship between oxidation reduction potential and methylene blue reduction time (Jenitta *et al*., 2014). 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 reduction time reduces the reduction time since the activity of some organisms 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 (Frazier and Westhoff, 1998).

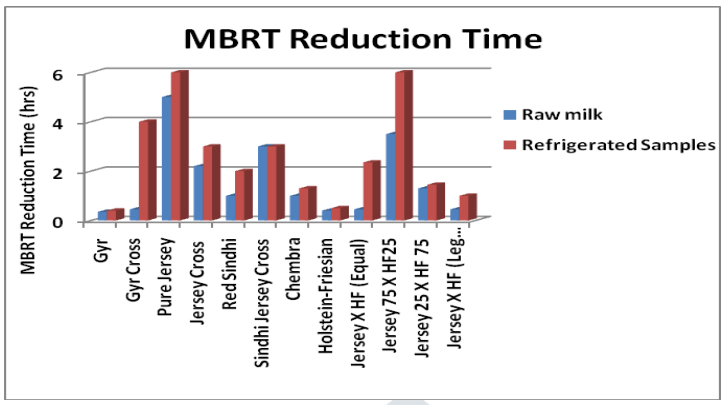

Fig.1: MBRT Reduction time of raw and refrigerated milk collected from different breeds of cattle.

It is well known that milk serves as a good medium for the growth of many microorganisms, especially, Lactobacillus, Streptococcus and Micrococcus sp. Bacterial contamination of raw milk can originate from different sources from animals such as air, milking equipment, feed, soil, faces and grass (Torkar and Teger, 2008). Milk microflora includes spoilage and pathogenic microorganisms. Many milk borne diseases such as tuberculosis, brucellosis and typhoid fever are known (Goff and Horst, 1997). Milk is spoiled by a wide range of microorganisms some of which are pathogenic and are responsible for milk borne diseases. The milk is very easily contaminated if collected unhygeinically and handled carelessly leading to quick spoilage (Prajapati, 1995; Chatterjee *et al*., 2006) and is often contaminated by Escherichia coli bacteria under poor sanitary conditions which can affect public health. The coliform group of bacteria is defined as the indicator of suitability of milk for consumption (Standard method committee, 1981).

V. CONCLUSION

The present study analysed the microbiological quality of different varieties of cattle including both exogenous and indigenous varieties and the result showed that the milk samples which were not processed was found to be in different grades. In fact, negligence in this area may result in serious contamination and the production of low quality product to the consumers. Thus to prevent from milk borne diseases, proper handling and processing of milk should be recommended for consumption of milk.

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