iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** VI **Month of publication:** June 2019

DOI: http://doi.org/10.22214/ijraset.2019.6346

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue VI, June 2019- Available at www.ijraset.com

Analysis of Anti-Microbial Effect of Curcumin in Milk from Different Breeds of Cattle

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Abstract: ***Curcumin is a phenolic component obtained from turmeric and which gives the yellow colour to turmeric. As well as being a natural colourant, curcumin has antioxidant, antimicrobial, anti-cancer, analgesic, anti-ulcer and anti-inflammatory effects. The antimicrobial effect of curcumin is stated to be effective both in wound therapy and against food pathogens. Nowadays, curcumin is widely used in dairy products, fats, oils etc. Dairy breeds or milch breeds are high milk yielding cows and we selected four breeds such as Sunandini, Sindhi, Holstein-Friesian (HF), Jersey, and three cross breeds such as HF X Swiss Brown, Jersey X Sunandini and Sunandini X HF from Aiswarya Dairy farm, Anchal and Thenginal Dairy farm, Chanapetta for analyzing the anti-microbial property of curcumin on milk samples. Among these Holstein-Friesian (HF), Jersey and Swiss Brown are popular exotic breeds reared in India.***
Keywords: Turmeric, MBRT, Refrigeration, Incubation

I. INTRODUCTION

Milk is a food of exceptional interest. Not only is milk an excellent food for the very young, but humans have also adapted milk, specifically cow's milk, as a food substance for persons of all ages. Many specialized milk products like cheese, yogurt, butter, and ice cream are staples of our diet. Milk is probably the most nutritionally-complete food that can be found in nature. This property is important for milk, since it is the only food young mammals consume in the nutritionally significant weeks following birth. Whole milk contains vitamins (principally thiamine, riboflavin, pantothenic acid, and vitamins A, D, and K), minerals (calcium, potassium, sodium, phosphorus, and trace metals), proteins (which include all the essential amino acids), carbohydrates (chiefly lactose), and lipids (fats) (Minard, 2000).

Production of maximum quantities of high quality milk is an important goal of every dairy operation. Poor quality milk affects all segments of the dairy industry, ultimately resulting in milk with decreased manufacturing properties and dairy products with reduced flavor quality and reduced shelf-life (Miller *et al*., 2007). Several different methods are used to assess milk quality. Recently, some milk buyers and dairy processing plants have made changes to their milk quality requirements for incoming raw milk. These changes have occurred, in part, by demands from retailers and major food service companies requiring milk with a higher quality to achieve a longer shelf-life (Miller *et al*., 2008).

A safe, wholesome, abundant and nutritious milk supply should be the goal of every dairy producer in the world. Safety and quality of dairy products start at the farm and continue throughout the processing continuum. To meet increased raw milk quality standards, producers must adopt production practices that reduce bacterial contamination of bulk tank milk. Use of effective management strategies to minimize contamination of raw milk will help dairy producers achieve these important goals (Gillespie *et al*., 2008). Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. Curcuma longa is a medicinal plant that botanically is related to Zigberiacea family, (Chattopadhyay *et al*, 2005). Turmeric powder, derived from the rhizome of *Curcuma longa*, is commonly used as a spice, food preservative, and food coloring agent, (Aggarwall *et al*, 2007; Menon and Sudheer, 2007). It also has a long history of therapeutic use, (Chattopadhyay *et al*., 2005). There is a lot of natural ingredient which we can use as natural remedy nowadays. Most of natural remedy that have been used since long ago are honey, turmeric, and milk. Those ingredients still being used until today and people believe that they provide many great benefits for health. Among these turmeric contains many active ingredients which have biological effect to human body. Curcumin is the main substance contained in turmeric and it used to be called as "holy powder" and it's the most active component in turmeric.

Turmeric is a spice obtained from the *Curcuma longa* L. plant (Stankovic, 2014). It has an important place in Indian medicine (Aggarwal *et al*., 2007). Curcumin is a phenolic component obtained from turmeric (Sharma *et al*., 2005; Stankovic, 2014). Curcumin, which gives the yellow colour to turmeric, was first isolated almost two centuries ago (Aggarwal *et al*., 2007). As well as being a natural colourant, it has been reported that curcumin has antioxidant, antimicrobial, anti-cancer, analgesic, anti-ulcer and anti-inflammatory effects (Aggarwal *et al*., 2007; Akpolat *et al*., 2010; Stankovic, 2014). The antimicrobial effect of curcumin is

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stated to be effective both in wound therapy and against food pathogens (Pattaratanawadee *et al.*, 2006; Wang *et al.*, 2012). Curcumin is a food additive (E100) used as a colourant. Curcumin has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the EU Scientific Committee on Food (SCF). Curcumin is widely used to colour many foods. Curcumin is listed for use in dairy products, fats, oils and fat emulsions, edible ices, fruit and vegetable products, confectionery, cereal products, bakery wares, meat and meat products, fish and fish products, eggs and egg products, spices, soups, sauces and protein products, foodstuffs intended for particular nutritional uses, beverages, ready to eat savouries and composite foods. Used levels of curcumin are in the range from 5 to 500 mg/kg depending on the food category. JECFA specifications define only curcumin extracted from natural source materials. It can also be produced by chemical synthesis but synthetic curcumin is not used as a food additive (Stankovic, 2014).

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. 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. We investigated whether the external application of turmeric reduces the growth of bacteria in different milk samples. Dairy breeds or milch breeds are high milk yielding cows and we selected four breeds such as Sunandini, Sindhi, Holstein-Friesian (HF), Jersey, and three cross breeds such as HF X Swiss Brown, Jersey X Sunandini and Sunandini X HF from Aiswarya Dairy farm, Anchal and Thenginal Dairy farm, Chanapetta for analyzing the anti-microbial property of curcumin on milk samples. Among these Holstein-Friesian (HF), Jersey and Swiss Brown are popular exotic breeds reared in India.

II. OBJECTIVES

1) To check the quality as well as anti-microbial effect of curcumin on non- refrigerated and refrigerated milk samples from different breeds of cattle,
2) To compare the breed wise quality and effect of curcumin on quality of milk samples from different breeds of cattle.

A. Materials Required

Milk samples to be analyzed, Methylene blue reductase test (MBRT) dye solution (dye concentration 0.005%), Powdered curcumin, Test tubes, Test tube rack, Measuring cylinders (10 ml), Dropper, Water bath (42±1^{0}C), Cotton

B. Procedure

Obtain sterilized test tubes to begin the experiment. Fill each test tube with 10mLof milk. Add 1mg of turmeric into each test tubes creating turmeric milk solution. Add a drop of Methylene blue to each test tube. 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±10C. 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. Measure and record the amount of time taken for the contents of each test tube to turn white. The control (normal) group in this experiment are the milk samples without turmeric.The contents of the test tubes with turmeric took an average of 2 to 7 hours longer to turn white in MBRT compared to the control test tubes with plain milk.



Control and Turmeric milk samples

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Decolourisation in different milk samples

III. RESULTS OBTAINED

MBRT-Starting time of incubation at 10 AM (Without Refrigeration)

Name of breed	Total incubation time of normal milk	Quality of normal milk	Total incubation time of turmeric milk	Quality of turmeric milk
Sunandini	1 Hour 40 Minutes	FAIR	Slight colour variation after 4 Hours	VERY GOOD
Sindhi	1 Hour 30 Minutes	FAIR	Slight colour variation after 3 Hours	GOOD
Holstein-Friesian (HF)	3 Hours	GOOD	No colour change up to 6 Hours	Excellent
Jersey	2 Hour 40 Minutes	GOOD	No colour change up to 6 Hours	Excellent
HF X Swiss Brown	2 Hour 55 Minutes	GOOD	No colour change up to 6 Hours	Excellent
Jersey X Sunandini	3 Hour 15 Minutes	GOOD	No colour change up to 6 Hours	Excellent
Sunandini X HF	1 Hour 35 Minutes	FAIR	No colour change up to 6 Hours	Excellent

MBRT- Starting time of incubation at 10 AM (After refrigeration for 24 Hours)

Name of breed	Total incubation time of normal milk	Quality of normal milk	Total incubation time of turmeric milk	Quality of turmeric milk
Sunandini	40 Minutes	POOR	Slight colour variation after 2 Hours	GOOD
Sindhi	10 Minutes	VERY POOR	45 Minutes	POOR
Holstein-Friesian (HF)	30 Minutes	VERY POOR	Slight colour variation after 2 Hours	GOOD
Jersey	2 Hour 30 Minutes	GOOD	Slight colour variation after 3 Hours	GOOD
HF X Swiss Brown	2 Hour 45 Minutes	GOOD	Slight colour variation after 3 Hours	GOOD
Jersey X Sunandini	3 Hours	GOOD	Slight colour variation after 4 Hours	VERY GOOD
Sunandini X HF	1 Hour 30 Minutes	FAIR	Slight colour variation after 2 Hours	GOOD

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IV. DISCUSSION

Turmeric has natural antibiotic properties and milk is a rich source of calcium. The inclusion of these two natural ingredients in everyday diet can prevent diseases, infections and is an effective remedy to fight hazardous environmental toxins. Curcumin have a wide spectrum of biological actions such as anti-inflammatory, antioxidant (Mohammadi *et al.*, 2013; Menon and Sudheer 2007), anticancer (Lotempio *et al.*, 2005) antidiabetic (Aggarwal *et al*., 2007), antiallergic, antiviral, antiprotozoal and antifungal activities (Chatopadhyay *et al*., 2005). The antiviral and antibacterial properties of turmeric can help fight the infections while the anti-inflammatory property helps relieve the symptoms. It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids and inhibits cancer at initiation, promotion and progression stages of tumor development (Hosny *et al*., 2011). The reaction in this instance is caused by an enzyme associated with the micro organisms and most probably is an oxidation-reduction in which the methylene blue acts as a hydrogen acceptor. Turmeric appears to inhibit microorganism growth in milk.

Milk quality control is the use of approved test to ensure the application of approved practices, standards and regulation concerning the milk and milk products. The tests are designated to ensure that milk products meet accepted standards for chemical composition and purity as well as levels of different micro organisms. 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). 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).

V. SUMMARY AND CONCLUSION

The reduction was observed simultaneously around one and half hour after in the non-refriferated milk samples taken from Sunandini, Sindhi and Sunandini X HF. But the samples with turmeric took three to four hours to impart a slight colour change, denoting the antimicrobial effect of turmeric in milk. The samples from Holstein-Friesian (HF), Jersey, HF X Swiss Brown and Jersey X Sunandini took an average of two hours to reduce the methylene blue, indicating that the milk is with good quality. The turmeric milk samples of the same breeds maintain an excellent quality with no colour change up to six hours. Among the refrigerated milk sampes the decolourisation was first observed in the normal milk sample of Sindhi within 15 minutes, indicating that the milk has very poor quality. The turmeric milk sample of Sindhi showed decolourisation after 45 minutes indicating a poor quality. Thus turmeric changes the very poor quality refrigerated milk sample from Sindhi to poor quality. Turmeric also changes the very poor quality refrigerated milk of HF and poor quality milk of Sunandini to good quality. Thus from our results it was concluded that turmeric maintains an excellent quality to non-refrigerated and good quality to refrigerated milk samples.

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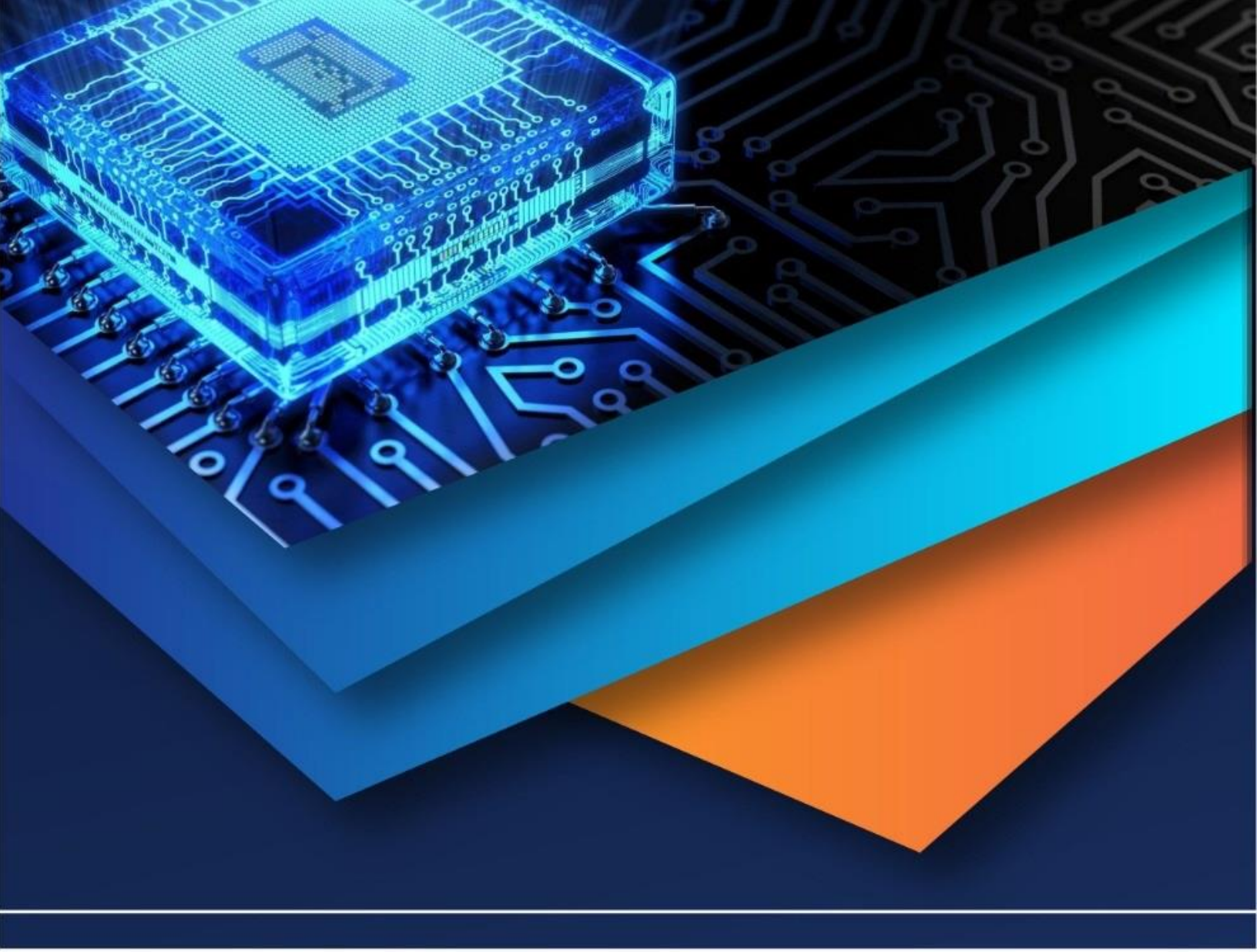
VI. ACKNOWLEDGEMENT

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.

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