CHAPTER 7

CONCLUSION

The review of literature and the results obtained in the present thesis suggest that food microorganisms isolated from food matrices, in particular of bacterial origin, can act on the nutrients contained in the food. These microorganisms could thus generate functional foods enriched in specific components which can influence the important physiological processes in human especially in the intestinal system, immune response and anti-genotoxicity. In this view, the present work explored the possibility to produce fermented milk with commercial fermented milks, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and obtain Casein Phosphopeptide (CPP) with gastroprotective, immunomodulatory and anti-genotoxic activity.

As a consequence, there is an increasing need to select the microorganism present in food matrices for their ability to produce functional food enriched in specific bioactivities on large scale. More research is thus needed to characterize the microorganisms and the associated bioactivities and to develop new methods for quantification of the bioactivity in the foodstuff and the identification of the food components responsible for such bioactivity. For example, it would be interesting to identify the presence of the peptides in milk fermented by *L. bulgaricus* to acquire better knowledge about the mechanisms determining the associated immunomodulatory activity. With this purpose, the Experiment chapters 4.3 and 4.4 have been performed. We aimed to study the immunomodulatory activity of the milk
fermented by two bacterial strains frequently found in dairy products of India and to explore the possible mechanism of action of a milk-derived peptide and compare with already documented immunomodulatory activity on lymphocytes, and considered as a model peptide.

The results we got demonstrate that the in vitro methods manifest some limitations in the characterization of immunomodulatory bioactivity and that an exhaustive view of the action of immunomodulatory peptides could be achieved only by a multi-view approach that should take into account the complexity of the interactions between the bioactive peptides and the different components of the immune system in vivo. In fact, the Experiment 4.5 and the section 7.1 evidenced the lack of knowledge about the interaction of the immunomodulatory peptides derived from food and the immune system dispersed along the GI tract (as GALT, Peyer’s Patches, antigen-presenting cells) that could represent a potential target of immunomodulatory peptides, even before to be absorbed at gut level and circulate in the body. At the moment the interactions between food-derived peptides and the gut associated immune system have been explored to elucidate the mechanisms underlying allergies but it would be interesting to apply the same approach to evaluate the bioactivities, considering both allergies and bioactivities as properties that could be displayed by peptides. The present thesis focused also on the physiology of absorption of bioactive peptides and demonstrated for the first time that a long hydrophobic bioactive peptide crossed intact a Caco-2 cell monolayer, a well recognized in vitro model for the intestinal epithelium. In fact, the fermented milk-
derived immunomodulatory peptide CPP was demonstrated to be resistant to the
digestion of gastrointestinal peptidases and to pass intact across Caco-2 cells. This
interesting result permits to suggest that even large peptides could be absorbed in
small quantities and that it cannot be excluded that at these concentrations the peptide
CPP could interact with the gut-associated immune system, as explained before.

The application of CPP isolated from milk fermented by *Lactobacillus acidophilus*
and *Lactobacillus bulgaricus* as an active probiotic can be studied with
human clinical trials which will yield immense health benefits and may be
commercialized into a product readily available in the Indian market as an immune
system booster. Fermented milk peptides seem to be having an effective anti-
genotoxic effect on the low ionizing background radiation, thus acting as a anti-
genotoxic agent. CPP functioned reasonably well with both albino mice and fish.
Development of low cost, high efficient anti-genotoxic agent especially for low
income radiation workers can be initiated using the fermented milk peptides as the
base. The approximate cost of fermented milk required to provide CPP for each
person would be only Rs.2 (market price of 100ml fermented milk).