SUMMARY

This thesis has aimed to document the indigenous knowledge of traditional processing of *Selroti* batter preparation in the Sikkim Himalaya; and to isolate, characterise and identify the dominant microorganisms in fermented batters collected from different sources. The objective of the thesis was also to analyse microbial load, testing for occurrence of pathogenic contaminants in the product as food safety, to study the effect of seasonal variation, technological properties of functional lactic acid bacteria (LAB) and yeasts such as acidifying capacity, enzymatic profiles, and their antimicrobial activities against pathogenic bacteria. Microbial population dynamics during *in situ* fermentation of Selroti batter were also examined including some physico-chemical changes. Strains of LAB and yeasts were selected and tested for production of fermented batters, and subjected to sensory evaluation. Proximate composition of raw materials and *Selroti* batters were also calculated.

Selroti is an important fermented rice food of the Sikkim Himalaya. Information on indigenous knowledge of *Selroti* was sought during survey, which was documented in this thesis. Per capita consumption of *Selroti* in Sikkim is 8

g/day. *Selroti* is mostly prepared at home (75.6 %) comparable to market purchase.

Scientific knowledge on *Selroti* of the Sikkim Himalaya is unknown outside this region. A total of 125 samples of *Selroti* batters were collected from different villages and markets of the Sikkim Himalaya. A total of 167 isolates of LAB and 141 yeasts were isolated. The microbial population of *Selroti* batters revealed that LAB present in viable numbers above 10^8 cfu/g, followed by yeasts around 10^5 cfu/g, respectively. On the basis of a combination of phenotypic properties and the API sugar profile data, strains of LAB isolated from *Selroti* batters were identified as *Leuconostoc mesenteroides, Enterococcus faecium, Pediococcus pentosaceus* and *Lactobacillus curvatus.* Based on the detailed characterizations and identification profiles of yeasts, *Saccharomyces cerevisiae, Saccharomyces kluyveri, Debaryomyces hansenii, Pichia burtonii* and *Zygosaccharomyces rouxii* were identified. The most prevalent LAB and yeasts in *Selroti* batters were *Leuc. mesenteroids* (42.9 %) and *S. cerevisiae* (35.6 %), respectively. Food borne pathogens *Bacillus cereus, Listeria* sp., *Salmonella* sp. and *Shigella* sp. were not detected in any sample of fermented

batters, due to slight acidic nature of the products. It was observed that seasons affect the development and prevalence of microorganisms in the fermented batters. During summer, the microbial load of LAB increased, whereas, winter was favourable for yeasts.

LAB and yeast strains isolated from *Selroti* batters were screened for their acidifying and coagulating capacity, and found that most of the LAB strains acidified with lowering of pH up to 4.3. About 63.6 % of LAB strains caused coagulation of skim milk at 30° C. Among yeasts, only *S. cerevisiae* and *D. hansenii* showed acidification characters, though decrease in pH was limited to 5.6. The use of the API-zym technique has relevance for selection of strains as potential starter cultures. Absence of proteinases and presence of strong peptidase activities produced by the predominant LAB strains are possible traits of desirable quality for their use in production of typical flavour and aroma. Yeasts strains from *Selroti* batters showed no proteinase activity. High activity of phosphatase by yeast strains showed their possible role in phytic acid degradation in cereal-based fermented foods. It was also shown that strain of *Leuc. mesenteroides* isolated from *Selroti* batters had

high α-galactosidase and β-galactosidase activities, indicating their ability to hydrolyse oligosaccharides. Though, all strains of LAB showed antimicrobial activities against pathogenic bacteria, none of them produced bacteriocin under the applied condition.

The proximate composition of unfermented rice and samples of *Selroti* batters were analysed. Moisture content in *Selroti* batters was higher due to soaking prior to fermentation. There was a remarkable increase in water-soluble and TCA-soluble nitrogen in *Selroti* batters due to solubilisation of proteins, indicating its protein digestibility. The food value of fermented batters was found to be increased. Samples of fermented batters had comparatively higher mineral contents.

During *in situ* fermentation of *Selroti* batter, indigenous lactic acid bacteria and yeasts changed spontaneously. As expected in a typical lactic fermentation, the pH of the fermenting substrates decreased and the titratable acidity increased as the batter fermentation progressed due to growth of LAB. Bacterial contaminants were associated with initial fermentation and finally disappeared during *Selroti* batter fermentation. By averting the invasion of these

potential contaminants, lactic acid fermentation imparts attributes of robust stability and safety in the product like *Selroti.* Another safety aspect of *Selroti* is deep frying prior to consumption. There was no remarkable increase in physical properties of fermenting cereal such as batter temperature, volume and weight during *in situ* fermentation of *Selroti* batter.

Batters prepared during *in situ* fermentation of *Selroti* from each hour ranging from 0 to 10 hour were deep-fried in edible oil to make *Selroti*, and served to consumers for sensory evaluation. *Selroti* batter prepared by *in situ* fermentation for 8 hour had significantly ($P<0.05$) high sensory properties.

Starter cultures of LAB and yeasts, previously isolated from native *Selroti* batters were tested singly or in combination for their ability to ferment rice flour to produce *Selroti.* Sensory evaluations were carried out in order to choose the best culture combinations. It was found that *Selroti* batters produced using a mixture of pure culture strains of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2, selected on the superior technological property, at 28° C for 4 hour had organoleptically scored the highest

acceptability among the consumers. This was also correlated by decrease and increase in pH and titratable acidity of the fermenting batters, respectively from 0 hour to 4 hour. None of the other strains combinations, used as starters could produce organoleptically acceptable *Selroti.* The consumers' preference trial showed that *Selroti* batter prepared by cell suspension mixture of *Leuc. mesenteroides* BS1:B1 and *S. cerevisiae* BA1:Y2 was more acceptable than *Selroti* batters prepared by conventional method. This fried *Selroti* had desirable sweet taste, a typical *Selroti* flavour and soft texture with golden-brown colour. *Selroti* prepared by using a starter culture had thus advantages over the traditional method, which resulted in a shorter fermentation time that eliminates the chance of growth of contaminants, hygienic conditions, maintaining consistency with better quality and flavour.