



## **EVALUATION OF PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF FERMENTED MILK (CURD) PREPARED BY USING *Capsicum annum* (CHILLI) AS A STARTER FOR FERMENTATION**

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### **ABSTRACT**

*In India curd can be prepared by adding chili (fruits of *Capsicum annum*) instead of adding curd starter. Milk was fermented in the laboratory by adding green chili as a starter for fermentation. After curdling, the fermented milk was subjected to physico-chemical and Microbiological analysis. The physicochemical analysis was carried out by performing organoleptic tests, determination of pH, titratable acidity, protein content, fat content, total solids etc. The microbiological analysis of curd was carried out by determination of Total Viable count (lactics), Determination of Yeast and Mold count, Coliform count and by isolation of microorganisms. The curd was formed after 9 h. The curd was found to be thick, white in color, having pleasant smell, and sweet taste. The pH of the curd was found to be 4.5. The titratable acidity was found to be 1.28%. The Protein content of the curd was found to be 3.2%. The coliform count was < 2. Strains of *Lactobacillus acidophilus* and *Streptococcus thermophilus*, and *Saccharomyces cerevisiae* were isolated from the curd sample. The results of this study reveal that chili can be used as a starter material for curd making. The curd formed was found to be tasty and safe for consumption. However, further study including the development of inoculum from chilli should be carried out to explore the use of Chili (fruits of *Capsicum annum*) as a starter material for curd making.*

**Keywords:** *Fermented milk, Chili, Curd making, Lactobacillus*

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### **INTRODUCTION**

Curd, is a Indian fermented milk product. Ayurvedic scripts, 6000 BC, have referred the benefits of curd (fermented milk) for human health being. Microbial Fermentation gives flavour and texture to milk and also adds therapeutic benefits. Curd is having prominent position in Indian diet. Curd helps in maintaining a health of digestive system due to the presence of the probiotic bacteria.

In India generally curd is made at home by adding small amount of previous day's curd (starter) into the milk. It can be observed that sometimes, curd is made by adding chilli or chilli stalks because of unavailability of the starter culture. In some region of India curd required for making Kadhi (curd Curry) is made by adding red, green chillis as a starter for making curd. In recent years, there has been increasing interest in lactic acid bacteria isolated from non-dairy products due to their diverse metabolic profile, unique flavor – forming activities and potential for use in starters or starter adjuncts for the dairy industry [1].

There have been claims that the calyx of capsicum fruits such as chilli, are often rich in various lactobacilli. These natural bacteria create a starter for lacto-fermentation of milk. In addition capsaisin from the chilli appears to increase the metabolic rate of the lactobacilli [2,3].

The freeze dried starter cultures are not easily available in the market. The effectiveness of the store bought yogurt are not always guaranteed due to certain hidden additives from the manufacturers and the fact that the active culture tends to decrease over time<sup>4</sup>. Despite several claims on the use of chilli stalks (calyx) in yogurt making, there have been paucity of scientific documentation of its effectiveness in terms of pH change and the percent lactic acid content of the yogurt produced with it [4]. This work was undertaken to study the physico-chemical and microbiological quality of curd made by adding chilli as a starter culture.

## MATERIAL AND METHODS

### A. Collection of samples

Chillis (*Capsicum annum*) were collected from vegetable market of Karad. Medium size fresh green chillis were selected for the study. Pasteurised milk was purchased from local market.

### B. Preparation of curd

Curd was prepared in the laboratory. The green chilli was washed with water and wiped with a clean cloth. 50ml pasteurized milk was collected in sterile beaker. Milk was boiled and cooled to 45°C. Two medium sized Green chilli's along with stalk were added to it and kept covered for incubation at 30°C temperature (Test). 50ml pasteurized milk was collected in sterile beaker, boiled and cooled to 45°C. 1 mL of homemade curd was added to it and kept covered for incubation at 30°C temperature (positive control). Both the beakers were observed after every hour. The curd formed was subjected to physico-chemical and microbiological analysis.

**C. Study of Physico-chemical Analysis of Fermented milk (Curd):** Both the curd samples were studied for their physicochemical tests i) color was determined by visual observation. ii) Determination of odour & taste by organoleptic testing of the curd samples. iii) Texture & consistency were determined by cutting the curd sample with knife and picking with the spoon. iv) Determination of pH: pH was determined by using pH meter [5]. v) Determination of Titratable acidity: Titratable acidity was determined by method described in Lab manual of FSSAI vi) Determination of total solid content was carried out by Gravimetric method, [15] vii) Determination of specific gravity was done by using specific gravity bottles viii) Determination of fat content of curd samples was carried out by using Gerber's method [7,8]. iii) Determination of lactose was carried out by Benedict's reagent method ix) determination of protein content was carried out by Folin lowry method [5,6]

### D. Microbiological Analysis of fermented milk samples

Serial ten fold dilutions of both the curd samples were made in sterile saline and subjected to

a. Determination of total viable count by Standard Plate Count Method [5]

b. Determination of number of Coliforms by Most Probable number (MPN) method [5,15].

c. Determination of yeast and mold count were carried out by SPC method [5].

d. Microscopic examination of curd sample was carried out by performing gram staining.

**e. Evaluation of microbiological quality of curd:** Loopful of diluted suspension of curd was spread inoculated on sterile MRS agar plates, Sterile MacConkeys agar plates, Sterile Sabouraud's agar plates & Sterile Nutrient agar plates. One of each inoculated agar plate was incubated at room temperature for 48 h. one from each plate was incubated at 37°C for 48 h. MRS agar plates were incubated under microaerophilic conditions. All the plates were observed for the growth, well isolated distinctive colonies were sub cultured, purified and subjected to their cultural, morphological and biochemical characterization. The isolates were identified according to their cultural, morphological and biochemical characteristics [10].

## RESULTS AND DISCUSSION

The time required for the curd (added with chilli as a starter) formation was found to 10 h. while the time required for the curd made by traditional method formation was found to 8h. (Table 2) The curd formed by (adding chilli (*Capsicum annum*) was found to be having same physical characteristics as soft, white, smooth having pleasant odour as of curd formed by traditional method. The taste of the curd made by using chilli as a starter was found to be creamy and spicy. The pH of the curd (added with chilli as a starter) was 4.5 which is as per the standards The titratable acidity was found to be 1.28%. specific gravity was found to be 0.930g/mL, fat content was 6.1%. lactose content was 2.7% and protein content was 3.2 %. (Table 2) All the physical and chemical properties of the curd are found to as per the standards [11].

Microscopic study of curd samples showed the presence of Gram positive rods, Gram positive cocci and oval yeast cells. The SPC for yeast and molds of the curd made by adding chilli was found to be  $2.6 \times 10^5$  and of curd made by traditional method was  $1.2 \times 10^5$ . The coliform count of both the curd samples was found to be 0. Total viable count of the curd made by adding chilli was  $3 \times 10^5$  while of curd made by traditional method was  $4 \times 10^5$  (Table 4)

Study of microflora of curd (Made by using chilli) showed the luxuriant growth on MRS agar and Sabouraud's agar. Moderate growth on Nutrient agar was observed. MacConkeys agar showed no growth even after 48 h incubation. In all three isolates (two bacterial and one yeast) were found from the curd made by adding chilli as a starter material. From the cultural, morphological and biochemical characteristics (Table 5) the bacterial isolate LB1 was tentatively identified as strain of *Lactobacillus acidophilus* isolate LB2 was tentatively identified as Strain of *Streptococcus thermophilus*. From the study

of Cultural and Morphological characteristics yeast isolate was tentatively identified as a strain of *Saccharomyces cerevisiae*.

The curd made by adding chilli as a starter culture was found to be free from gram negative bacteria and moulds.

Components	Quantity of components in Curd made from Whole milk	Quantity of components in Curd made from Skimmed milk
	%	%
Water	85-88	90-91
Fat	5 - 8	0.05 - 0.1
protein	3.2-3.4	3.3-3.5
Lactose	4.6-5.2	4.7-5.3
Lactic acid	0.5-1.1	0.5-1.1
Ash	0.7-0.75	0.7-0.75

**Table 1: Chemical composition of Curd.[9]**

**Table 2: Physicochemical properties of curd samples**

Sr. No	Name of the Test	Curd Made by adding Chilli as a starter Material	Curd made by traditional method
1	Odor	Pleasant	Pleasant
2	Taste	Creamy with spicy	Creamy
3	Body	Firm	Firm
4	Consistency	Soft	Soft
5	Color	White	White
6	Texture	Smooth	Smooth
7	pH	4.5	4.1
8	Acidity	1.28 %	1.26%
9	Specific gravity	0.930g/mL	0.940 g/mL
10	Fat	6.1%	6.5%
11	Sugar	2.7%	2.65%
12	protein	3.2%	3.3%

**Table 3: Results of Microscopic study of curd samples**

Curd sample	Gram Positive Rods	Gram Positive cocci	Gram negative cocci	Gram negative Rods	Yeast cells	Molds
Made by adding chilli	+	+	-	-	+	-
Made by traditional method	+	+	-	-	+	-

**Table 4: Microbiological analysis of Fermented milk**

Type of Curd	SPC for Yeast and Molds	Coliform Count.	TVC
Made by adding Chilli as a starter culture	2.6×10 <sup>5</sup>	0	3×10 <sup>5</sup>
Made by traditional method.	1.2x10 <sup>5</sup>	0	4 x10 <sup>5</sup>

**Table 5 : Morphological and Biochemical characteristics of the Bacteria isolated from curd made by using chilli as a starter material**

Isolate	Gram nature	Spore formation	Catalase	oxidase	Sugar Fermentation test			
					Glucose	lactose	Maltose	mannitol
LB1	Gram positive rod	Nonspore former	negative	negative	positive	positive	positive	negative
LB2	Gram positive cocci	Nonspore former	negative	negative	positive	positive	positive	negative

**Fig. 1 Photoplate of fermented milk [curd]made by adding chilli as as a starter culture**



## CONCLUSIONS

1. The Curd formed by using chilli(*Capsicum annum*) was found to be tasty and safe for consumption.
2. The result of this study reveals that chilli can be used as a starter material for curd making
3. However, further study including the development of inoculum and more study to explore the microbiology of curd should be carried out to explore the use of Chilli as a starter material for curd making .

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## CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interests.

## REFERENCES

1. Teneva-Angelova T, Beshkova D. Non-traditional sources for isolation of lactic acid bacteria. *Ann Microbiol.* 2016;66(1):449-59
2. Sharma SJ, Nair GN, Ramachandran S. *Capsicum annum* enhances L-lactate production by lactobacillus acidophilus: implication in curd formation. *J Dairy Sci.* 2013;96(7):4142-8.
3. Harini, P., 2013. Vegan Recepies. A blog in tongueticklers. com, 4.
4. Tech, O.M.M., Arawande, J.O. and Tech, A.A.M., Pilot study on chilli stalks as a source of non-dairy lactic acid bacteria in yogurt making.
5. Chellapandi, P., 2007. Laboratory Manual in Industrial Biotechnology. Pointer Publishers.
6. Classics Lowry, O., Rosebrough, N., Farr, A. and Randall, R., 1951. Protein measurement with the Folin phenol reagent. *J biolChem*, 193(1), pp.265-75.
7. Lab. Manual 1 Manual Of Methods Of Analysis Of Foods Food Safety And Standards Authority Of India Ministry Of Health And Family Welfare Government Of India New Delhi 2015 Milk And Milk Products.pp90
8. Jayaraman, J. and Jayaraman, J., 1981. Laboratory manual in biochemistry (pp. 75-76). Delhi, India:: Wiley Eastern.
9. Ranganadham, M., MH, S.K., Devraja, H.C. and Garg, F.C., 2016. Traditional dairy products.
10. Whitman, W.B. ed., 2015. Bergey's manual of systematics of Archaea and Bacteria (Vol. 410). Hoboken, NJ: Wiley.
11. Magdi EO. Yogurt and your health. Star Base Publication. Washington. 2004;4-6
12. Kanawjia, S.K., 2006. Developments in the manufacture of lassi. Developments in Traditional Dairy Products, lecture compendium of the 21st Short Course. CAS in Dairy Technology, national Dairy Research Institute, Karnal, India. Pp, pp.55-63.
13. Aneja, R.P., Mathur, B.N., Chandan, R.C. and Banerjee, A.K., 2002. Technology of indian milk products: handbook on process technology modernization for professionals, entrepreneurs and scientists. Dairy India Yearbook. IS 9617, 1980. Dahi
14. Sivakumar, N. and Kalaiarasu, S., 2010. Microbiological approach of curd samples collected from different locations of Tamilnadu, India. *International Journal of Current Research*, 2(10), pp.027-030.

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