



Microencapsulation of *L. acidophilus* and assessing its efficacy in probiotic curd preparation

S. Radhai Sri¹, R. Sangami¹, R. Rajendran² and S. Karthik Sundaram²

¹Department of Nutrition, PSG College of Arts & Science, Coimbatore-14

²Department of Microbiology, PSG College of Arts & Science, Coimbatore-14.

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ABSTRACT

Recently, the focus of scientific investigations has moved from the primary role of food as the source of energy and body-forming substances to the more subtle action of biologically active food components on human health. There has been an explosion of consumer interest in the active role of food in the well-being and life prolongation, as well as in the prevention of initiation, promotion, and development of non-transmissible chronic diseases. As a result, a new term—functional food—was proposed. Among these foods, probiotics exert positive effects on the composition of gut micro biota and overall health, and the market is increasing annually. In the current study the probiotic bacterium *Lactobacillus acidophilus* was encapsulated in microcapsules of sodium alginate. These encapsulated microbes were used in the fermentation and production of curd with high nutritional values. On comparing the curd fermented with microencapsulated bacterium with that of the non-encapsulated forms (traditional fermentation technique) it was found out that there was a considerable reduction in the fat content of the sample prepared with the microencapsulated form (2.20 gm) to the non-encapsulated organisms (3.90 gm). The protein and the vitamin-C content were found to be more or less the same in both the cases. The iron content of the sample prepared with microencapsulated form was found to be 7.2 gm to 3 gm of iron content for the sample produced through non-encapsulated bacterium. It was found out that the curd fermented through the microencapsulated *L. acidophilus* was found to be nutritionally rich and that the organism could also act survive as a probiotic in the gut in the presence of a protective microencapsulation.

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Introduction

In the recent past, there is increasing consumer awareness throughout the globe on the potential influence of various food stuffs on health benefits. Recently, there has been increased interest in bacterial food supplements, which are now referred as probiotics (Prem Prakash Dewani, 2004). Probiotics – Bacterial preparations that impart clinically verified beneficial effects on the health of the host when consumed orally.

Generally dairy products are selected as a carrier in probiotic foods due to its efficient delivery of probiotic organism to the gut. The organism selected for production of probiotic foods should satisfy certain specific criteria. Of the different probiotic strain, *Lactobacillus acidophilus* establishes an intimate relationship with man throughout life (Binita Rani et al., 1997). Acidity, pH, concentration of lactic acid and acetic acid, hydrogen peroxide and dissolved oxygen content have been identified to have an effect on viability during manufacture and storage of cultured milk products (Dave and Shan, 1997).

Application of microencapsulation - probiocap technology is now being evolved to improve the stabilization of probiotics to escape processing conditions such as heat treatments, microwave treatments, sterilization etc there by enhancing the micro organisms to extend shelf life, increase heat resistance, improves and enhances acid tolerance (Suita and Goulet, 2001).

Considering the nutritional and therapeutic value of probiotic foods, in the present study *L. acidophilus* was

microencapsulated as a matrix and curd was prepared with encapsulated *L. acidophilus* beads. Further its quality characteristics were compared with the prepared curd to evaluate the efficiency of probiotic *L. acidophilus* in encapsulated form.

Methodology

Sub culturing of *L. acidophilus*

The lyophilized form of the culture (*L. acidophilus*) was sub cultured with the suitable medium using standard procedure (MTCC, Chandigarh). From the sub cultured mass to confirm the presence of *L. acidophilus* its morphological, physiological and biochemical characteristics were screened.

Preparation of microcapsules

The microcapsules were prepared by encapsulating the probiotic *L. acidophilus* in a matrix of sodium alginate. The core material is the pelleted. *L. acidophilus* cells and the wall material selected was sodium alginate. The capsules (beads) were collected in calcium chloride solution and were recovered and dried (Shah and Ravula, 2000).

Preparation of curd with microencapsulated *L. acidophilus* and free bacterial cells

Encapsulation improves probiotic viability in fermented dairy products. Efficiency of encapsulated *L. acidophilus* was evaluated by inoculating it into pasteurized cow's milk. About 5 gm of microencapsulated *L. acidophilus* beads were inoculated into 100 ml of pasteurized cow's milk and allowed for setting. The quality characteristics such as nutrient content and physico-

Tele:

E-mail addresses: skarthiksundaram1984@yahoo.com

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chemical properties were determined to assess the efficiency of encapsulated *L. acidophilus* and compared with that of the curd produced traditionally (Adhikari et al., 2001).

Results and Discussion

The morphological characteristics of sub cultured *L. acidophilus* were studied using gram staining technique. It was clear that the organism was a Gram positive rod, with a size of 1.5-6µm, non-flagellated, non motile, non-spore forming, occurs in pairs and in short chains. The organism was able to utilize a host of many sugars (fructose, galactose, glucose, lactose, maltose, sucrose, and mannose) though it was not able to utilize arabinose (Table 1). These characteristics were in accordance with the characteristics of *L. acidophilus* illustrated in the Bergy's manual of determinative bacteriology (Bergey and Breed, 1957).

Preparation of curd with microencapsulated *L. acidophilus* - Quality characteristics

The curd prepared with microencapsulated *L. acidophilus* was found to set at a time period of 25 ½ hours. The quality characteristics such as physico-chemical characteristics and nutrient contents were analyzed to evaluate the efficiency of *L. acidophilus* in probiotic curd prepared. Table 2 gives the physico-chemical characteristics of the curd samples.

The pH and titrable acidity of the curd prepared with microencapsulated *L. acidophilus* was 4.0 and 0.81 respectively. The value was less than that of the pH (3.8) and titrable acidity (0.9) of normal curd. Microencapsulation has found to be very effective and this approach could be used to increase the stability of curd during storage (Sultana *et al.*, 2000). Similarly according to the work of Srinivas *et al.*, (1997) it was observed that the acidity of the curd gets increased on storage. The prepared curd's acidity (0.81 and 0.9) satisfied the standard value of 0.7 – 10.0 percent (Champak Palit, 2001)

The protein content has an important role in assessing the quality characteristics of any product. The protein content of microencapsulated curd sample was 1.81 gm which was slightly higher than that of the protein content of normal curd (1.75 gm) (Table 3).

This could be attributed to synthesis of amino acid and protein by *L. acidophilus* as stated by Hou *et al.*, (2000) and Blandino *et al.*, (2003) who reported that bacterial synthesis raises the food protein value and improves the balance of amino acids.

The fat content of the curd prepared with microencapsulated *L. acidophilus* was 2.20 gm against 3.90 gm of fat in normal curd (Table 3).

The triglyceride and free fatty acid content of the curd gets reduced on fermentation due to fat reducing property of *L. acidophilus* which reflected in the fat content of cultured curd (Gupta and Prabhu, 2004).

The curd prepared with microencapsulated *L. acidophilus* had an ash content of about 0.41 gm. The iron and phosphorus content were found to be 7.2 mg and 99.85 mg respectively (Table 3). Abdalla *et al.*, (1998) stated that bacterial action can break down components such as iron and phosphorus and due to this the prepared probiotic curd with microencapsulated *L. acidophilus* had high mineral content than normal curd (0.2 gm).

The curd prepared with microencapsulated *L. acidophilus* had Vitamin-C content of 6 mg which was similar to Vitamin-C

content of normal curd (6mg) and higher than the standard (ICMR) value of 1 mg. Calcium content was found to be 116 mg against 128 mg in normal curd (Table 3).

Conclusion

From the present investigation, it is understood that the probiotic curd prepared with microencapsulated *L. acidophilus* had more nutritional properties. Microencapsulated process was found to increase the viability of probiotic strain *L. acidophilus* and there was a slight increase in the nutrient content than the normal curd. It had protein of high biological value and reduced fat content compared to ordinary curd and also had relatively high level of iron and phosphorus and calcium content. The consumption of such probiotic products induces measurable health benefits linked to the presence of live bacteria, which supports healthy intestinal balance, enhances immune function, helps in easy lactose digestion and improves the better utilization of nutrients.

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Table 1: Morphological, Physiological and Biochemical characteristics of *L. acidophilus*

S. No	Characters analyzed	Results observed
1	Gram Staining	Positive
2	Size	1.5-6µm
3	Shape	Rod
4	Motility	Non flagellated non motile
5	Spore formation	Non spore forming
6	Arrangement	Pairs or short chains
7	Arabinose	-
8	Fructose	+
9	Galactose	+
10	Glucose	+
11	Lactose	+
12	Maltose	+
13	Sucrose	+
14	Mannose	+

(+) - Positive reaction; (-) – Negative reaction

Table 2: Physico-chemical characteristics of prepared curd samples

S. No	Criteria	pH	Titration acidity
1	Curd with microencapsulated <i>L. acidophilus</i>	4.0	0.81
2	Normal curd	3.8	0.9

Table 3: Nutrient content of the prepared curd samples

S.No	Nutrient content	Curd with microencapsulated <i>L. acidophilus</i>	Normal curd
1	Protein (gm)	1.81	1.75
2	Fat (gm)	2.20	3.90
3	Ash (gm)	0.41	0.2
4	Vitamin-C (mg)	6	6
5	Iron (mg)	7.2	3
6	Phosphorus (mg)	99.8	92.8
7	Calcium (mg)	116	128