



Physicochemical and Microbiological Study of Fermented Milk [Curd] Prepared by Using Silver Coin as a Catalyst for Fermentation.

Jayashri P. Nanaware *, **Aparna S. Pawar**, **Raviraj S. Pawar** and **Girish R. Pathade**
Krishna Institute of Allied Sciences, Krishna Vishwa Vidyapeeth, "Deemed to be University", Karad

ABSTRACT

In India there is practice of curd making by adding Silver Coins (presoaked in "Panchamruta", mixture of Curd, honey, milk, sugar/honey and cow ghee) in the milk instead of adding curd starter. Milk was fermented in the laboratory by adding Silver Coins presoaked in "Panchamruta" (mixture of Curd, honey, milk, sugar/honey and cow ghee) 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, Determination of Yeast and Mold count, Coliform count and by isolation of microorganisms. The curd was formed after 8h. 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 4.8. The titratable acidity was found to be 1.28%. The Protein content of the curd was found to be 5.7%. The coliform count was < 2. Strains of Lactobacillus acidophilus, Streptococcus thermophilus, and Saccharomyces cerevisiae were isolated from the curd sample. The curd formed was found to be tasty and safe for consumption. The silver coins presoaked in "Panchamruta" were subjected to isolate microorganisms and found that they contained lactic acid bacteria and yeasts. The microbial isolates obtained from silver coins (lactic acid bacteria and yeasts) were used with and without silver coins in curd making. It was observed that the quality of curds formed with silver coins was better to that without silver coins and fermentation time was just 6-h. There are reports that silver enhances activity of curd forming microorganisms. Hence present project was undertaken to study effect of silver coins in curd making.

Keywords: Fermented milk, silver coin, Curd, Lactobacillus.

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INTRODUCTION

Curd, is an Indian fermented milk product. Microbial Fermentation gives flavor 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 silver coin because of unavailability of the starter culture. In some region of India curd required for making Kadhi (curd Curry) is made by adding silver coin 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].

These natural bacteria create a starter for lacto-fermentation of milk. In addition of silver coin appears to increase the metabolic rate of the lactobacilli [2].

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 the time. Despite several claims on the use of silver coin 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. This work was undertaken to study the physico- chemical and microbiological quality of curd made by adding silver coin as a starter [3].

MATERIAL AND METHODS

Collection of Soil Samples: Silver coins were collected from jewelry shop Karad, Maharashtra, India. Medium size silver coins were selected for study. Pasteurized milk was purchased from local

market. The homemade "Panchamruta" was collected (prepared in the laboratory -10-mL milk, 5mL curd, 3-g sugar, 3-mL honey and 3-mL cow ghee).

Isolation of Lactic Acid Bacteria And Yeasts From Sample of Panchamruta Curd[7]

The panchamruta was serially diluted to 10^6 dilution using sterile saline water and plated on deMann-Rogosa-Sharpe(MRS agar), Nutrient agar, MPN test set(Brilliant Green Lactose bile Broth) and Sabouraud's agar for isolation of lactic acid bacteria, total viable count of bacteria, Coliform count and isolation of yeasts, respectively.

Preparation of Curd

Curd was prepared in the laboratory. The silver coins (2 per set) were washed with water and soaked in the Panchamruta for 15- min wiped with a clean cloth. Four sets of 50 mL pasteurized milk were prepared in sterile beakers. Milk was boiled and cooled to 45° C. Silver coins (two per beaker) and were added to three sets (triplicate experiment) with 1-mL of homemade curd in each set (which was used for Panchamruta making) and kept covered for incubation at 30°C temperature (Test). In the fourth set silver coins were not added only 1-mL of homemade curd (which was used for Panchamruta making) was added and the set was kept covered for incubation at 30°C temperature (control). The beakers were observed hourly for curd formation without disturbing the contents of beakers. Then curd formed was subjected to physicochemical and microbiological analysis.

Study of Physicochemical Analysis of Fermented Milk (Curd) [8]

The curd samples of both sets (Test and Control) were studied for their physicochemical test:

Color was determined by visual observation.

- i. **Determination of odour & taste** by organoleptic tasting of curd samples.
- ii. **Texture & consistency** were determined by cutting the curd sample with knife and picking with the spoon.
- iii. **Determination of pH:** pH was determined by using pH meter [4]
- iv. **Determination of Titratable acidity:** Titratable acidity was determined by method described in Lab manual of FSSAI.
- v. **Determination of total solid content** was carried out by Gravimetric method [5].
- vi. **Determination of specific gravity** was done by using specific gravity bottles.
- vii. **Determination of fat content of curd** samples was carried out by using Gerber's method [8].
- viii. **Determination of lactose** was carried out by Benedict's reagent method.
- ix. **Determination of protein content** was carried out by Folin Lowery method [6].

Microbiological Analysis of Fermented Milk Samples

Serial tenfold dilution of both the curd samples were made in sterile saline (10^4 , 10^5 and 10^6 dilutions were used for plating) 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].
- c) Determination of yeast count were carried out by SPC method [5].
- d) Microscopic examination of curd sample was carried out by performing Gram's staining.

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 plates were incubated at 30°C for 48h. 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 [8].

RESULTS AND DISCUSSION

The time required for the curd (added with silver coin as starter) formation was found to 6-h while the time required for the curd made by traditional method (no silver coins) formation was found to 10-h (Table 1). The curd formed by (adding silver coin) was found to 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 silver coin as a catalyst was found to be creamy. The pH of the curd (added with silver coin) was 4.8 which is as per standards. The titratable acidity was found to be 1.28%, specific gravity was found to be 0.940g/mL, fat content was 4.8%, lactose content was 2.7% and protein content was 5.7% (Table 1). All the physical and chemical properties of the curd are found to as per the standards [9].

Microscopic study of curd sample showed the presence gram positive cocci, gram positive rods and oval yeast cells (Table-2). The SPC for yeast and molds of the curd made by adding silver coin was found to be 2.6×10^5 and the curd made by traditional method was 1.2×10^5 . The coliform count of both the curd

samples was found to be zero. Total viable count of the curd made by adding silver coin was 4×10^5 while of curd made by traditional method was 5×10^5 (Table 3).

Study of microflora of curd (Made by adding silver coin) showed the growth on MRS agar and Sabouraud's agar. Moderate growth on nutrient agar was observed. MacConkeys agar showed no growth even after 48h incubation. In all three isolates (two bacterial one yeast) were found from the curd made by adding silver coin as a starter material as well as set with no silver coins. From the cultural, morphological and biochemical characteristics (Tables-2,3 and 4) the bacterial isolate LB1 was tentatively identified as *Lactobacillus acidophilus* and isolate LB2 was tentatively identified as *Streptococcus thermophilus* and from the above study of cultural, morphological characteristics yeast isolate was tentatively identified as species of *Saccharomyces cerevisiae*. The curd made by adding silver coin as a starter culture was found to be free from gram negative bacteria like coliforms but curd without silver coins showed some coliform count.

Thus it is evident from the results that addition of silver coins in the milk along with curd starter:1) Enhances the activity of lactic acid bacteria and yeasts resulting in shortening of curdling time(from 10-h of milk without silver coins to 6-h with silver coins).

2) Also inhibits the growth of gram-negative bacteria like coliforms.

The above effects of silver coins may be attributed to oligodynamic action of silver ions. [8].

Table 1: Quality of Curd Samples Prepared

Sr. No	Name of the taste	Curd made by adding silver coin as a starter material	Curd made by Traditional method (without silver coins)
1	Odour	Pleasant	Pleasant
2	Taste	Creamy	Creamy
3	Body	Firm	Firm
4	Consistency	Soft	Soft
5	Color	White	White
6	Texture	Smooth	Smooth
7	pH	4.8	4.1
8	Acidity	1.28%	1.26%
9	Specific gravity	0.940g/mL	0.930g/mL
10	Fat	4.8%	8%
11	Sugar	2.8%	2.67%
12	Protein	5.7%	5.9%
13	Time of curdling(h)	6-h	10-h

Table 2: 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 silver coin	+	+	-	-	+	-
Made by Traditional methods	+	+	-	-	+	-

Table 3: Results of Quantitative Microbiology Study of Curd Samples

Type of curd	SPC for Yeast and Molds	Coliform Count MPN9coliforms/100mL)	TVC
Made by adding silver coin as a starter culture	2.6×10^5	0	4×10^5
Made by Traditional method(No silvercoins)	1.2×10^5	>2	5×10^5

Table 4: Morphological and Biochemical characteristics of the Bacteria isolated from curd made by using Silver Coin as a starter material

Isolate	Gram nature	Spore formation	Catalase	Oxidase	Sugar fermentation Test			
					Glucose	Lactose	Maltose	Mannitol
LB1	Gram Positive Rod	Non spore former	Negative	Negative	Positive	Positive	Positive	Negative
LB2	Gram positive Cocci	Non spore former	Negative	Negative	Positive	Positive	Positive	Negative

Photoplate-1 of Fermented Milk (Curd) & Photoplate-2 of colonial growth of made by adding Silver Coin as a starter organisms on MRS agar material



CONCLUSIONS

The curd formed by using silver coins was found to be tasty and safe for consumption. The result of this study reveals that silver coins can be used with curd starter as a catalyst for fast making of safe curd.

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