OVERALL CONCLUSIONS AND FUTURE STUDIES
8.1 CONCLUSIONS

- During development of functional beverages, consideration of the eventual retention, bio-accessibility and bio availability of nutrients has been a major priority. Our initial screening confirmed that seabuckthorn juice matrix had a high content of total phenolics and ascorbic acid. It also contained a strong antioxidant activity in comparison to other selected fruit juices (cherry, wild apricot, apple, apricot and pear).

- Inspite of its high nutritional value, the seabuckthorn juice was not accepted by panelist during sensory evaluation. Due to the acidic taste, these berries required processing either through fermentation or fortification for development of new brands of products with consumer appeal.

- This study resulted in the standardization of process for development of two functional beverages: an antioxidant rich wine (Functional beverage I) and probiotic fortified beverage (Functional beverage II).

- In order to develop Functional beverage I, the wine making process by co-fermentation was standardized. For co-fermentation process an appropriate yeast strain is mandatory which should cope with stress conditions like high ethanol-, osmo- and thermo-tolerance. We were able to select one strain Saccharomyces cerevisiae with high ethanol-, osmo- and thermo-tolerance which could be compared to the industrial strains (‘I-1’ and ‘I-2’). Issatchenkia orientalis, an acidophilus yeast was used to degrade malic acid along with Saccharomyces cerevisiae. The co-fermentation process was standardized with an emphasis on the retention of polyphenolic content of seabuckthorn wine because wine phenolics have gained recent attention due to proven health benefits. The co-fermentation process also resulted in another significant achievement of reduction of malic acid by 55 %.
The standardized co-fermentation process resulted in a seabuckthorn wine with comparable physico-chemical characteristics in comparison to commercial red wine, Cabernet Shiraz. As quality of wine is of utmost importance one of the fundamental motivations behind the consumer choice is a product with acceptable organoleptic qualities. The product showed high total phenolics and flavonoid content in comparison to Cabernet Shiraz. The standardized process resulted in an antioxidant rich seabuckthorn wine with acceptable organoleptic qualities.

One of the important steps in design and development of a functional food is the identification of a specific function (i.e., cellular, biochemical, or physiologic) that is potentially beneficial to maintenance of well-being and health, and may reduce the risk of a disease. The protective effect of seabuckthorn wine against phorone-induced oxidative stress and high-cholesterol diet-induced hypercholesterolemia was evaluated in mice studies. The administration of seabuckthorn wine reduced hepatic lipid peroxidation and increased the superoxide dismutase (SOD) activity indicating improved resistance to oxidative stress. The seabuckthorn wine performed better with respect to reduced glutathione (GSH), catalase, glutathione peroxidase (Gpx) and glutathione-S-transferase (GST) as compared to commercial red wine, Cabernet Shiraz. The observed protective effects may be due to the synergistic effects of bioactive compounds present in seabuckthorn wine which also contributes to the uniqueness of the product.

The results of this study proved that the adverse effect of phorone could be significantly curtailed by administering seabuckthorn wine to the male LACA mice. It is interesting to observe similar in vivo protective effects both in case of seabuckthorn wine and commercial red wine, Cabernet Shiraz inspite of the fact that resveratrol (which is one of the most important health improving factor of red wine) is absent in seabuckthorn wine.
A significant degree of cardioprotection has been attributed to ingestion of wines rich in flavonoids. This study provided similar important evidence that seabuckthorn wine exerts protective actions by reducing the total cholesterol and LDL-cholesterol in hyperlipidemic conditions. To the best of our knowledge the impact of seabuckthorn wine on lipids and lipoproteins in diet-induced hypercholesterolemic has not been reported so far. In addition, high-cholesterol-fed mice administered with seabuckthorn wine exhibited a 197% increase in the HDL-C/LDL-C ratio compared to high cholesterol diet treated mice. From this study it can be concluded that inclusion of seabuckthorn wine in a diet may be an effective way of reducing the total cholesterol and LDL-cholesterol under hyperlipidemic conditions.

This study also resulted in a standardization of a process for development of probiotic fortified beverage from seabuckthorn juice (Functional beverage II). To the best of our knowledge, there is no probiotic beverage based on seabuckthorn juice so far in the Indian market.

*L. plantarum* (ATCC 8014) and *L. rhamnosus* (ATCC 7469) were evaluated for their probiotic efficiency. Both strains were identified as robust probiotic cultures. The strains exhibited high gastric juice tolerance, higher degree of resistance in the presence of streptomycin and ampicillin, moderate bile salt hydrolase activity and cholesterol-lowering ability. From the results of the probiotic evaluation, it was inferred that *L. plantarum* (ATCC 8014) and *L. rhamnosus* (ATCC 7469) have potential to be used as functional supplements in seabuckthorn juice.

*L. plantarum* and *L. rhamnosus* showed poor survivability in fortified seabuckthorn juice of pH 2.5. This poor survival could be due to the lack of growth factors which were generally present in the MRS medium but absent in the seabuckthorn juice. Hence, juice was fortified with whey protein concentrate
(WPC), soy protein isolate (SPI) and skim milk (SM) at specific pHs to support the viability of probiotic strains.

- The fortification of juice with whey protein concentrate (WPC) and adjustment of pH with tri sodium citrate positively affected the viability of *L. plantarum* and *L. rhamnosus* during cold storage with a stable cell count of $9 \log_{10}$ CFU/mL. The adequate fortification and pH maintenance of juice rendered it a suitable medium for the delivery of probiotics. The evaluation of chemical changes during 4 weeks of cold storage suggested that the best storage condition were 4 °C or below and storage in sealed glass container resulted in minimum changes. In addition, this product combines ascorbic acid other phyto-stimulants and antioxidant compounds. The probiotic fortified juice containing whey protein concentrate (WPC) was well accepted by panel members in comparison to other product (pH adjusted to 4.0-without any fortifying agent). The shelf life of the product was 4 weeks without addition of synthetic components or preservatives. **This is the first study to provide a standardization procedure for production of probiotic fortified seabuckthorn beverage.**
8.2 FUTURE DIRECTIONS FOR THIS STUDY

The importance of consuming functional foods/beverages for the improvement of the quality of life is on the rise since the last decade. The diversification of the market from this point of view could be strongly expected.

- These studies will focus on large scale production of two new functional beverages, as there is a need to evaluate the economic value of the process technology.

- With respect to seabuckthorn wine (Functional beverage I), further studies are required to evaluate additional health benefits of wine, to gain an understanding of the underlying mechanisms and to determine the chemical identity of the bioactive constituents.

- With respect to the probiotic fortified beverage (Functional beverage II), the in vivo studies are needed to evaluate the efficacy of probiotic-fortified beverage to ameliorate pathogenic conditions.