Effect of *Lactobacillus plantarum* and *Bifidobacterium lactis* on the Physical Parameters of Curd

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Abstract
This study was conducted to examine the changes in the physical parameters of homemade curd on introduction of known Probiotic strains, obtained from external source. Curd is one of the most commonly used fermented foods for decades because of its health benefits. Traditional curd contains Lactobacilli and Streptococcus species but the proportion of the bacteria differs vividly in every case. This proportion also affects various characteristics considerably, such as acidity, aroma, taste, smoothness, texture, etc. The objective of this study was to compare various essential parameters which are required to be fulfilled for production of better quality curd. For this, curd samples were prepared, the first one traditionally, taken as reference and the other with additionally added Lactobacillus plantarum and Bifidobacterium lactis species, known as potential Probiotics and also, not very commonly found in natural conventional curd. Significant improvement in firmness, taste and smoothness was observed in the sample containing the added Probiotics, which also gives positive results of their viability. Results also showed much improved antimicrobial activity of the latter, indicating greater than before health benefits than traditional curd.

IndexTerms – Curd, Lactobacillus plantarum, Bifidobacterium lactis, Probiotics.

I. INTRODUCTION

With sudden increase in number of health disorders in the recent years, scientists have shifted their attention towards diet-related treatments as the side effects of the drugs is not acceptable at all times. Hence, a lot of studies are conducted on Probiotics, with an intention to explore their benefits on health.

Probiotics can be helpful in disorders like inflammatory intestinal diseases, diarrhea, gastroenteritis, hypercholesterolemia, inadequate lactase digestion, cancer, immunosuppressive states, allergies, liver diseases, hyper-lipidemia, *Helicobacter pylori infections*, and genitourinary tract infections, etc. [1,2]. Fermented foods are known to have lactic acid bacteria (LAB), Streptococcus sp, etc which are known to have beneficial effects on health. They can be as considered Probiotics, provided they fit the criteria.

All *S. thermophilus* and most *L. bulgaricus* strains are known to have high lactase activity [3]. Yoghurt consumption improves lactose digestion and also eliminates symptoms of lactose intolerance. Various human studies have demonstrated the physiological effects of yoghurt consumption, comparing live cultures with pasteurized product having heat-killed bacteria. [4, 5, 6].

LAB species are known to show symbiotic relations during their growth in milk [7]. Thus, a carefully selected mixture of LAB species is used to balance each other and to achieve a significant fitness in acid production. Further, to increase the number of LAB that survive the low pH and high acidity of the gastrointestinal environment, some LAB species that are indigenous to the human intestine have been used in yogurt production. To meet the National Yogurt Association's criteria for "live and active culture yogurt," the finished yogurt product must contain live LAB in amounts 108 organisms/g at the time of manufacture [7], and the cultures must remain active even at the end of the stated shelf life, as ascertained with the use of a specific activity test. In today's time, fermented dairy products make up a substantial proportion of the total daily food consumption. Further, for long it has been believed that consuming yogurt and other fermented milk products provides various health benefits [8]. Various studies in recent past have confirmed this belief [9, 10].

Yogurt was firstly introduced to the USA in the early 20th century in the form of tablets especially designed for those with digestive intolerance [11]. However, it became popular in the North America only when Dannon, a small-scale yogurt factory started manufacture of yogurt in New York in 1940 [12]. Even though, yogurt has been evolved for centuries, it was subjected to a significant and dynamic evolution process in the 20th Century to originate a vast array of products [13,14]. For instance, fruit yogurts, yogurts with fruit on bottom and blended yogurts were introduced in 1937, 1947 and 1963 respectively [15,16]. It seems that the evolution process of yogurt has taken place in different regions of the world once it had been originated in the Central Asia.

Understanding the benefits from the above, attention to yoghurt or curd is being given by industries as well. Keeping all this in mind, the study undertaken aims at comparing the normally made conventional home curd with the curd, modified by introduction of Probiotic strains known for their enhanced health benefits and encouraging more studies on improving curd quality further.

II. MATERIALS AND METHODS

Source for curd and Sample collection

Buffalo milk was used to form curd, obtained from local dairy and was given heat treatment (pasteurization) of 65 °C for 30 minutes. Milk was filled in sterile tubes while transport.

Bacterial strains

Bacterial strains – *Lactobacillus plantarum* and *Bifidobacterium lactis* strains, obtained in lyophilized form, were cultured repetitively in de Man, Rogosa, and Sharpe (MRS) and Bifidobacterium selective media respectively for a week. Mild heat treatment was conducted to keep them viable even at slightly high temperature, while preparing curd. This process of keeping the culture at 65 °C for 30 minutes in water bath makes it heat adaptive [17].

25 mL of the two cell cultures were taken in four falcon tubes and were centrifuged at 1200 ×g for 15 minutes. The supernatants were carefully discarded, without disturbing the pellet formed, which contains cells. The pellets formed were washed carefully with autoclaved distilled water. These cells were then cultured on respective selective growth media to test their viability. Colonies were obtained and the count was found to be around 7.9 log cfu/mL.

Curd Preparation

The milk was slowly stirred to distribute the culture organisms uniformly and the inoculated milk was poured into 250 mL capacity beakers which were then incubated at 42°C for about 6 h. After fermentation, the beakers were shifted to refrigerator to cool the curd for about 24 h so that it was cooled to about 5°C. The curd was then analysed for sensory quality, rheological attributes and physico-chemical attributes. Following parameters are checked on curd formation:

Texture of Curd*:* The milk was cooled to about 40^{o}C and inoculated with the two cultures and incubated at 40^{o}C for about 6 hours. The curd formed was chilled to 5^{o}C and evaluated for quality. Firmness, consistency and index of viscosity as measured by Texture Analyser increased with increased heat treatment and the highest values were observed in curd prepared from boiled milk. Boiling treatment of milk resulted in least syneresis of whey in the curd.

Sensory: The chilled curd (5°C) was served to a panel of seven judges and its colour and appearance, flavor, body and texture and overall acceptance were evaluated on 9- point Hedonic scale [19]. The sensory acceptance data were tabulated by taking average of scores awarded by all the judges for different treatments in three replications.

Syneresis: The set curd at 5°C was slowly transferred to 15 mL capacity centrifuge tubes causing minimum disturbance to the coagulum. The centrifuge tubes were balanced by adjusting their weights and centrifuged at 2000 rpm in a Remi centrifuge for 5 min. The quantity of whey separated at the top of the coagulum inside centrifuge tubes was recorded as mL. The higher the volume of whey separated, the higher will be the syneresis and vice versa.

pH: pH was determined by potentiometric method i.e. by potential difference between the sample and electrolyte solution present inside the electrode of pH meter, using digital pH meter (Systronic Co., Bangalore). The electrode of the pH meter was directly dipped in the set curd and the pH was recorded (5°C). pH was recorded to check the lactic acid secreting capability of the culture in specific duration with favorable temperature. Curd was prepared in cups with 100 mL volume and was incubated. pH was recorded in periods.

Rheological: Firmness, consistency and viscosity of curd are important rheological or textural parameters that govern the quality of curd. These attributes can be measured objectively by Texture Analyzer or cone penetrometer. The following method was employed for measuring firmness, consistency and index of viscosity by Texture Analyzer (Stable Microsystems, UK). The working of Texture Analyser is based on the principle that a cylindrical steel probe penetrates into the curd samples and experiences resistance during the penetration. The resistance offered by the curd sample (5ºC) during the penetration of the probe up to a specified distance is recorded as firmness of curd.

III. RESULTS AND DISCUSSION

Texture analysis of curd

The modified curd was found to have better texture physically with more firmness and smoothness of the top layer. The top layer remained intact and undisturbed in comparison with the traditionally formed curd. The shelf life of the modified curd was 3 days more than the reference curd, which stayed stable usually only for one day.

Viability

Presence of Bifidobacterium species was tested in both reference and the modified curd. The reference sample did not have the species. The presence of Bifidobacterium was confirmed by preliminary and biochemical tests. The distinct Y-shaped purple colonies showed viability of Bifidobacterium in the curd, giving a positive result.

Antimicrobial Activity

Antimicrobial activities of both curd samples were tested against *S.aureus* and remarkable difference was noted in the zone of inhibition produced.

Table1. Parameters observed in curd

Parameters	Reference (Traditional)	Modified Curd
Firmness (g)	81.6	149.4
Consistency (g.sec)	221.2	967.3
Cohesiveness (g)	-9.3	-20

IV. Conclusion

This study showed that introduction of Probiotic strains like *L. plantarum* and *B. lactis* in milk to prepared curd had a significant impact on its texture and quality. The improvement in these properties due to change in proportion of strains in curd, which had a uniform and synchronized impact on curd setting. The results also show possible positive impact of the modified curd on human heath as Probiotic Bifidobacterium sp are known for their cholesterol lowering activity and their viability in the above experiment gives hope for their future use. Further studies can be done regarding introduction of more species and testing their clinical effects as Probiotics are harmless.

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