Physicochemical and microbiological properties of probiotic microencapsulated enriched infant formula

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Abstract Breast milk is the natural optimal source of nutrition for the infants. In case of its non availability or mother's inability to feed her child, nutrient fortified infant formula can be considered as its substitute. Infant formula can be described as "A food which claims to be or is represented for special dietary use solely as a food for infants by reason of its simulation and as a complete or partial substitute for human milk". Recently researches are carried out to improve the chemical and microbiological value of infant formula in terms of formulation and techniques. This study was carried out to check prebiotic (5% Partially Hydrolyzed Guar Gum (PHGG)) effect in the matrix of microcapsules on physico-chemical and microbiological qualities of probiotic microorganism containing infant formula. Gross composition of control (excluding prebiotic in matrix material) and experimental infant formula (including 5% PHGG in matrix material) prepared were meeting with the Indian standard. No significant difference was found in physicochemical properties i.e. dispersibility, bulk density, free fat and solubility index of control and experimental infant formula. Initial probiotic count in both control and prebiotic containing microcapsules were $10^8$-$10^9$ cfu/gm. Prepared infant formula was containing 5-6 log cfu/gm of viable count in control powder and experimental infant formula was containing 6-7 log cfu/gm of viable count. Prebiotic containing probiotic microcapsules had positive effect on the viability of probiotic bacteria and had no adverse effect on physico-chemical property of infant formula.

Keywords Prebiotic, probiotic, *lactobacilli*, microencapsulation, infant formula

Introduction

Milk, a biological fluid, contains the nutrients for the growth and development of the new born. Human infant should ideally be nursed on mother's milk, which constitutes nature's best food. However, in case of lactation failure, insufficient milk secretion, and where mothers are suffering from transmittable diseases, human milk substitutes serve as savior of precious life during vulnerable stages of infancy (Thompkinson and Mathur, 1997). Human milk feeding stimulates the growth of both *Lactobacilli* and *Bifidobacteria*. These strains inhibit the growth of pathogens such as *E. coli* and *C. difficile* (Krebs et al, 1994), protect children from many contagious diseases as well as improve immunity.

Qualitative and quantitative differences appear to exist between the microflora of human milk fed and formula fed infants. Hence, recent innovations to infant formulas have involved the inclusion of probiotics and prebiotics as a measure of making the intestinal flora of formula fed infants similar to that of the breast fed infants. Neither probiotics nor prebiotics have a negative influence on infant growth, nitrogen balance, mineral bioavailability or hydration (Ghisolfi et al., 2002). The probiotic microorganism in infant formula must be able to transit the stomach and small intestine and exert its health benefits in the lower segment of the small intestine. Prebiotic count in the food at the time of consumption should be at least $10^7$ viable cells per gram or milliliter of a product (Ishibashi and Shimamura, 1993). The prebiotic in the symbiotic mixture improves survivability of the probiotic bacteria and enhances the activity of the host's endogenous bacteria (Mugambi et al, 2012). Prebiotics are non digestible food ingredients that may beneficially affect the host by promoting the growth of and/or the activity of a limited number of bacteria in the colon (Roberfroid, 1998). Along with prebiotics,
Microencapsulation of probiotic bacteria is helpful in enhancing the viability during processing, and also for the targeted delivery in gastrointestinal tract (Amir et al., 2007). Microencapsulation of probiotic bacteria in hydrocolloid beads is one of the 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). There are different methods to encapsulate the probiotic microorganism using hydrocolloid and prebiotic. Because of decrease in viability of probiotic organism during processing and gastro intestinal transit, there is need to have certain developments where in the probiotic bacteria reach the gut without deleterious effect during transit. Kent and Doherty (2014) reviewed in detail how microencapsulation of probiotic organisms using milk and pea proteins in Infant formula helped to amplify the survival rate of probiotic bacteria. This process also enhances the survivability of probiotic organism during process and storage (Doherty et al., 2011; Klemmer et al., 2011). The present investigation was carried out to check the effect of combination of prebiotic (PHGG) with a particular hydrocolloid (sodium alginate) using spray nozzle method for microencapsulation on the viability of probiotic Lactobacillus acidophilus (NCDC-15) and the physico-chemical properties of the prepared encapsulated probiotic containing infant formula.

Materials and Methods

Probiotic culture: The probiotic microbial strain of Lactobacillus acidophilus (NCDC-15) was obtained from Dairy Microbiology Division of National Dairy Research Institute (NDRI), Karnal. Milk products and other ingredients: Condensed skimmed milk (32% TS) and cream (50% fat) were obtained from the Experimental Dairy, National Dairy Research Institute, Karnal. Groundnut oil (Ginni brand) and sugar were procured from local market; Maltodextrin (DE-16) was procured from M/s Good Rich Carbohydrates, Karnal.

Chemicals: All the chemicals required for this investigation were of Analytical Grade reagents and procured from standard suppliers.

Microbiological Media: Microbiological media and materials were obtained from M/s Hi-Media, Mumbai.

Product preparation: Infant formula was prepared using the method developed by Thompkinson (1984). Control infant formula containing L. acidophilus NCDC15 was prepared by excluding PHGG in the encapsulation matrix material. The experimental infant formula with prebiotic was prepared by using 5% PHGG in the matrix material.

Control and prebiotic containing microcapsules were having 10⁸-10⁹ cfu/ml and the microencapsulated biomass was added in the condensed milk before drying. Compositional, physico-chemical and microbiological analysis of control and experimental infant formula was prepared at Experimental Dairy, National Dairy Research Institute, Karnal using spray drying method. The drying temperature of condensed milk was kept 165-170°C and 75-85°C for inlet and outlet air respectively.

Gross compositional analysis of developed infant formula: Moisture was analysed using method described in Indian Standards (SP: 18 Part XI)1981, Fat estimation was done using Rose-Gottlieb illustrated as per Indian Standards (SP: 18 part XI) 1981, Protein content was determined using method used by Menefee and Overman (1940), Total ash content was estimated as per the method described by AOAC (1995).

Physico-chemical analysis: Free fat was determined by method suggested by Hall and Hedrick (1971), Dispersibility was measured by the method described by American Dry Milk Institute (ADMI, 1965), Solubility index was determined by the method described in ADMI (1965), Bulk density (Loose and Packed) were estimated as described by Sjollema (1963).

Microbiological analysis: Depolymerisation method to enumerate the microencapsulated probiotic organism, suggested by Sheu and Marshall (1993) was followed. Enumeration of probiotic organism was carried out by pour plate technique described by Houghtby et al. (1993).

Preparation and maintenance of media used during the entire investigation was done following the method suggested by Marshall (1993) and prepared infant formula both experimental and control samples were evaluated for the microbiological analysis as per the method suggested by Houghtby et al., (1993).

Results and Discussion

The prepared infant formula with control and experimental probiotic organism were stored at room temperature and analyzed for composition, physico-chemical and microbiological tests.

Gross composition of control and experimental infant formulas are given in Table 1. From the table it is observed that there is no significant difference in gross composition of the control and experimental infant formulas. The prepared infant formulas were meeting with FSSA standard, 2011.

In Table 2 physico-chemical properties of control and experimental infant formula is given. Student "t"-test was applied to compare the control and experimental infant formula. It was observed that there was no significant (p<0.05) difference in free fat content of both infant formulas but significant difference (p<0.05) was found in dispersibility, bulk
density and solubility index. Dispersibility was increased, bulk density was decreased and solubility index was increased in case of experimental sample compared to the control sample.

Microbiological count (log cfu/gm) given in Table 3 shows that control infant formula had less SPC, higher Coliform count (log cfu/gm) and lower probiotic count compare to experimental infant formula.

Therefore, from the microbiological data it was pertained that addition of PHGG in the matrix had given good resistance to the probiotic organism and because of microencapsulation the probiotic organism were more viable than the un-encapsulated probiotic organism. These results are similar with the results reported by Salaria et al, (2013). A detailed review by Kent and Doherty (2014) also reported that addition of prebiotic contents improves the microbiological quality of Infant powders.

### Conclusions

Addition of prebiotic in microcapsules enhances the viability of probiotic organism in infant formula and has no adverse effect on physico-chemical properties. Further studies are to be carried out to check for its storage stability and its effectiveness basing on animal studies. Based on these test results feasibility for the production at pilot scale at industrial level has to be carried out.

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