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RESEARCH ARTICLE

MICROBIOLOGICAL APPROACH OF CURD SAMPLES COLLECTED FROM DIFFERENT LOCATIONS OF TAMILNADU INDIA

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ABSTRACT

Microbiological and biochemical analysis of different curd samples collected from different locations of Tamilnadu India were enumerated for their total bacteria. The higher bacterial population $3.1x10^5$cfu $M1^{-1}$ was found in Ruby and lower count $1.3x10^5$ cfu $M1^{-1}$ was recorded in Bhavani. The six different curd samples collected estimated for their fungi and all of them have no mold population. The total lactic acid bacteria in six different curd sample were enumerated. The higher total lactic acid bacterial population ($1.8x10^5$ cfu $M1^{-1}$) in Ruby and lower count (1.0 x 105cfu ml^{-1}) in Bhavani. The result of the nutritional value of curd estimating the protein and lactose were studied which indicated the highest value of protein and lactose in Bhavani sample notified was 18.20 g/500ml and 24.60 g/500ml respectively and least protein in Raja and Ruby sample notified was 17.60 g/500 ml and lactose content in Ruby sample notified was 23.40 g/500 ml respectively. The phosphatase test indicated that the microbial contamination was under limit in Bhavani and the pasteurization carried out during processing was good and microbial contamination was move that indicated the absence of phosphatase enzyme. As there are Good Manufacturing Practices, the curd produced by various dairies on commercial sale in generally good. From study Bhavani curd sample shows best result among the six samples.

INTRODUCTION

Dahi is most popular indigenous milk product on our country. Dahi is more palatable. easily digestable and faster assailable in body in comparison with milk on prolonged storage it becomes highly acidic. This makes Dahi unfit for human consumption. Curd contain all the macronutrients (protein, carbohydrates and fat supplies energy), minerals, calcium and phosphorus are one of the major dietary sources of calcium. Calcium is needed for proper development and maintenance of strong, healthy bones and teeth. Curd protein is of high biological value providing all the essential amino acids.

The presence of calcium ions to form white creamy lumps. As it is rich in its nutritive content, it is easily subjected to contamination by many kinds of microorganisms making the curd unfit for consumption. The nutritive value and the kinds of microorganisms present are highly interrelated. *Bacillus licheniformis* and *Bacillus cereus* were the most commonly isolated species of *Bacillus* found in curd at all stages of processing. The presence of pathogens (*Salmonella* and *Listeria monocytogenes*), indicators of hygiene deficiencies (*Staphylococcus aureus* and *Escherichia coli*) and of microorganisms associated with deterioration in flavour and appearance of the product were detected. The most important index of microbiological quality is total bacterial count, coliforms, yeast and moulds count and detection of specific pathogens and their toxins. Lactic acid bacteria produce antimicrobial metabolites that the shelf life of the food. The strain of CAB, belonging *Lactococcus, Lactobacillus, Streptococcus, Leuconostoc* and *Pediococcus* species. The major sources of contamination of raw milk are the milking machine, the bulk tank lack of cooling facilities and unsatisfactory transportation.

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MATERIALS AND METHODS

The curd sample of Bhavani, Manju, Chakra, Anantha, Raja and Ruby were collected from in and around Chidambaram, Cuddalore District, Tamilnadu, India.

Estimation of total bacterial and fungal populations

The total bacterial populations were estimated in curd samples by using the nutrient agar medium. To estimate total bacterial populations in the curd samples, they were collected in presterilized test tubes and serially diluted to 10^{-5} in sterile water blanks. One ml of dilutions of 10^{-4} and 10^{-5} were pour plated on nutrient agar medium and then incubated at 39˚C for 24 hrs. After the period of incubation the plates were observed from appearance of

colonies and counted them. The results were expressed as colony forming unit (cfu) per ml of sample.

Total number of Bacterial population
per ml = Average no. of colonies x dilution factor.

The total fungal populations were estimated in curd samples by using the RBA (Rose Bengal Agar) medium. To estimate total fungal populations in the curd samples, the samples were collected in pre-sterilized test tubes and serially diluted to 10^{-4} dilution in sterile water blanks. One ml of dilutions of 10^{-2} and 10^{-1} were pour plated on Rose Bengal agar medium and the incubated at 34˚C 24 hrs. After the period of incubation the plates were observed for appearance of colonies and counted them.

Estimation of total lactic acid

The total lactic acid bacterial populations were estimated in the curd samples by using the yeast extract lactose agar medium. To estimate total lactic acid bacterial populations in curd samples were collected in pre-sterilized test tubes and serially diluted to10^{-6} dilution in sterile water blanks. One ml of dilutions at 10^{-5} and 10^{-6} were pour plated on yeast extract lactose agar medium and incubated at 34˚C for 24 hrs. After the period of incubation the plates were observed for appearance of colonies and counted them. The results were expressed as colony forming unit (cfu) per ml of sample.

Total number of lactic acid bacterial population per ml=Average no. of colonies x dilution factor.

Coliform test

Prepare the 500 ml of MacConkey broth and distribute in 10ml quantities in test tubes. Fill the Durham's tube with MacConkey broth and carefully push them into the test tubes containing MacConkey broth in inverted position with out allowing any air bubbles. Sterilize the test tube containing a MacConkey broth at 100˚C for 3 successive days. The curd samples serially diluted to (10^{-2}) each test tube adding 1ml of curd sample. Maintain 10^{-1}, 10^{-2} dilution (3 replication) test tubes of Macconkey broth without adding sample to have as control. Incubate all the test tubes at 27˚C for 24 hrs. The production of gas in observed by replacement of broth in the Durham's tube, which was the positive result.

Confirmed test for coliform's in curd samples

From the test tubes showing positive results one (or) two loops of culture were transferred to brilliant green lactose broth tubes with Durham's tubes. The tubes were gently rotated to mix the inoculums equally. Then the tubes were incubated at 37˚C for 48 hours. After incubation the formation of gas in any amount constitute the positive confirmed test. The positive confirmed test tubes reincubated to 40˚C for 24 hours. After incubation, the tubes were examined for gas production, which indicated positive result. From such tubes one loopful of inoculum was transferred to Eosin Methyle blue Agar (EMB) plate and streaked. These plates were incubated at 37˚C for 24 hours. After 24 hours of incubation, the colonies were observed for their characteristic morphology called metallic sheen indicating the growth of *E. coli*.

Coliform count

The presence of coliform indicates improper pasteurization or post-pasteurization contamination of the curd, because most coliforms will not survive pasteurization temperature. Serial dilutions of 10^{-1} and 10^{-2} of the curd sample were prepared using sterile water blanks. 1 ml of the dilutions was transferred into petriplates. 15 ml of sterilized and cooled MacConkey agar was added to the plates and rotated for uniform distribution. The plates were allowed to solidify and incubated at 37˚C for 24 hours. The coliform colonies were counted using Quebec colony counter.

No. of cells per ml = No of colonies x dilution factor
Standard coliform count per ml = Below 100 – Satisfactory

Estimation of protein

10 ml of curd sample was transferred into 250 ml conical flask. 10 drops of Phenolphthalein indicator and 0.4 ml of neutral saturated potassium oxalate solution was added. After 2 minutes, it was titrated against 0.1 N sodium hydroxide till the appearance of faint pink colour. Then 2 ml of formaldehyde was added and mixed well. Titration was done again to a faint pink colour with 0.1 N sodium hydroxide solutions. Volume of 0.1 N sodium hydroxide utilized after the addition of formaldehyde was recorded and used for further calculation.

Protein content of curd = Titre value x 1.7 = %

Estimation of Lactose percentage

20 ml of curd sample was pipette out into a 100 ml volumetric flask. 12 ml of 10% sodium tungstate and 12 ml of 2/3 N sulphuric acid was added to it and mixed thoroughly. The mixture was made up to the mark using distilled water. The mixture was filtered using a filter paper. The filter was than taken in a burette. In a separated 250 ml conical flask, 25 ml of Benedict's quantitative reagent with a pinch of sodium carbonate was added and a piece of porcelain. Lactose in the filtrate was determined by titrating it with boiling Benedict's reagent. End point was the appearance of a white precipitate with straw yellow coloration.

Calculation

Calculation of the above titration method is done bearing in mind that 25 ml of Benedicts reagent is completely reduced by 0.067 grams of lactose

$$100 \text{ g of curd} = \frac{0.067 \times 100 \times 100}{\text{Titration value} \times \text{specific gravity}}$$

20% of the curd

Determination of phosphatase

This test was used to determine the efficiency of pasteurization of curd. Curd contains an enzyme called phosphatase, which would be inactivated at 72˚C. The principle aim of pasteurization was to destroy the pathogens in curd. The most heat resistant human pathogen in curd was *Mycobacterium tuberculosis*, which would be destroyed by boiling at 71˚C for 15 minutes. So the efficiency of pasteurization was determined by detection of presence of phosphatase enzyme in curd. Buffer solution was prepared by adding 0.35g sodium carbonate and 0.15 g of sodium hydrogen carbonate in 1000 ml of water. The buffer substrate was prepared by adding 0.15g of P-Nitro phenyl disodium or thiophosphate salt and was made upto 100 ml using buffer solution. To 5 ml of buffer substrate, 1 ml of curd was added and shaken well and observed. 1 ml of curd with 5 ml buffer substrate was used as control. Tubes were incubated for 3-4 hours at 37˚C in a water bath and examined for colour change. Raw or improperly

pasteurized curd gives result within 1 hour. Yellow colour indicates raw or improperly pasteurized curd. No colour change indicates pasteurized curd.

Methylene blue reductase test

Curd that contains large number of actively growing bacteria will have a lowered oxidation – reduction potential due to the exhaustion of dissolved oxygen by microorganisms. Ethylene blue is a redox indicator that loses its colour in an anaerobic environment and is then said to be reduced. Large population of enteric microorganisms and *Streptococcus lactis* are potent reducers of the dye. The speed at which reduction occurs following addition of methylene blue to a sample of curd indicates the quality of curd.

(MBRT). Shorter the MBRT, the lower will be the quality of curd.

Keeping quality test of curd

Keeping quality was a bacteriological measurement of quality of curd samples. It was performed to check the shelf life of the curd samples. It helps to evaluate the quality of curd for the consumption. Curd samples were incubated at 18˚C for 24 hours. From the incubated sample, 10ml was transferred to a sterile test tube and Methylene blue reduction test was performed. One hour Methylene Blue Reduction Time (MBRT) is recommended as the minimum standard for this test, a reduction time less than this can be taken as an indication

Table 1. Total Lacticacid bacteria in curd samples

Sample	Total bacterial population	Total fungal population	Lactic acid bacterial population	Coliform count (cfu/M1) 10^{-1}	
				10^2	Grade
BHAVANI	13,00,000	NIL	10,00,000	NIL	Good
MANJU	18,50,000	NIL	13,00,000	NIL	Good
CHAKRA	26,00,000	NIL	12,00,000	NIL	Good
ANANDHA	23,80,000	NIL	11,00,000	NIL	Good
RAJA	28,00,000	NIL	15,00,000	42	Satisfactory
RUBY	31,00,000	NIL	18,00,000	21	Satisfactory

Table 2. Protein and Lactose content in curd samples

Sample	Protein	Lactose	Phosphatase activity	Reductase test	
				MBRT	Grade
BHAVANI	17.60	23.60	Negative	6.30hr	Good
MANJU	17.80	23.70	Negative	6.00 hr	Good
CHAKRA	18.20	24.60	Negative	5.15 hr	Fair
ANANDHA	18	24.20	Negative	5.30 hr	Fair
RAJA	18.16	24.30	Negative	4.40 hr	Fair
RUBY	17.60	23.40	Negative	1.50 hr	Fair

Table 3. Keeping quality test of curd samples

Sample	Incubation time (hrs)	Temperature(°C)	MBRT(hrs)	Grade
BHAVANI	24	18	5.50	Good
MANJU	24	18	5.25	Good
CHAKRA	24	18	5.00	Good
ANANDHA	24	18	4.15	Good
RAJA	24	18	2.38	Fair
RUBY	24	18	2.15	Fair

The determination was based on the following criteria:

With in 30 minutes – Very poor quality
Between 30 minutes – 2 hours – Poor quality
Between 2-6 hours – Fair quality
Between 6-8 hours – Good quality

10 mg of methylene blue was dissolved in 250 ml of distilled water. In this test 1 ml of methylene blue was added to 10 ml of curd. The tube was sealed and slowly inverted thrice to mix. It was placed in a water bath at 37˚C and examined every half-an hour upto six hours. Curd without dye was also incubated as blank on control. The time taken for the Methylene blue to become colourless is referred as the methylene blue reduction time of the need for improvement in the standard of processing (or) the initial quality of the curd.

RESULTS

The six curd samples collected from Chidambaram, Cuddalore district, Tamilnadu, India were enumerated for their microbial load of total bacteria and fungi presented in Table-1. The population of total bacteria ranged from 1.3 to 3.1 x 10^5 cfu ml^{-1}. The higher total bacterial population (3.1 x 10^5 cfu ml^{-1}) was found in Ruby and lower count (13 x 10^5 cfu ml^{-1}) was recorded in Bhavani. In this experiment 10^{-1} and 10^{-2} serial dilutions were taken for standard plate count of mold. The six samples (Bhavani,

Manju, Chakra, Anantha, Raja and Ruby) have no mold population. The microbial load in curd samples showed a total bacterial population ranging from 1.3 to 3.1 x 10^5 cfu per ml^{-1}.

The curd samples are collected and tested the coliform count out of the six curd samples four of them namely Bhavani, Manju, Chakra, Anantha is having no coliform. Hence the grade was good. Another two samples namely Raja and Ruby produced coliform counts of less than 100 cfu/ml, hence the grade was satisfactory. The curd samples were estimated nutritional value of Protein and Lactose presented in The nutritional value of protein and lactose in curd samples were showing Bhavani 18.20 g/500ml and 24.60 g/500 ml respectively. The highest protein and lactose content were present in Bhavani respectively. The lowest protein content was present in Raja and Ruby. The curd samples collected from Chidambaram, Cuddalore district were undergone phosphatase test. The six samples (Bhavani, Manju, Chakra, Anantha, Raja and Ruby) showed no colour change after the addition of buffer, which show no phosphatase enzyme is present in the samples tested. The results were tabulated in Table-2.

DISCUSSION

The microbial load in curd samples showed a total lactic acid bacterial population anging from 1.0 to 1.8 x 10^5 cfu ml^{-1}. Jayarao and Henning (2001) reported that total bacterial population counts ranged from 2.0 to 5.1 x 10^5 cfu per ml of dahi sample. The curd samples have no mold population. The standard plate count of curd samples indirectly indicated the storage temperature at which they were stored. Some of the brands of curd have counts of more than a lakhs which indicates the quality. Curd (skim and whole) was double-steamed and initially inoculated with 10^3 cfu/ml of each *Pseudomonas*. Curds were held at 5˚C for one month. Plate counts were conducted every three days to determine growth rates. Descriptive aroma analysis was conducted weekly using a trained panel. Experiments were replicated in triplicate. This was confirmed by Hayes et al., 2002.The total lactic acid bacteria in the curd samples were enumerated and presented. The population of total lactic acid bacteria ranged from 1.0 to 1.8 x 10^5 cfu ml^{-1}. The higher total lactic acid bacterial population 1.8 x 10^5 cfu ml^{-1} in Ruby and lower count 1.0 x 10^5 cfu ml^{-1} were estimated.

Esayas Assefe et al. (2008) reported that lacticacid bacteria produce antimicrobial metabolities that inhibit growth of food borne pathogenic and spoilage microorganisms, thereby enhancing the shelf life of the food.

The coliform count of certain types of curd was found to be zero in coliform count per ml. Some were found to contain coliform that indicate post/pasteurization contamination. In the present study, coliforms were recorded in 30% of the commercial curd samples tested. Dasilva et al., 2001 reported the presence of coliforms in 40% of samples. The study showed that raw curd can get contaminated with enteropathogenic strains of *E. coli*. The microbial contamination was under limit and the pasteurization process and other processes carried out during the processing were good, which were clearly indicated by the results of Phosphatase and Reductase test. Few samples had reduced Methylene Blue Reductase Tests and hence were of poor quality due to the multiplication of microbes present in the curd during storage at booths. (Bhavani, Manju, Chakra and Anantha) had Methelene Blue Reductase Test of four hours to six hours after the sample were stored at 24 hours at 18˚C, which indicated good quality respectively. Remaining two curd samples namely Raja and Ruby had Methelene Blue Reductase Test of two hours to three hours indicating fair quality.

The results were tabulated in Table-2. The keeping quality of all the curd samples was good, which clearly indicated that those dairies people have taken utmost care for the shelf life of their product. A long shelf-life with consistent high quality is desired for fluid curd. A more complete understanding of the microbiological and chemical characteristics of the organisms in curd may lead to better methods of detection and prevention. As there are good manufacturing practices (GMP), the curd produced by various Dairies on commercial sale is generally good. This study has also proved the same. From this study Bhavani curd sample shows best result among the six samples. So it is recommended for human consumption.

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