DISCUSSION

Documentation of traditional knowledge

Traditional knowledge of the ethnic people living in the Sikkim Himalaya on production of fermented cereal-based food was worth documenting, both as functional foods, and for socio-cultural reasons. *Selroti* is an important fermented cereal-based food in the local diet of the Sikkim Himalaya, and are prepared at households. Its production practiced by the ethnic people of Sikkim is a unique type of food production by a natural fermentation.

Information on indigenous knowledge and antiquity of *Selroti* was sought from village elders and older women during survey, which has been well documented in this thesis. *Roti* is a Nepali word for bread. Out of many kinds of bread the Nepalis consume, two of them have a special place in the society. One is *Babari* and the other is *Selroti. Babari* is round, solid pancake whereas *Selroti* is ring shaped pretzel-like bread. Both are prepared from grounded rice flour. It was recorded during interview that in olden days only *Babari* was prepared and consumed by the people instead of *Selroti.* Since, the consumers found it difficult to fry in a pan, especially to turn it upside down; they started making rings with the batter. And to turn this ring-shaped bread they

started using a poker locally called 'suiro' (a pointed bamboo stick). Anything lifted with a 'suiro' is called 'saela' in the Nepali language. Probably, the word *Selroti* might have derived from the word 'saela'. That is how preparation and consumption of *Babari* among the Nepalis was slowly replaced by *Selroti* preparation, which has become a distinct food culture of the Nepalis.

There is a hypothesis on the nomenclature of *Selroti.* The word 'seli' is a name for local variety of rice cultivated in foot hills of Nepal. The product prepared from 'seli' variety of rice might have been called as *Selroti.* In Nepali, the word 'saal' means a year. Since *Selroti* is prepared during *Tiwar,* one of the main festivals of the Nepalis which is celebrated once in a year. Some people believe that the word *Selroti* has originated from the word 'saal' meaning confectionary bread prepare during festival once in a year. The antiquity of *Selroti* remains a myth, no historical documents or monographs were available on this product. Documentation of ethnical information on antiquity of *Selroti* during our survey will provide vital information on the history and food culture of the Nepalis in the Sikkim Himalaya.

In olden days, *Selroti* preparation did not include the use of spices or condiments but now-a-days, because of the development of diversified taste, people prefer to add spices during *Selroti* preparation.

Selroti has a deeply rooted ethnic importance among the Nepalis. *Selroti* is served during marriage ceremony of various castes of the Nepalis along with other traditional food items. It is a customary to hand over a basket full of freshly fried *Selroti* to bride's parents by the groom during marriage among the Nepalis. This is probably to supplement the sweet-dish, which is traditionally not common among the Nepalis, for greetings. Traditionally, newly married Nepali bride visits her parent's house once in a year. When she returns back to her husband's house she should carry a 'thumsey' (local name for bamboo basket) containing freshly fried-*Selroti.* This traditional is known as 'pani roti' in Nepali.

Selroti is traditionally served along with other traditional food items during *Bhai Tika,* a Hindu festival of the Nepalis, which is observed to honour the brothers by their sisters. Beside this, it is also served during other festivals of the Nepalis like *chaitay dasai, maghay sakranti, bara dasai,* etc.

Fried *Selroti* products can be preserved for about 10-15 days without refrigeration and consumed as it is or slightly warmed up. People might have invented such preservation technique to feed themselves while traveling. Carrying fried *Selroti* is a traditional practice in the Sikkim Himalaya while traveling for long distances (Tamang, 2005a).

It was understood from the feeding frequency summarized in Table 3 that *Selroti* is prepared and consumed occasionally may be because its preparation is labour-intensive, time consuming and costly. *Selroti* is mostly prepared at home (75.6 %) comparable to market purchase. Among the ethnic groups in Sikkim, 93.4 % of Nepali prepared *Selroti* at home, justifying that *Selroti* has an important bearing in the dietary habits of the Nepalis in the Sikkim Himalaya.

Microorganisms

The microbial population of *Selroti* batters collected from different sources revealed that lactic acid bacteria (LAB), comprising lactobacilli, pediococci, leuconostocs and enterococci were the predominant microorganisms present in viable numbers above 10^8 cfu/g, followed by yeasts around

10^5 cfu/g, respectively. The identity of the LAB strains seems to be similar with that of LAB typically present in many traditional cereal-based fermented foods of other regions (Soni and Sandhu, 1990; Oyewole, 1997; Brandt, 2007). Taxonomically diverse species of LAB have been identified from *Selroti* batters of the Sikkim Himalaya. They represented the four genera of LAB - *Lactobacillus, Leuconostoc, Pediococcus* and *Enterococcus.* Classification of LAB into different genera is largely based on morphology, gas production from glucose (Kandler, 1983), mode of glucose fermentation, and growth at different temperatures (Mundt, 1986; Dykes, 1994).

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. Leuc. mesenteroides* has been reported in several cereal-based fermented foods such as *idli* of India (Mukherjee *et al.,* 1965), *enjera* of Ethiopia (Oyewole, 1997), *puto* of the Philippines (Kelly *et al.*, 1995); *mawè* of Togo and Benin (Hounhouigan *et al.*, 1993b). Enteroccoci play beneficial role in production of many fermented foods

(Bouton *et al.*, 1998; Cintas *et al.*, 2000). *E. faecium* appears to pose a low risk for use in foods, because these strains generally harbour fewer recognised virulence determinants than *E. faecalis* (Franz *et al.*, 2003). *P. pentosaceous* along with several species of lactobacilli were reported as predominant LAB strains in Tanzanian *togwa* (Mugula *et al.*, 2003a); in *mawè* of Togo and Benin (Hounhouigan *et al.*, 1993b) and in *kodo ko jaanr* of India (Thapa and Tamang, 2004). There are not many reports on the occurrence of *Lb. curvatus* in fermented cereal-based foods. *Lb. curvatus* has been reported in *mawè*, a fermented maize food in lesser percentage of prevalence than other LAB (Hounhouigan *et al.*, 1993b).

Though the dominant microflora in fermented *Selroti* products was LAB, a sizable number of yeasts were also reported. Based on the detailed characterisations and identification profiles, the following yeasts were isolated and identified from *Selroti* batters: *Saccharomyces cerevisiae*, *Saccharomyces kluyveri*, *Debaryomyces hansenii*, *Pichia burtonii* and *Zygosaccharomyces rouxii*. *Saccharomyces cerevisiae* is the principal yeast of most bread fermentations (Jenson, 1998). Yeast fermentation serves several functions

in sourdough production (Hammes and Ganzle, 1998). Gas production causes expansion and leavening of the dough, ultimately affecting the texture, density and volume of the bread (Hammes *et al.*, 2005). *Saccharomyces kluyveri* has been reported in *nan*, a leaved bread of North India (Batra, 1986). *Debaryomyces hansenii* has been isolated from *idli* along with several other yeasts (Soni and Sandhu, 1991). *Pichia burtonii* has been reported in some Asian rice-based alcoholic starters such as *loog-pang* of Thailand (Limtong *et al.*, 2002) and *marcha* of Sikkim (Tsuyoshi *et al.*, 2005). However, *Pichia burtonii* produces visible, white or chalky discoloration in sourdough (Legan and Voysey, 1991). Acid-tolerant *Zygosaccharomyces rouxii* has not been reported in cereal-based fermented foods, though it has been reported in many fermented soybean foods of Asia which contribute aroma to the product (Aidoo *et al.*, 2006). Origin of *Z. rouxxii* is usually from sugar, honey and confectionery (Kreger-van Rij, 1984). Probably recovery of *Z. rouxii* in *Selroti* batters was their entry through sugars and honey which are added during *Selroti* batter preparation to make it sweet.

The most prevalent LAB and yeasts in all samples of *Selroti* batters were *Leuc. mesenteroids* and *S. cerevisiae*, respectively which were recovered in all samples analysed as predominant organisms. Predominance of *Leuc. mesenteroids* and *S. cerevisiae* have been common in other cereal-based fermented foods (Steinkraus, 1996; Brandt, 2007).

Occurrence of bacterial contaminants

Food borne pathogens *Bacillus cereus, Listeria* sp., *Salmonella* sp. and *Shigella* sp. were not detected in any sample of fermented batters of *Selroti*, due to slight acidic nature of the products. Fermentation of cereals reduces contamination of weaning foods in Ghana (Mensah *et al.*, 1990). These contaminants might have introduced during handling of raw materials for preparation when pH was not low enough to inhibit their growth. High population (>10^8 cfu) of LAB in *Selroti* batters (Table 4 and 5), could restrict the growth of other organisms simply by their physical occupation of available space and uptake of most readily assimilative nutrients (Adams and Nicolaides, 1997). It is also a fact that lactic acid produced by LAB may reduce pH to a

level where pathogenic bacteria may be inhibited (Holzapfel *et al.*, 1995; Tsai and Ingham, 1997; Adams and Nout, 2001).

Effect of seasonal variation on microbial load

It was observed that seasons affect the development and prevalence of microorganisms in the fermented batters. During summer, the microbial load of LAB increased due to rise in temperature which may accelerate fermentation rate. Whereas, winter was favourable for yeasts. Similar observation on effect of seasonal variation was made during *idli* fermentation favouring the bacterial load (Soni *et al.*, 1986).

Technological properties of isolates

Technological or technical properties of LAB strains isolated from fermented foods are important criteria for selection of starter cultures to be used in the manufacture of functional foods (Durlu-Ozkaya, 2001; Badis, 2004). Acidification is an important technological property in relevance of selection for starter culture among the LAB (de Vuyst, 2000). 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 (Table 14). About 63.6 % of LAB strains caused coagulation of skim milk at 30° C (Table 14). Coagulation of milk by LAB strains shows their potential as starters or adjunct cultures in the production of fermented products. Among yeasts strains, only *S. cerevisiae* and *D. hansenii* showed acidification characters, though decrease in pH was limited to 5.6 (Table 15). These strains although originating from plant sources, appeared to be adapted to the milk ecology, since they coagulated and acidified the skim milk used in the applied method. The casein degradation initiated with milk clotting peptidases and proteinases, which produce peptides and amino acids (Mäyra-Mäkinen and Bigret, 1998).

The use of the API-zym technique has been reported (Arora *et al.*, 1990) as a rapid and simple means of evaluating and localising 19 different hydrolases of microorganisms associated with dairy fermentations. This method is also of relevance for selection of strains as potential starter cultures based on superior enzyme profiles, especially peptidases and esterases, for accelerated maturation and flavour development of other fermented products (Tamang *et al.*,

2000; Kostinek *et al.*, 2005). Absence of proteinases (trypsin) and presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) activities produced by the predominant LAB strains isolated from *Selroti* batters are possible traits of desirable quality for their use in production of typical flavour and aroma. *Leuc. mesenteroides* isolated from Armada cheese showed the highest aminopeptidase activity (Herreros *et al.*, 2003). Yeasts strains from *Selroti* batters showed no detectable proteinase activity in the applied method. High activity of phosphatase by yeast strains (Table 17) showed their possible role in phytic acid degradation in cereal-based fermented foods. Anti-nutritive factors such as phytic acids and oligosaccharides are of particular significance in unbalanced cereal-based diets (Fredrikson *et al.*, 2002). Due to these nutritional consequences, the degradation of anti-nutritive factors in food products by fermentation is desirable as reported for a number of foods of plant origin (Chavan and Kadam, 1989; Mbugua *et al.*, 1992; Svanberg *et al.*, 1993). It was also shown that strain of *Leuc. mesenteroides* isolated from *Selroti* batters had high α-galactosidase and β-galactosidase activities. Presence of high activity of α-galactosidase (Table 16); probably indicated their ability to

hydrolyse oligosaccharides of raffinose family (Holzapfel, 2002). High activity of β-galactosidase exhibited by LAB strains are essential features in fermented milk products (Mathara *et al.*, 2004).

All strains of LAB showed antimicrobial activities against a number of pathogenic Gram-positive and Gram-negative bacteria in the applied method. However, the cell-free supernatant fluid extracts of LAB strains isolated from *Selroti* batters could not produce bacteriocin under the applied condition. Lactic acid bacteria compete with other microorganisms by screening antagonistic compounds and modifying the micro-environment by their metabolism (Lindgren and Dobrogosz, 1990; Tagg, 1992). Production of bacteriocin depends on a number of intrinsic and extrinsic factors including redox potential, water activity, pH and temperature (Yang and Ray, 1994; Delgado *et al.*, 2005). Species of LAB strains isolated from several cereal-based fermented foods have the antimicrobial activities including bacteriocins production (Olukoya *et al.*, 1993; Omar *et al.*, 2006).

Proximate composition

The proximate composition of unfermented rice and wheat flour and samples of *Selroti* batters collected from different sources were analysed (Table 19 and 20). Moisture content in *Selroti* batters was higher than that of raw materials due to soaking prior to fermentation and also due to addition of water and milk during its preparation. There was a remarkable increase in water-soluble and TCA-soluble nitrogen in *Selroti* batters due to solubilisation of proteins, indicating its protein digestibility. Increase in free amino acids in *tarhana* has been reported (Erbas *et al.*, 2005b). The food value of fermented batters was found to be increased slightly than the unfermented raw materials. Samples of fermented batters collected from Sikkim Himalaya had comparatively higher mineral content. Fermentation may have enhanced the nutritional and mineral contents of cereals (Amoa and Muller, 1973; Blandino *et al.*, 2003; Umeta *et al.*, 2005). Proximate and food value of *Selroti* batters are almost same as reported in other cereal-based fermented foods such as *idli* (Soni and Sandhu, 1989a), *rabadi* (Gupta *et al.*, 1992b) and *tarhana* (Erbas *et al.*, 2005a).

Fermentation dynamics *in situ*

During *in situ* fermentation of *Selroti* batter, indigenous lactic acid bacteria and yeasts changed spontaneously. Spontaneous change in LAB as well as yeast population during several cereal fermentations were reported (Soni and Sandhu, 1990; Mugula *et al.*, 2003a). As expected in a typical lactic fermentation (Vaughn, 1985; Lee, 1997), the pH of the fermenting substrates decreased and the titratable acidity increased as the batter fermentation progressed due to growth of LAB which, converts fermentable sugars into lactic acid (Buono *et al.*, 1990). Bacterial contaminants *Bacillus cereus* and enterobacteriaceae were associated with initial fermentation and finally disappeared during *Selroti* batter fermentation. Such similar observation was also made in *masa* fermentation (Efiuvwevwere and Ezeama, 1996). The LAB produced sufficient acid for inhibition of pathogenic microorganisms in foods (Adam and Nicolaides, 1997). 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 has been no report of any food poisoning or infectious disease infestation

by consuming *Selroti.* The results showed that various microbial genera occur at the early stages of *Selroti* batter fermentation. This may be partly attributed to the microbial diversity often associated with rice grains and plant materials (Sneath *et al.*, 1986; Efiuvwevwere and Ezeama, 1996). Rice grains contain a numerous microflora including yeasts *P. burtonii* (Kreger-van Riz, 1984), leuconostocs and pediococci (Wood and Holzapfel, 1995). 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. However, increase in batter volume during fermentation has been reported in *idli* and *dosa* (Soni *et al.*, 1986) and in *puto* (Tongananta and Orillo, 1996). Numerous chemical and physical factors influence the rate and growth of various microorganisms, as well as their sequence of appearance during cereal fermentation (Cartel *et al.*, 2007).

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 due to soft texture, sweet taste and aroma, acceptable to consumers. It is generally noted that a soft texture and sweet-taste, with golden brown colour fried *Selrori* is considered the best to the consumers. Yeasts play vital roles in production of many traditional fermented foods mostly enhancing sensory quality of the foods (Boekhout and Robert, 2003; Romano *et al.*, 2006).

Selroti batter preparation by selected starter cultures

Use of standard starter culture is not a practice in the Sikkim Himalaya except in alcoholic beverage production (Thapa and Tamang, 2004). 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.* The rationale behind is to use starter culture in order to supplement the natural microflora of *Selroti* batter. 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 *Leuconostoc mesenteroides* BS1:B1 and *Saccharomyces cerevisiae* BA1:Y2, selected on the superior technological property as mentioned in the result section, 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. Yeast contributes in flavour development to fermented maize product and LAB are responsible for acidification (Nche, 1995). None of the strains combinations of *E. faecium*, *Lb. curvatus*, *P. pentosaceus*, *D. hansenii*, *P. burtonii*, *S. kluyveri*, and *Z. rouxii*, used as starters could produce organoleptically acceptable *Selroti* product. The consumers' preference trial showed that *Selroti* batter prepared in the laboratory 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. The principle requirements of the strains are rapid production of CO_2 from maltose and glucose, and generation of good bread flavours (Decock and Cappelle, 2005), which were performed by both isolates (*Leuc. mesenteroides* and *S. cerevisiae*) in *Selroti* batters (Table 7 and 9).

Application of starter cultures may appear appropriate in *Selroti* batter production at household level since it is cost-effective and may contribute to effective control and safeguarding of the fermentation process. *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. The final product is not always consistent in natural fermentation; the use of a mixed starter culture could provide more consistent fermentations and products of higher quality (Gardner *et al.*, 2001; Zorba *et al.*, 2003). Modern starter cultures are selected, either as single or multiple strains, especially for their adaptation to a substrate or raw material, for example cereals, milk, meat, legumes, roots, and tubers (Buchenhüskes, 1993; Holzapfel *et al.*, 2003). Commercial starter cultures of the yeast-bacterial combinations are now available for sourdough production (Decock and Cappelle, 2005). The authentic identity of indigenous LAB isolated from traditional fermented cereal products and their detailed technological characters should be considered for development of starter culture.

Though, optimised process condition is always superior and advantageous than the conventional method, however, introduction and replacement of natural and easily operated traditional technology may be difficult to change for the producers or rural populace (Holzapfel, 1997). Authentic identity of functional microbes in fermented foods is necessary to develop the starter cultures isolated from conventionally prepared foods (Geisen and Holzapfel, 1996; Tamang and Holzapfel, 1999). Preservation and safeguarding of foods are still major objectives of fermentation (Holzapfel, 2002). Yet, other aspects such as wholesomeness, acceptability and overall quality have become increasingly important and valued features to account of the substrate, technical properties of the strain, food safety requirements and quality expectations (Holzapfel *et al.*, 2003).

Conclusion

Selroti is a cultural food of the Nepalis in the Sikkim Himalaya prepared from rice. The traditional knowledge of ethnic people for *Selroti* batter preparation was worth documenting. Scientific knowledge on *Selroti* of the Sikkim Himalaya is unknown outside this region. Species of

functional microorganisms associated with fermented batters of *Selroti* collected from different sources of the Sikkim Himalaya were LAB: *Leuconostoc mesenteroides, Enterococcus faecium, Pediococcus pentosaceus* and *Lactobacillus curvatus;* Yeasts: *Saccharomyces cerevisiae, Debaryomyces hansenii, Pichia burtonii, Zygosaccharomyces rouxii* and *Saccharomyces kluyveri.*

This study showed that strains of LAB and yeast play important and partly complex role in this traditional fermentation process by their functional properties related to a specific and partly a wide enzyme spectrum, their acidifying capacity and antimicrobial activities of LAB, though bactericion production was not observed. Microbial dynamics *in situ* fermentation of *Selroti* batter was studied to understand the microbial composition of the product. Some of these LAB strains possess interesting protective and functional properties, which render them interesting candidates for use as starter culture for controlled and optimised production of fermented cereal batters typical of Sikkim. *Selroti* batters prepared by a mixed pure culture strains of LAB had many advantages over the conventionally prepared products.