

## Survival of Free and Encapsulated Probiotic Bacteria and their effect on the Sensory Properties of Quarg Cheese

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### Abstract

The survival and effect of free and calcium-induced alginate-starch encapsulated probiotic bacteria (*Lactobacillus casei* NCDC 298 and *Lactobacillus acidophilus* NCDC 15), on sensory attributes of quarg cheese were studied over 30 days of storage. The results showed that there were significant difference ( $p < 0.05$ ) in free and encapsulated probiotic cells for both the probiotic bacteria after 30 days of storage. There was increased survival of 1.5 and 1 log cell Number chargess of both *Lactobacillus casei* NCDC 298 and *Lactobacillus acidophilus* NCDC 15, respectively due to protection of cells by microencapsulation. The high survivability of encapsulated probiotic cells 8.88 log in case of NCDC 298 compared to 8.14 log cells for NCDC 15 during 30 days of storage of quarg cheese, indicates that *Lactobacillus casei* NCDC 298 can be effectively used as therapeutic dose in encapsulated form for the manufacture of probiotic quarg cheese. The addition of probiotics either in free or encapsulated states did not significantly ( $p < 0.05$ ) affected various sensory attributes like flavor and taste, body and texture and color and appearance of the quarg cheese over the storage period. This study has shown that incorporation of free (M3) and encapsulated (M4) probiotic bacteria do not substantially alter the overall sensory characteristics of quarg cheese and microencapsulation helps to enhance the survival of probiotic bacteria in quarg cheese during storage.

**Keywords:** Quarg cheese, Probiotics, Microencapsulation, Free cells, Sensory attributes

*Vedic sanskriti* recognized the role of diet in health and nutrition and now after several thousand years, this is an era when we are proving the linkages among health, nutrition and diet with science based investigations. Food serves a much wider role than just satisfying hunger needs and providing a sense of satiety (Saguy and Moskowitz, 1999). Functional foods have emerged as a new approach to improve human nutrition and well-being. A fermented dairy product constitutes one of the most important functional foods and is a vital component of the human diet in India, as in many other regions of the world. Introduction of products containing probiotics are on the rise, with many new product introductions occurring in yogurt, smoothies, spreads, fresh cheeses, cereals and shelf stable dressings.

Probiotic bacteria are defined as ‘living microorganisms, which upon ingestion in certain Number chargess exert health benefits beyond inherent basic nutrition’ (Ross *et al.*, 2002). A Number charges of health benefits for product containing live probiotic bacteria have been claimed including alleviation of symptoms of lactose intolerance, treatment of diarrhea, anticarcinogenic properties, reduction of blood cholesterol and improvement in immunity (Shah and Wu, 1999; Shah, 2000a). High levels of daily consumption of probiotic bacteria, however, are required to confer health benefits. For dietary cultures to be beneficial in food systems, they are expected to be viable in the food until the time of consumption and present at levels of at least  $10^7$  viable cells per gram or milliliter of a product (Ishibashi and Shimamura, 1993). For this reason, it is important to know the changes in the Number chargess of viable bacteria during storage period. A few studies have shown that many commercial products have failed to successfully deliver the required level of viable cells of probiotic bacteria (Shah and Lankaputhra, 1997; Dave and Shah, 1997).

The utmost challenge in the development of probiotic products is to maintain the adequate Number charges of viable cells through the shelf life of the product as well as during the gastrointestinal (GI)-tract transit after consumption, so that the claimed health benefits can be delivered to the consumer (Shah *et al.*, 1997). However, there are still several problems with respect to the low viability of probiotic bacteria in dairy foods. Several factors have been reported to affect the viability of probiotics in fermented dairy products, including titratable acidity, pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains of associative fermented dairy product organisms, concentration of lactic and acetic acids and even whey protein concentration (Dave and Shah, 1997; Kailasapathy and Supriadi, 1996; Lankaputhra, Shah, and Britz, 1996). Survival is, of course, essential for organisms targeted to populate the human gut – one of the most important issues in health benefit provision by probiotic bacteria. Different approaches that increase the resistance of these sensitive microorganisms

against adverse conditions have been proposed, including appropriate selection of acid- and bile-resistant strains, use of oxygen-impermeable containers, two-step fermentation and stress adaptation, incorporation of micronutrients such as peptides and amino acids and microencapsulation (Gismondo *et al.*, 1999).

Microencapsulation of probiotic bacteria in hydrocolloid beads is one of the recent techniques studied to improve the viability and activity of the cells under unfavorable conditions by entrapping the bacteria within a bead matrix (Chandramouli *et al.*, 2004). It has been found to improve the viability and activity of probiotics in food products and the intestinal tract by entrapping the cells within a bead matrix, thus, segregating them from adverse environmental conditions as well as protecting them against bacteriophages (W. Krasaekoopt *et al.*, 2003).

Cheeses have a number of advantages over fresh fermented products such as yoghurt. As a delivery system for viable probiotic carriage to gastrointestinal (GI)-tract, cheeses have a higher pH and a more solid consistency, where the matrix of the cheese and its relatively high fat content may offer protection to probiotic bacteria during passage through the gastrointestinal tract. Cheeses also have higher buffering capacity than yoghurt (Gardiner *et al.*, 1999).

Quarg is a natural, soft, white and un-ripened variety of fresh cheese originated from Central Europe where it is generally manufactured from cow milk only. India is the largest manufacturer of buffalo milk in the world, so there is a need to develop an appropriate technology for manufacture of good quality Quarg cheese from buffalo milk. Further, the health status of Quarg cheese can be enhanced by incorporation of functional ingredients like probiotics bacteria and prebiotic like inulin. Several well established probiotics in terms of health benefits have been used in various dairy products including Quarg cheese. However, the combined use of prebiotic like inulin and probiotic bacteria in Quarg cheese and that are also in encapsulated form has seldom been reported. Therefore, in this study, probiotic Quarg cheese was manufactured from buffalo milk by incorporation of probiotic bacteria in free as well as in encapsulated form.

## Materials and Methods

Fresh, chilled, raw buffalo milk was used in this study. Microbial rennet (Meito) commercially produced in granular form from *Mucorpusillus var. Lindt* was procured from M/s Meito Sangyo and Co. Ltd., Tokyo, Japan. Freeze dried lactic mesophilic cheese culture NCDC-149 (*Lactococcus lactis ssp. lactis*, *Lactococcus lactis ssp. cremoris*, *Lactococcus lactis ssp. diacetylactis*) for manufacturing Cultured Quarg Cheese was procured from the National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, NDRI, Karnal. The probiotic strains *Lactobacillus casei* NCDC 298 and *Lactobacillus acidophilus* NCDC

15 were used in this experiment obtained from the National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, NDRI, Karnal. All probiotic strains had previously been shown to possess properties required of probiotic microorganisms including bile salt and low pH tolerance, various antibiotic resistances and antagonistic activity (Mandal, 2006). Prebiotic inulin Raftline®ST manufactured by Orafit Active Food Ingredients, Belgium was procured from S.A. Pharmachem Pvt. Ltd., Goregaon, Mumbai. Iodized sodium chloride salt manufactured by Tata chemicals Ltd., Mumbai was procured from local market.

### **Cultivation and Harvesting of Probiotic *Lactobacillus***

The MRS broth was prepared in 500 ml quantities and sterilized at 15 psi for 15 min followed by cooling at room temperature and inoculation with selected probiotic culture @ 1 percent with subsequent incubation at 37°C for 48 hrs. After the incubation period was over, the contents were transferred aseptically to sterilized 100 ml centrifuge bottles. The content transferred in centrifuge bottles of 100 ml were concentrated by centrifuging the cultivated cell biomass at 10000 × g for 10 min at 4°C using refrigerated centrifuge. The supernatant in the form of spent MRS broth was disposed and cell biomass sticking at the bottom of the tubes was washed thrice using sterile saline water followed by centrifugation under same conditions and decantation of the supernatant and thereafter the biomass was collected in sterile petri dishes under sterile condition and stored at refrigeration temperature up to the final use.

### **Total Viable *Lactobacillus* Count**

Total viable count of cell biomass obtained as pellets upon centrifugation was enumerated to quantify cell biomass to be incorporated in the product (approx.  $10^7 - 10^8$  cfu/gm) to maintain the required therapeutic dose of probiotics. Roughly 1.0 gm cell pellets were dissolved in 10 ml of sterile saline water test tube. In order to ensure complete dispersion, the suspension was vortexed at maximum speed for 2 min. The serial dilutions were made from this suspension by taking one ml aliquot and appropriate dilutions were plated out in MRS agar media using the standard procedure. The plates after solidification were incubated at 37°C for 24-48 hrs.

### **Microencapsulation and enumeration of probiotic bacteria**

The probiotic cells (NCDC 298 and NCDC 15) were microencapsulated in sodium alginate matrix as described by Sheu *et al.*, (1993). Sodium alginate solutions (2, 3 and 4%) were prepared, sterilized by autoclaving (120°C for 15 min.) and cooled to 38-40°C. Twenty milliliters of sodium alginate solution and 4 ml of cell suspension  $\sim 10^{11}$  cfu/ml cell concentration were transferred to a centrifuge tube (40

ml) and the content was mixed for homogeneity. One hundred milliliters soybean oil, containing 0.2 per cent Tween 80 as emulsifier, was taken and the alginate-cell mixture was added drop wise to the continuous phase that was magnetically stirred. Within 5 min a uniformly turbid emulsion was obtained to which 0.1M calcium chloride (100 ml) was added quickly to break the emulsion and to harden the alginate microcapsules. The capsules were harvested by gentle centrifugation at 350xG for 10 minutes at 4°C and washed with distilled water. The beads were separated by filtration and stored in refrigerator (7±1°C). Schematic flow diagram of Encapsulation process is given in figure 1. The viability of alginate-encapsulated lactobacilli was evaluated after depolymerisation of beads in phosphate buffer followed by plating using MRS agar (Sheu and Marshall, 1993).

### **Manufacture of Cultured Quarg cheese**

- Quarg cheese was manufactured by using method described by (Milanovic *et al.* 2004) with some modifications as shown in figure 2. Good quality standardized buffalo milk (Fat- 3.0±0.30%) was taken in a vat and pasteurized at 85°C for 15 min. The cheese milk was then cooled to 35 to 37°C and inoculated with cheese culture (NCDC-149) @ 1.0 percent followed by incubation at temperature 37°C. Two and half hrs (2.5 hrs) after the addition of starter culture (when the pH reaches up to 6.3), Meito rennet @ 250mg/100kg milk was added and mixed thoroughly. Thereafter the vat content was left undisturbed for curd setting, which took around 14-16 hrs (pH 4.5-4.6) (time starting from culturing). The coagulum was then cut using 1/3 inch cheese knives and it was again left undisturbed for about 10-15 min. The temperature of the contents was then gradually increased to 60°C @ 1°C per minute and the curd was held for 10 min at 60°C as per the requirement for thermo-quarg manufacture. Cooked curd was then cooled to room temperature and filled in muslin cloth and hung for 3.5 to 4 hrs for whey drainage. Thereafter, salt was added in curd and homogenization of total mass was carried out in Hobart mixer. The quarg cheese was then filled in PS cups and stored at 6±1°C.

### **Incorporation of Probiotic Cultures and Prebiotic**

Probiotic cultures (NCDC 298 and NCDC 15) were incorporated into quarg cheese in three different forms and at two different stages. Accordingly probiotic quarg cheese was manufactured using three different methods with control Cultured Quarg cheese namely M2, M3, M4 and M1 methods respectively. Prebiotic ingredient (Inulin) was added at mixing stage in powder form without giving any additional treatment.

**M1** : Control Cultured Quarg Cheese

**M2** : Probiotic added with Traditional Starter Culture (TSC)

**M3** : Probiotic Cell Bio-mass added at mixing stage

**M4** : Encapsulated Probiotic added at mixing stage

### **Sensory analysis**

The experimental quarg cheese was evaluated by a trained panel of seven judges selected from the faculty of Dairy Technology Division, NDRI, Karnal (India), for sensory evaluation using quarg cheese score card (Total score-100). Out of 100, the maximum marks of 50 were allocated for flavour, while 35 and 15 marks were allocated for body and texture, and colour and appearance, respectively. The flavour was evaluated by smelling and tasting. The body and texture was assessed by inserting the spoon and scooping the portion of the sample from sample container, by mouth feel and by spreading a layer on the bread slice.

### **Data analysis**

The data were statistically analyzed using the SYSTAT Software (version 6.01). In all experiments, two-way analysis of variance (ANOVA) with subsequent least significant difference (LSD) test was applied for multiple sample comparison. This was done to test for any significant differences ( $P < 0.05$ ) in the mean value of all the groups as described by Snedecor and Cochern (1989).

### **Results and Discussion**

The survival and effect of free and calcium-induced alginate-starch encapsulated probiotic bacteria (*Lactobacillus casei* NCDC 298 and *Lactobacillus acidophilus* NCDC 15), on sensory attributes of quarg cheese were studied over 30 days of storage.

#### ***Effect on survivability of probiotics NCDC 298 and NCDC 15***

The survivability of two probiotics NCDC 298 and NCDC 15, obtained for free as well as encapsulated cells in quarg cheese during storage is shown in figure 3. The results showed that there were significant difference ( $p < 0.05$ ) in free and encapsulated probiotic cells for both the probiotic bacteria after 30 days of storage. There was increased survival of 1.5 and 1 log cell Number chargess of both *Lactobacillus casei* NCDC 298 and *Lactobacillus acidophilus* NCDC 15, respectively due to protection of cells by microencapsulation. The survivability of NCDC 298 and NCDC 15 did not differ significantly between the experimental

samples M2 and M3 up to 7 days of storage. Significant ( $p < 0.05$ ) increase was found after 14 days of storage. The high survivability during the whole storage period (up to 30 days), not less than 8.88 log cfu/gm was observed in case of Quarg cheese manufactured by M4 method using NCDC 298 as probiotic culture. However, we can disregard the variation found and strongly consider the viable counts found as excellent. The probiotic population significantly ( $p < 0.05$ ) decreased during storage for probiotic NCDC 15. Moreover population was above  $10^7$  cfu per gm during the complete storage period (up to 30 days). For the experimental samples M3 and M4, the minimum counts suggested by several authors to produce beneficial health effects on the gut, is  $10^7$ - $10^9$  cfu per 100 g of daily product consumption (Hoier *et al.*, 1999; Vinderola, Prosello, Ghiberto and Reinheimer, 2000, Vinderola and Reinheimer, 2000). Other authors also reported satisfactory probiotic viability when producing probiotic fresh cheese (Buriti *et al.*, 2005a; Gomes and Malcata, 1999; Vinderola *et al.*, 2000), corroborating the use of fresh cheese like Quarg cheese as vehicle for probiotics.

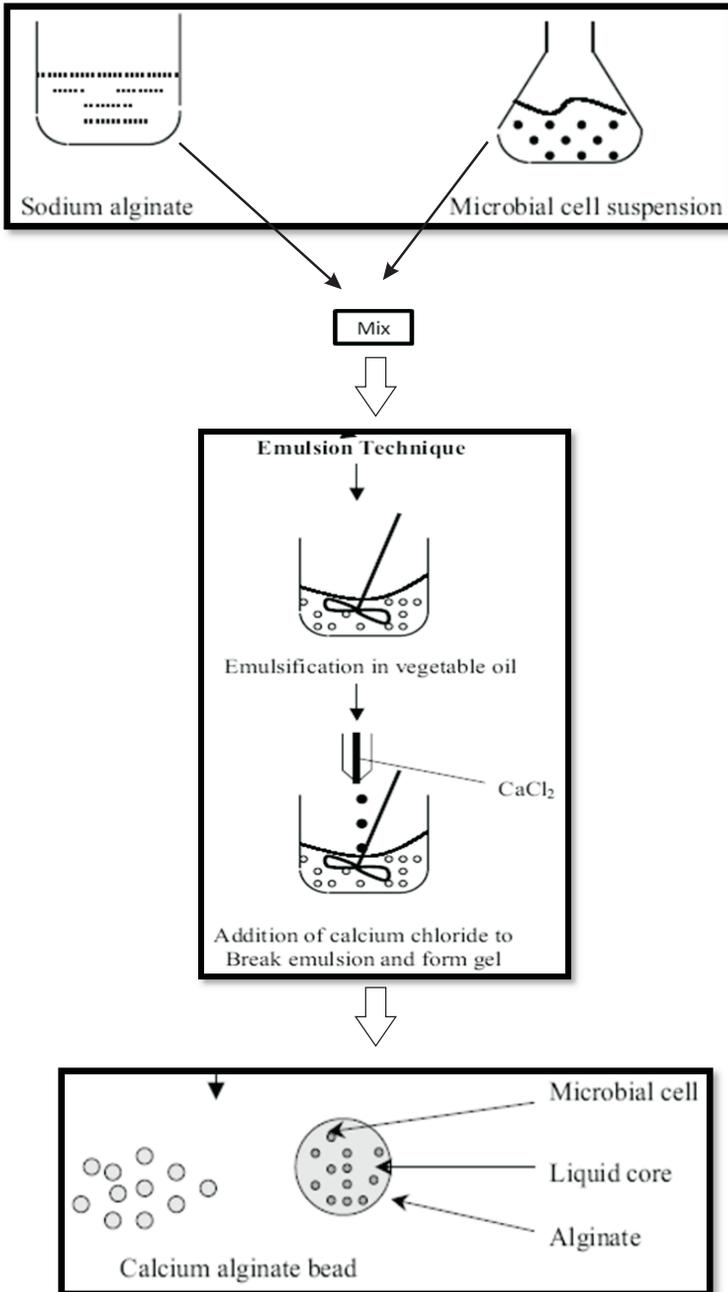
Besides the satisfactory probiotic population (viable count) a protective behavior by prebiotic (inulin) added to the cheese trials was expected. In fact, the fructan type prebiotic inulin and oligo-fructose may also aid survival of probiotic organism during processing and storage of dairy products, particularly increasing or at least retaining, the viability of *Bifidobacterium spp.* and *L. acidophilus* (Bruno, Lankaputhra, and Shah, 2002; Capela, Hay, and Shah, 2006; Ozer, Akin, and Ozer, 2005; Shin, Lee, Pestka, and Ustunol, 2000). Moreover in present study, the presence of prebiotics in experimental samples M2, M3 and M4 lead to a slight increase in viable counts of NCDC 298 and NCDC 15 during storage. Thus, incorporation of encapsulated probiotic NCDC 298 in Quarg cheese improves the viability well above the recommended therapeutic dose level  $10^7$ cfu per gm during storage period.

### ***Effect on sensory properties of quarg cheese***

The effects of addition of probiotics in free as well as microencapsulated form, on sensory properties of quarg cheese were also studied over storage period of 30 days. The addition of probiotics either in free or encapsulated states significantly ( $p < 0.05$ ) affected various sensory attributes like flavor and taste, body and texture and color and appearance of the quarg cheese over the storage period.

### ***Effect on flavor***

The effect of incorporation of two probiotics NCDC 298 and NCDC 15 in free and encapsulated forms, on flavour of quarg cheese during storage period is presented in Table 1. Regardless of the type of method, initial flavour score of fresh sample found to be ranges from 42.33 to 46.52. Methods of manufacturing



**Fig. 1.** Encapsulation of probiotic bacteria by the emulsion technique (Krasaekoopt *et al.*, 2003)

and free and encapsulated probiotics appeared to appreciably affect the initial flavour score. Table 1 clearly depicts that flavour score of samples significantly decrease ( $P<0.05$ ) with increase in storage period. Up to 21 days, control (M1) and M2 samples remains acceptable. Thereafter control (M1) and sample M2 showed rapid deterioration. Both samples were judged and found unacceptable on sensory evaluation due to prominent putrefactive odour being formed as yeasty or moldy. However, samples M3 and M4 reasonably achieved higher ( $p<0.05$ ) scores which on subsequent storage declined to 42.27 and 42.03 for probiotic culture NCDC 298 and 40.96 and 40.421 for NCDC 15, respectively upon 21 days storage of quarg cheese. However, samples M3 and M4 still possessed significantly higher flavour ( $P<0.05$ ) scores up to 30 days of storage.

**Table 1. Effect of free and encapsulated probiotics NCDC 298 and NCDC 15 on flavour of quarg cheese during storage**

Probiotic	Days	Production Method				CD <sub>0.05</sub>
		M1 (Control)	M2	M3	M4	
NCDC 298	0	46.52±0.72 <sup>p</sup>	42.33±0.83 <sup>q</sup>	45.20±0.58 <sup>p</sup>	45.85±0.28 <sup>p</sup>	1.732
	7	44.45±0.47 <sup>p</sup>	39.83±0.38 <sup>q</sup>	44.48±0.62 <sup>p</sup>	44.35±0.61 <sup>p</sup>	
	14	40.91±0.82 <sup>q</sup>	37.35±0.21 <sup>r</sup>	43.28±0.32 <sup>p</sup>	44.15±0.70 <sup>p</sup>	
	21	35.64±1.04 <sup>q</sup>	35.21±0.67 <sup>r</sup>	42.27±0.49 <sup>p</sup>	42.03±1.19 <sup>p</sup>	
	30	-----	-----	40.52±0.52 <sup>p</sup>	40.97±0.25 <sup>p</sup>	
NCDC 15	0	46.52±0.72 <sup>p</sup>	41.30±0.94 <sup>q</sup>	44.91±0.88 <sup>p</sup>	45.58±0.32 <sup>p</sup>	1.864
	7	44.45±0.47 <sup>p</sup>	38.90±0.39 <sup>q</sup>	44.14±0.74 <sup>p</sup>	44.03±0.64 <sup>p</sup>	
	14	40.91±0.82 <sup>q</sup>	36.86±0.42 <sup>r</sup>	42.86±0.48 <sup>p</sup>	42.41±0.73 <sup>p</sup>	
	21	35.64±1.04 <sup>q</sup>	34.23±0.64 <sup>r</sup>	40.96±0.82 <sup>p</sup>	40.41±0.82 <sup>p</sup>	
	30	-----	-----	38.79±0.63 <sup>p</sup>	39.27±0.61 <sup>p</sup>	

Mean±S.D, n=3

pqr Mean with different superscripts within row differ significantly ( $p<0.05$ )

Upon further storage it was observed that flavour of M3 and M4 samples slightly deteriorated but still had significantly ( $p<0.05$ ) higher flavour score as compared to both control (M1) and M2 samples and were kept well up to 30 days of storage without any perception of off flavour.

**Table 2. Effect of free and encapsulated probiotics NCDC 298 and NCDC 15 on body and texture of quarg cheese during storage**

Probiotic	Days	Production Method				CD <sub>0.05</sub>
		M1 (Control)	M2	M3	M4	
NCDC 298	0	33.69±0.85 <sup>p</sup>	31.36±0.62 <sup>r</sup>	34.16±0.80 <sup>p</sup>	33.98±0.32 <sup>p</sup>	1.036
	7	32.49±0.82 <sup>p</sup>	30.16±0.31 <sup>r</sup>	33.72±0.77 <sup>p</sup>	32.91±0.28 <sup>p</sup>	
	14	31.13±0.65 <sup>p</sup>	28.93±0.60 <sup>r</sup>	32.82±0.62 <sup>q</sup>	32.20±0.86 <sup>q</sup>	
	21	27.36±0.63 <sup>p</sup>	26.49±0.87 <sup>p</sup>	31.13±0.72 <sup>q</sup>	31.07±0.75 <sup>q</sup>	
	30	-----	-----	30.93±0.63 <sup>q</sup>	30.44±0.49 <sup>q</sup>	
NCDC 15	0	33.69±0.85 <sup>p</sup>	31.02±0.44 <sup>r</sup>	33.97±0.48 <sup>p</sup>	33.76±0.62 <sup>p</sup>	1.104
	7	32.49±0.82 <sup>p</sup>	30.29±0.58 <sup>r</sup>	33.71±0.84 <sup>p</sup>	33.06±0.43 <sup>p</sup>	
	14	31.13±0.65 <sup>p</sup>	28.35±0.32 <sup>r</sup>	32.40±0.72 <sup>q</sup>	32.10±0.65 <sup>q</sup>	
	21	27.36±0.63 <sup>p</sup>	26.61±0.82 <sup>p</sup>	30.76±0.66 <sup>q</sup>	30.58±0.67 <sup>q</sup>	
	30	-----	----	29.82±0.74 <sup>q</sup>	29.55±0.35 <sup>q</sup>	

Mean±S.D, n=3

pqr Mean with different superscripts within row differ significantly (p<0.05)

***Effect on body and texture***

Type of probiotic organism and method of manufacturing were found to apparently affect the body and texture score of the fresh quarg cheese samples. Table 2 indicates that no significant difference (p<0.05) was observed in body and texture score of fresh samples of control (M1), M3 and M4 on 0 day for both probiotic cultures NCDC 298 and NCDC 15. It was observed that quality of M3 samples as well as M4 samples were relatively same in terms of body and texture up to 30 days. Control (M1) and M2 samples were judged and found acceptable body and texture score up to 21 days. After 21 days of storage, body and texture quality of both the control (M1) and M2 samples absolutely deteriorated and lost the identity of smooth and spreadable body and texture. However, Quarg cheese samples M3 and M4 made using probiotic NCDC 298 showed relatively small deterioration in body and texture as compared to that for NCDC 15.

***Effect on colour and appearance***

The consequence of method of manufacturing using two different probiotic NCDC 298 and NCDC 15 on the colour and appearance of quarg cheese during storage are detailed in Table 3. It was observed that type of probiotic in free and encapsulated

forms had significant effect ( $P<0.05$ ) on colour and appearance of quarg cheese. Up to 7 days of storage, colour and appearance of quarg cheese, regardless of the method of manufacture, showed no marked variation and the average score of different samples dropped steadily and slowly for both the probiotic NCDC 298 and NCDC 15. On 14 days, control (M1) as well as the experimental sample M2 achieved significantly ( $P<0.05$ ) inferior score as compared to M3 and M4 samples on subsequent storage. After 21 days, quality of the control as well as the experimental sample M2 speedily deteriorated in comparison to the M3 and M4 samples. However, the samples M3 and M4 were still quite good in terms of colour and appearance up to 30 days.

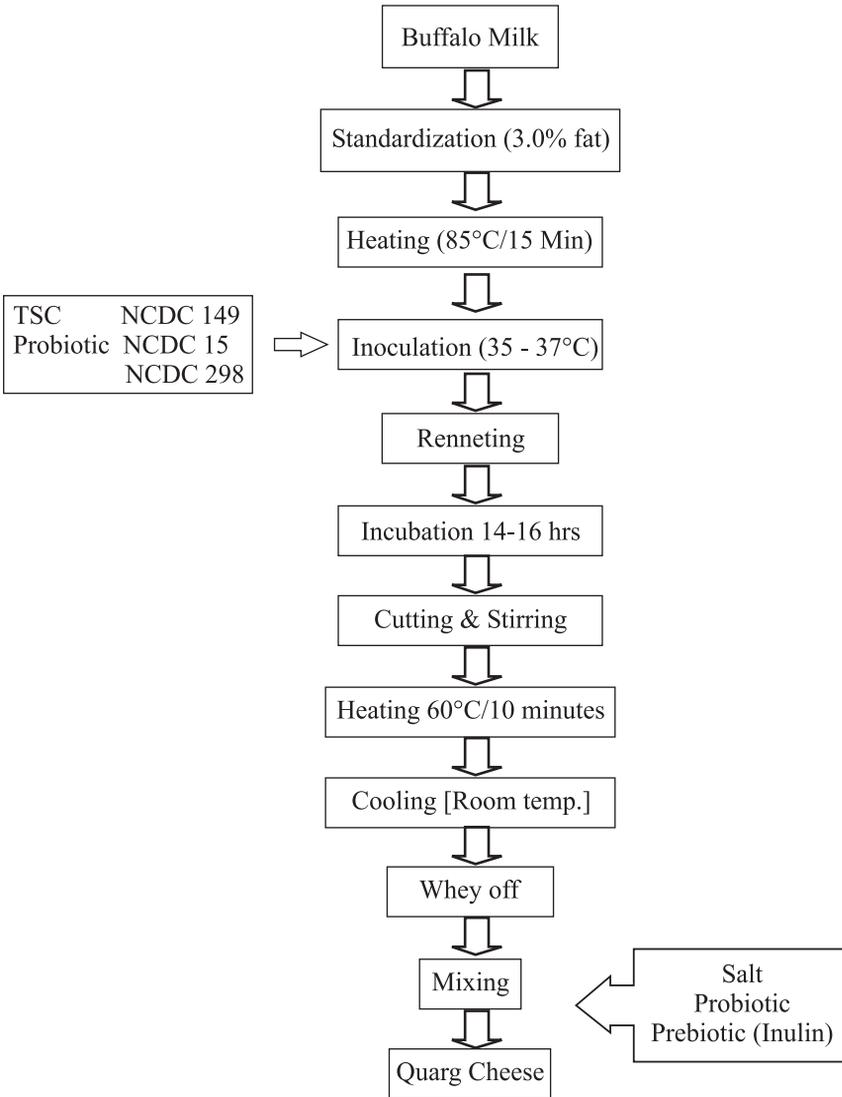
Table 3 also illustrates that deterioration in colour and appearance occurred rapidly towards the end of the storage period because of the rapid proliferation of yeast and mold growth which resulted in slimy appearance and discoloration of surface. Whereas the samples M3 and M4 has got acceptability up to 30 days in terms of colour and appearance.

**Table 3. Effect of free and encapsulated probiotics NCDC 298 and NCDC 15 on colour and appearance of quarg cheese during storage**

Probiotic	Days	Production Method				CD <sub>0.05</sub>
		M1 (Control)	M2	M3	M4	
NCDC 298	0	13.29±0.45 <sup>p</sup>	12.30±0.34 <sup>r</sup>	14.13±0.30 <sup>q</sup>	13.96±0.44 <sup>q</sup>	0.430
	7	13.09±0.42 <sup>p</sup>	12.00±0.44 <sup>r</sup>	14.02±0.24 <sup>q</sup>	13.52±0.50 <sup>p</sup>	
	14	11.78±0.38 <sup>p</sup>	10.29±0.48 <sup>r</sup>	13.17±0.18 <sup>q</sup>	12.93±0.66 <sup>q</sup>	
	21	8.06±0.76 <sup>p</sup>	9.78±0.58 <sup>r</sup>	12.30±0.38 <sup>q</sup>	12.27±0.42 <sup>q</sup>	
	30	-----	-----	11.58±0.46 <sup>q</sup>	11.52±0.35 <sup>q</sup>	
NCDC 15	0	13.29±0.52 <sup>p</sup>	10.93±0.58 <sup>r</sup>	14.19±0.40 <sup>q</sup>	14.25±0.34 <sup>q</sup>	0.441
	7	13.09±0.40 <sup>p</sup>	10.71±0.62 <sup>r</sup>	13.90±0.38 <sup>q</sup>	13.49±0.45 <sup>p</sup>	
	14	11.78±0.36 <sup>p</sup>	9.95±0.42 <sup>r</sup>	12.97±0.48 <sup>q</sup>	12.86±0.28 <sup>q</sup>	
	21	8.06±0.60 <sup>p</sup>	9.28±0.48 <sup>r</sup>	12.18±0.55 <sup>q</sup>	12.22±0.48 <sup>q</sup>	
	30	-----	-----	11.50±0.65 <sup>q</sup>	11.11±0.54 <sup>q</sup>	

Mean±S.D, n=3

pqr Mean with different superscripts within row differ significantly ( $p<0.05$ )

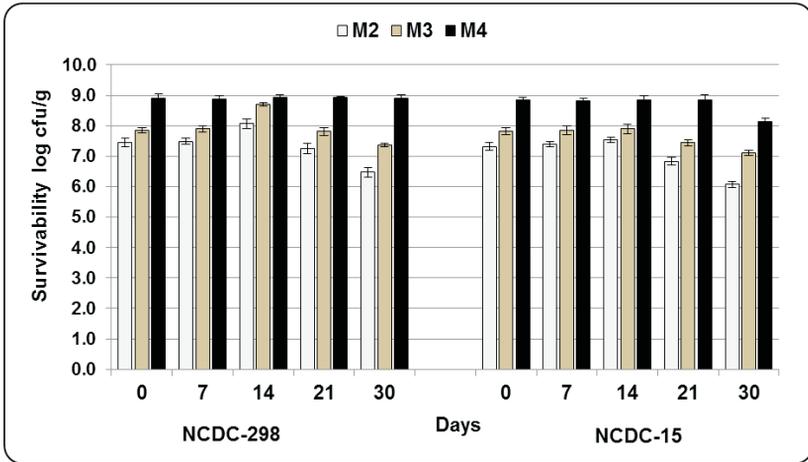


**Fig. 2.** Flow diagram for manufacture of probiotic quarg cheese

***Effect on overall acceptability***

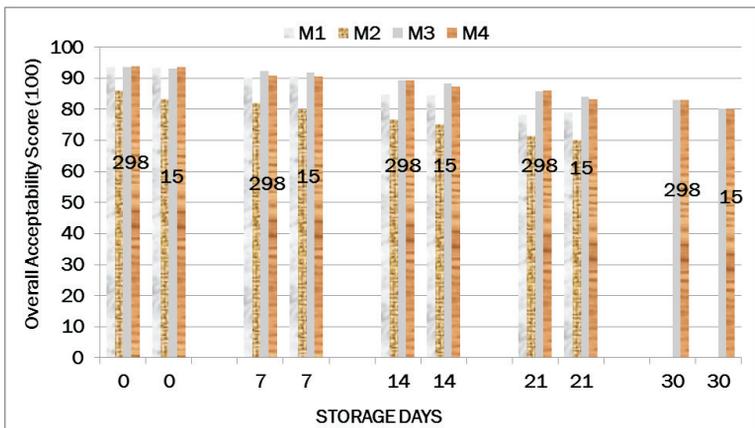
Figure 4 shows overall acceptability results from the comparative study of different manufacturing processes of quarg cheese using probiotics NCDC 298 and NCDC 15 in free as well as encapsulated form and it shows significant impact on overall acceptability score, by affecting the various sensory attributes of quarg cheese during storage. It was observed that the overall acceptability score of experimental samples M3 and M4 were significantly higher than those of the other two cheeses

M1 (control) and M2 almost at all sampling ages.



**Fig. 3.** Survival of free and encapsulated probiotics NCDC 298 and NCDC 15 during storage of quarg cheese

As the storage days progressed, the overall acceptability score decreased for all experimental samples as well as for control samples. However control (M1) and experimental sample M2 got acceptability up to 21 days as compared to experimental samples M3 and M4 which got acceptability up to 30 days. Among the two probiotic, quarg cheese with NCDC 298 in encapsulated form was found to have good sensory attributes up to 30 days as compared to that made from NCDC 15.



**Fig. 4.** Effect of free and encapsulated probiotics NCDC 298 and NCDC 15 on overall acceptability of quarg cheese during storage

## Conclusion

This study showed that quarg cheese incorporating probiotic cultures could be made without much modification from the traditional quarg making technology. Sensory analysis of the quarg cheese showed that addition of probiotic in free and capsule forms did not significantly alter the flavor and taste, body and texture and color and appearance of the quarg cheese, however microencapsulation is required to enhance the survival of probiotic cells compared to free cells in quarg cheese stored over 30 days.

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## References

- Bruno, F., Lankaputhra, W.E.V. and Shah, N.P. (2002). Growth, viability and activity of *Bifidobacterium* spp. in skim milk containing prebiotics. *Journal of Food Science*, **67**(7): 2740-2744.
- Buriti, F. C. A., Rocha, J. S., Assis, E. G. and Saad, S. M. I. (2005). Probiotic potential of Minas fresh cheese prepared with the addition of *Lactobacillus paracasei*. *Lebensmittel-Wissenschaft und-Technologie*, **38**(2): 173–180.
- Buriti, F. C. A., Rocha, J. S. and Saad, S. M. I. (2005). Incorporation of *Lactobacillus acidophilus* in Minas fresh cheese and its implications for textural and sensorial properties during storage. *International Dairy Journal*, **15**(12): 1279–1288.
- Capela, M., Hay, T. K. and Shah, N. P. (2006). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze dried yoghurt. *Food Research International*, **39**(2): 203-211.
- Chandramouli, V., Kailasapathy, K, Peiris, P. and Jones, M. (2004). An improved method of microencapsulation and its evaluation to protect *Lactobacillus* spp in simulated gastric conditions. *Journal of Microbiology Methods*, **57**: 27-35.
- Dave, R. I. and Shah, N. P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal*, **7**: 31-41.
- Gardiner, G., Ross, R. P., Wallace, J. M., Scanlan, F. P., Jagers, P. P.J. M., Fitzgerald, G., Collins, J. K. and Stanton, C. (1999). Influence of a probiotic adjunct culture of *Enterococcus faecium* on the quality of Cheddar cheese. *J. Agric. Food Chem.*, **47**: 4907-4916.

- Gismondo, M. R., Drago, L. and Lombardi, A. (1999). Review of probiotics available to modify gastrointestinal flora. *International Journal of Antimicrobial Agents*, **12**: 287-292.
- Gomes, A. M. P. and Malcata, F. X. (1999). Bifidobacterium spp. and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science and Technology*, **10**(4-5): 139-157.
- Hoier, E., Janzen, T., Henriksen, C. M., Rattray, F., Brockmann, E. and Johansen, E. (1999). The production, application and action of lactic cheese starter cultures. In B. A. Law (Ed.), *Technology of cheesemaking*. Boca Raton: CRC Press, pp. 99–131.
- Ishibashi, N. and Shimamura, S. (1993). Bifidobacteria: Research and development in Japan. *Food Technol.*, **47**: 126-134.
- Kailasapathy, K. and Supriadi, D. (1996). Effect of whey protein concentrate on the survival of Lactobacillus acidophilus in lactose hydrolysed yoghurt during refrigerated storage. *Milchwissenschaft*, **51**: 565-569.
- Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: Technology and potential applications. *Current Issues in Intestinal Microbiology*, **3**: 39–48.
- Krasaekoopt, W., Bhandari, B. and Deeth, H. (2003). Evaluation of encapsulation technique of probiotics for yoghurt: A review. *International Dairy Journal*, **13**: 3-13.
- Lankaputhra, W. E. V., Shah, N. P. and Britz, M. L. (1996). Survival of Bifidobacteria during refrigerated storage in the presence of acid and hydrogen peroxide. *Milchwissenschaft*, **51**: 65-70.
- Mandal, S. (2006). Microencapsulation of probiotics and their application in manufacture of biodynamic milk chocolate. Ph. D. Thesis, National Dairy Research Institute, Karnal-132001, Haryana, India.
- Milanovic, S., M. Panic and M. Caric (2004). Quality of Quarg Produced by Probiotics Application. *Acta Periodica Technologica*, **35**: 37-48.
- O'zer, D., Akin, S., and O' zer, B. (2005). Effect of inulin and lactulose on survival of Lactobacillus acidophilus La-5 and Bifidobacterium bifidum BB-02 in acidophilus-bifidus yoghurt. *Food Science and Technology International*, **11**(1): 19–24.
- Ross RP, Fitzgerald GF, Collins JK and Stanton C. (2002). Cheese delivering biocultures-probiotic cheese. *Aust J Dairy Technol*, **57**: 71–8.
- Saguy I. S., Moskowitz H. R. (1999). Integrating the consumer into new product development. *Food Technol*, **53**(8): 68–73.
- Shah, N. P. and Lankaputhra, W. E. V. (1997). Improving viability of Lactobacillus acidophilus and Bifidobacterium ssp. in yoghurt. *International Dairy Journal*, **7**(5): 349–356.
- Shah, N. P. and Wu, X. (1999). Aflatoxin B1 binding abilities of probiotic bacteria. *Bioscience and Microflora*, **18**: 43–48.

- Shah, N. P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. *Journal of Dairy Science*, **83**: 894–907.
- Shah, N.P. and R. Ravula (2000). Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. *Aust J Dairy Technol*, **55**: 139-144.
- Sheu, T.Y. and Marshall, R.T. (1993). Microentrapment of Lactobacilli in calcium alginate gels. *Journal of Food Science*, **54**: 557–561.
- Sheu, T.Y. and Marshall, R.T. and Heymann, H. (1993). Improving survival of culture bacteria in frozen desserts by microentrapment. *Journal of Dairy Science*, **76**: 1902-1907.
- Shin H. S., Lee J. H., Pestka J. J. and Ustunol, Z. (2000). Growth and viability of commercial Bifidobacterium spp. in skim milk containing oligosaccharides and inulin. *J. Food Sci.*, **65**: 884–887.
- Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods, 8<sup>th</sup> Ed., Iowa state university press. Blackwell Publishing Limited, Ames.
- Vinderola, C. G., Bailo, N. and Reinheimer, J. A. (2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. *Food Research International*, **33**(2): 97–102.
- Vinderola, C. G., Prosello, W., Ghiberto, D. and Reinheimer, J. A. (2000). Viability of probiotic (Bifidobacterium, Lactobacillus acidophilus and Lactobacillus casei) and nonprobiotic microflora in Argentinian fresco cheese. *Journal of Dairy Science*, **83**(9): 1905-1911.