5. SUMMARY AND CONCLUSION

Present investigation was planned and executed to standardize a method to prepare a whey-based tropical fruit beverage containing proven probiotic organisms and evaluation of its suitability for feeding to the children so as to modulate their gastrointestinal microbiota. As such the whey is the major dairy by-product, which causes enormous environmental problems leading to imposition of stringent laws by the Authorities on dairy industry regarding whey disposal. As a result, dairy industry is looking for ways and means for whey disposal. Having realized the nutritional potential, the utilization of whey in more economical way is the need of the hour. Use of whey in its liquid state is one of the best ways for its utilization. India is emerged as a major fruit producer in the world. Value-added food commodities including dairy products are the new milestones being achieved in the food industry as a result of decades of research in microbial fruition in the form of functional foods. Probiotic foods can play an important role in promoting health and reducing public health care costs in a developing country like
India. This chapter deals with the salient features of the investigation conducted and embodied in this thesis.

The paneer whey was prepared in the laboratory, fig pulp was also prepared in laboratory while Alfonso pulp obtained from the market. These three basic ingredients were used as the major raw materials in this investigation. Fresh fig is a rich source of carbohydrates, calcium, iron, and vitamin A and C. while Alfonso is rich in carbohydrates, essential minerals, vitamins, other nutritive factors and excellent flavour, attractive fragrance, beautiful shades of colour, delicious taste and high nutritive value made it one of the ingredients in value-addition of the product under investigation. A proven strain of probiotic Viz. *L. rhamnosus*-LBKV3, which is a human origin organism, was used after confirmation of its purity. Morphological, physiological and biochemical characteristics of the probiotic organism were studied.

Growth pattern of the probiotic strain was studied to achieve maximum viable counts. It was observed that a viable count of 8.27 CFU/ml was obtained at 24h
incubation at 40°C however about 0.7% titratable acidity was obtained at this stage of growth.

While standardizing the methodology utmost importance is to be focused on the viable counts of the finished product. It is earlier suggested by numerous workers that such a product should contain at least $10^5$ CFU of the proven probiotic culture per single dose to achieve the possible health benefits. Keeping in view such frequent indications, the method was standardized to obtain a probiotic product acceptable to the target group of volunteers and containing maximum number of the viable cells of the probiotic organism. It was observed that the whey-based probiotic beverage containing 15% fig pulp and that with 15% Alfonso pulp was attracted highest score from the sensory evaluators. The panel of judges assigned the score in the range of “extremely liked” category in the 9-point Hedonic scale.

The success of any functional food containing probiotics is based on the positive implantationability of the strain in the GIT of the test subjects/consumers. The microflora of GI tract is a key for the nutrition and health of the host. Microflora modulation can occur
through diet that contains probiotics. The approach of using a diet to induce microbial change offers a very straightforward loom towards the improved health i.e. consumer-friendly and effective. To study the impact of feeding whey-based tropical fruit beverage containing probiotics on overall health of the kids involved in this investigation, their general health in terms of body weight, GI ailments, etc. was regularly checked/monitored through pediatricians. It was observed that none of the test group volunteers suffered from any of the GI or related ailments during the feeding trials. This is an indication of safety and probiotic benefits of the culture consumed.

Twenty one randomly selected children of 2-5 years were grouped in three comprising of 7 children in each of the groups. Among the two test groups (the group F3 receiving whey-based fig beverage containing 15% fig pulp and M3 receiving whey-based mango beverage containing 15% Alfonso pulp) of the children was receiving 200 ml/volunteer/day of the freshly prepared whey-based tropical fruit beverage containing $10^5$ cfu/100 ml of the *L. rhamnosus*-LBKV3 and the control
group of the volunteers was receiving 200 ml freshly prepared whey-based tropical fruit beverage containing same quantum of fig and Alfonso pulp (15%) containing no any probiotic organisms in it. This feeding trial was uninterruptedly continued for a period of 21 days. The faecal microbiota of lactobacilli, total bacterial counts and coliform organisms was determined before commencing the feeding trial and 7 days intervals during the twenty one days feeding trial and at same interval after stopping the feeding trial.

It was observed that the mean initial LAB counts in the faecal matter of the control group children were 1.18 CFU/g, the coliform counts were 5.76 CFU/g and the total bacterial counts in the faecal matter was 7.98 CFU/g. Feeding of the whey-based tropical fruit beverage without any probiotic organisms could not impact on the LAB counts.

It was observed that feeding of whey-based probiotic beverage containing 15% fig pulp for seven days resulted into three-fold increases in LAB counts and at the same time the coliform counts were declined to almost 50% of the initial value. The standard plate
counts showed increase by one log cycle. The faecal LAB counts after 21 days of feeding were 6.83 CFU/g, which is six-fold more than the counts prior to commencement of the feeding trials. The coliform counts at this stage were just 0.68 CFU/g i.e. nine-times less than the initial values. It was further observed that termination of feeding trial resulted into declines in the LAB counts and gradual increases in the coliform counts.

Feeding of whey-based tropical fruit beverage containing probiotics and 15% Alfonso pulp for seven days resulted into a dramatic shift in the microbial counts of friendly types of organisms namely the LAB as against very sharp declines in coliforms. Terminating the feeding trial showed three-fold higher LAB counts as recorded before commencement of the feeding trials. The coliform counts were 45% of the initial value. These findings indicate positive implantationability of the probiotic culture in the GIT of children.

To study the in vitro antimicrobial activity, the CFC of the whey-based fig beverage and mango beverage containing L. rhamnosus-LBKV3 was obtained. It was seen that the pathogenic strains of Ps. aeruginosa and
Y. enterocolitica, E. coli and Staph. aureus were inhibited by the CFC. The antibacterial trend observed initially was continued with the zone of inhibition of 15.9 mm, 18.2 mm, 19.8 mm, 20.4 mm and 22.0 respectively at 24h, 36h, 48h, 60h and 72h in case of E. coli. It was followed by Ps. aeruginosa with the highest zone of inhibition at 72h of 21.8 mm. The CFC of probiotic beverage containing 15% Alfonso showed highest inhibitory effect against E. coli. Probiotic beverage containing 15% Alfonso pulp exhibited highest antibacterial activity against E. coli followed by Ps. aeruginosa and Y. enterocolitica while Staph. aureus showed the least inhibition.

It is therefore concluded that an acceptable quality beverage can be obtained by utilizing paneer whey and fortifying it with 15% fig pulp/Alfonso pulp and 10% cane sugar and a probiotic strain of L. rhamnosus-LBKV3 can be incorporated. Feeding of such product has demonstrated its positive implantationability in children of 2-5 years.

Further studies aimed at scaling up the technology for production of this value-added functional product to explore its industrial production are needed.