CONCLUSION AND FUTURE SCOPE OF WORK

7.1. Salient findings

The thesis includes studies on the viability and stability of the four different strains of probiotic *Lactobacilli* and the quality of the probiotic fruit juices developed from litchi, pineapple, guava and orange. The present study determined the potential probiotic strains and suitable carriers for their delivery. The present study also investigated the changes in phytochemicals, antioxidant properties and mineral content of two selected probiotic fruit juices during cold storage. Optimization was carried out by response surface methodology (RSM) to optimize the spray dying conditions for microencapsulation of probiotic bacteria in selected fruit juices with maltodextrin to extend survivability and enhance recovery. The viability, physicochemical, and morphological properties of the spray dried powders and reconstituted fruit juices were also examined. The effect of different coating materials on the survival of probiotic bacteria in litchi juice and characterisation of encapsulates for their morphological, physicochemical and toxicological properties were also done. The stability of the optimized product in simulated gastric environment condition was also evaluated.

The salient findings of the thesis are summarized below:

1. **Viability and stability of probiotics in fruit juices during storage**
   - All the three strains of *Lactobacillus* ie, *L. plantarum*, *L. rhamnosus* and *L. acidophilus* have the capacity to survive in freshly prepared juices from four different fruits (pineapple, orange and guava juice) that are locally available in Assam.
   - *L. plantarum* had maintained the required $\log 10^8$ count in litchi and pineapple juices up to 6 weeks under refrigerated condition (4 ± 1°C)**.**
   - The change in total soluble sugar, pH and titratable acidity was minimum in fruit juices fortified with *L. plantarum* (Lp) than other fortified juices.
   - Juice of litchi and pineapple appeared to be better carriers compared to orange and guava for delivery of probiotic due to acceptable changes in their pH, TSS and titratable acidity on probiotication.
   - Overall acceptability of the probiotic juices was found to be in the range between 7.4 and 7.6. on a 9-point Hedonic scale.

- *L. plantarum* (Lp) was found to be the superior species and litchi juice was found to be the suitable carrier for probiotic bacteria for developing health promoting functional fruit drink.

2. **Changes in phytochemicals, antioxidant properties and mineral content of two probiotic fruit juices during cold storage**
   - Among the probiotic fruit studied, highest TPC and FRAP activity was observed in litchi juice.
   - Probiotic litchi juice showed good amount phenolic acids like rutin hydrate and kaempferol which were present in normal litchi and pineapple juice reduced to detectable quantities on long storage.
   - Gallic acid and catechin were found in higher quantities in fermented juices whereas the quercetin quantity reduced during fermentation.
   - Probiotication increased the lactic acid in both juices and the content was found higher in probiotic pineapple juice
   - Other organic acid like malic acid in litchi juice decreases and citric acid increases with time on probiotication.
   - Addition of probiotics and storage at refrigerated condition did not significantly affect mineral content of the fruit juice.

3. **Optimization of spray drying conditions for enhanced survivability and recovery of *Lactobacillus plantarum* MTCC2621 in fruit juices**
   - Inlet temperature had a positive effect on % recovery of the product during spray drying of probiotic litchi juice.
   - Feed rate and juice solid to maltodextrin ratio also influenced the cell viability and product recovery.
   - Maltodextrin ratio had a major role on recovery and survivability of the bacterial cells in orange and litchi juices but was not found significant in pineapple juice powder.
   - Flow rate had positive effect on recovery in litchi juice and had negative effect on survivability of the cells in spray dried powder of pineapple juice.

- Among the juice powders, litchi juice powder showed the maximum recovery and cell survival.
- The optimization data revealed that 130^{o}C inlet temperature, 1:1 juice solid content to maltodextrin ratio and 60 mL/min would give highest recovery and desirable survivability of the spray dried litchi juice powder.

4. Effect of coating materials on the survival of *Lactobacillus plantarum* MTCC2621 in litchi juice and characterisation of morphological, physicochemical and toxicological properties

- Spray drying of probiotic *L. plantarum* in litchi juice with different prebiotics had both positive and adverse effects on the powder quality.
- The physicochemical properties such as yield, colour, solubility, water activity and hygroscopicity of the final product varied depending on carrier material used.
- Maltodextrin 10% (w/v) plus 5% (w/v) FOS gave highest yield and viability of the encapsulated bacteria then the other combinations.
- Flowability and solubility were found superior in case of maltodextrin plus FOS coated spray dried powder.
- Cytotoxicological study showed that spray drying of probiotic *L. plantarum* in litchi juice has no cytotoxic effect on human kidney cells.
- Spray drying is a convenient method to enhance the viability of the probiotics in fruit juice powder. It has also the convenience of reconstitution just prior to consumption

5. Effect of addition of maltodextrin and fructooligosaccharide in litchi juice on the survival of microencapsulated probiotic *L. plantarum* MTCC2621 in simulated digestive system

- The viability of the bacterial cells dropped sharply in the first 30 min of exposure to simulated gastric environment due to the acidic effect of gastric juice and then decreased slowly until the end of the study period (120 min).
- When the same cells were exposed to *in-vitro* simulated intestinal environment the number of cells increased due to conducive environment and high pH of intestinal fluid.

- Microencapsulation of probiotics with maltodextrin and fructooligosaccharide in litchi juice was found to give better protection to probiotic bacterial cells from the harsh conditions of gastric environment.
- It also enhanced the growth of these microorganisms in simulated gastric environment.
- The synbiotic effect of coating material, probiotics and the delivery medium enhances the survivability of probiotics in gut environment.

7.2. Future scope of work

The results obtained from the current thesis can form the basis for further studies on stability and quality parameters of probiotic fruit with free and encapsulated probiotic bacterial bacteria.

The following scope for future study can be considered from the findings of the present work.

- Screening and selection of robust strains (with respect to physiological stress) over sensitive ones and tools to assess the fitness of the resistant strains must be developed for more versatile industrial application of these probiotics.
- Health benefits must be validated in the presence of food matrix dosage rather than simply with isolated pure culture.
- The compatibility and stability between several bioactives when held together in the same microcapsule should be investigated, thereby widening the range of functional components that can be encapsulated.
- Microencapsulating agents' interaction between the protein-carbohydrate-probiotics formulation should be studied to ensure minimal toxicity and better bioavailability.
- Scale up of novel hybrid drying technologies with artificial intelligence, enhanced recovery (product and microbes) and energy efficiency needs to be developed
- Effect of different types of packaging materials on the viability of probiotic fruit juice can be studied
- The physiological response with respect to the heat stress at the molecular level has not been firmly established in probiotics. The molecular physiology of

proteins involved in this stress tolerance could be a potential future perspective of this study.

- More extensive *in vitro* and *in vivo* studies are vital in order to authenticate the probiotic potential and safety of such cultures.
- Health claims must be defendable and must be scientifically substantiated. Randomized and controlled placebo *in vivo* trials can explore more functional benefits and hence wider applications.