INTRODUCTION

A fermented food is defined as an edible product prepared from raw or cooked materials of plant or animal origins by microorganisms either spontaneously or by adding mixed or pure culture (Hansen, 2002; Hulse, 2004). The essential objective of food fermentation is to carry over supplies from the time of plenty to those of deficit (Kwon, 1994; Tamang, 2000). Bacteria, mostly lactic acid bacteria, yeasts and filamentous moulds constitute the microbiota of fermented foods and beverages, which are present in or on the ingredients, utensils, environment, and are selected through adaptation to the substrates (Hesseltine, 1983, Steinkraus, 1997). Functional microorganisms play important roles in bringing some remarkable physico-chemical changes in the substrates during fermentation such as enrichment of the human dietary with acceptable flavour, texture and aroma, biopreservation of food, bioenrichment of substrates with vitamins, protein and essential amino acids, improved digestibility, detoxification of undesirable and anti-nutritive compounds, enhancement of bioactive compounds and stimulation of probiotic functions (Campbell-Platt, 1994; Holzapfel and Schillinger, 2001; Shah, 2001; Ammor *et al.*, 2007).

In India, mostly due to wide variation in agro-climatic conditions and diverse dietary cultures of the multi-ethnic groups of people, microorganisms associated with more familiar and less familiar traditional fermented foods and beverages may contribute significant microbial resources (Batra, 1986; Soni and Sandhu, 1990; Tamang, 1998). Eleven genera of lactic acid bacteria with several species have been reported from fermented foods and beverages which include *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* (Stiles and Holzapfel, 1997; Axelsson, 1998; Carr *et al.*, 2002). Lactic acid bacteria have been traditionally used as starter cultures in the production of fermented foods which contribute to the organoleptic, rheological, nutritional properties of the products, resist spoilage and food toxins by their antimicrobial properties (Nout and Ngoddy, 1997; Salminen and Wright, 1998; Leroy and de Vuyst, 2004). Functional yeasts genera associated with fermented foods and beverages are mostly *Brettanomyces* (its perfect stage, *Dekkera*), *Candida, Cryptococcus, Debaryomyces, Galactomyces, Geotrichum, Hansenula, Hanseniaspora* (its

asexual counterpart *Kloeckera*), *Hyphopichia, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Torulopsis, Trichosporon, Yarrowia* and *Zygosaccharomyces* (Kurtzman and Fell, 1998; Pretorius, 2000; Nout and Aidoo, 2002; Tsuyoshi *et al.*, 2005). Yeasts play vital roles in production of many traditional fermented foods and beverages across the world (Aidoo *et al.*, 2006) mostly enhancing sensory quality of the foods (Boekhout and Robert, 2003).

In developing countries, a global interest in rice and its fermented product is increasing due to their calorigenic value, unique quality characteristics and high acceptability (Steinkraus, 1994; Efiuvwevwere and Ezeama, 1996). Traditional cereal-based fermented foods are frequently used as complementary foods for infants and young children in Africa (Tou *et al.*, 2006). In most of the countries, rice is fermented either by using mixed-culture(s) into alcoholic beverages, or by natural fermentation into leavened batter formed dough breads which are usually baked or steamed (Sugihara 1985; Yokotsuka, 1991). Some of the common cereal-based fermented foods across the world have been

extensively studied. The well-documented Indian cereal-based non-alcoholic fermented foods are *idli* (Mukherjee *et al.* 1965; Steinkraus *et al.* 1967; Soni and Sandhu, 1991), *dosa* (Soni *et al.*, 1985, 1986), *jalebies* (Batra, 1986), *rabadi* (Gupta *et al.*, 1992a); and Indian cereal-based alcoholic beverages are *bhaati jaanr* (Tamang and Thapa, 2006) and *kodo ko jaanr* (Thapa and Tamang, 2004, 2006). Several cereal-based fermented foods of Africa and other parts of the countries have been well investigated which include *masa* of South Africa (Efiuvwevwere and Ezeama, 1996), *puto* of South-East Asia (Kelly *et al.*, 1995), *mawé* or *ogi* of Benin (Onyekwere *et al.*, 1989; Hounhouigan *et al.*, 1993a), *kisra* of Sudan (Mohammed *et al.*, 1991), *sourdough* of America and Europe (Brandt, 2007), *ben-saalga* of Burkino Faso (Tou *et al.*, 2007), *kenkey* of Ghana (Nche *et al.*, 1994; Nout *et al.*, 1996), *togwa* of Tanzania (Mugula *et al.*, 2003a), *tarhana* of Turkey (Erbas *et al.*, 2006), etc.

Sikkim is a mountainous state of India with an area of 7096 sq. km and has four administrative districts - North, East, South and West. Darjeeling is a picturesque hill district of West Bengal state with an area of 3075 sq. km. Three sub-divisions of Darjeeling district viz. Darjeeling, Kalimpong and

Kurseong are hilly, commonly known as the Darjeeling hills or officially as Darjeeling Gorkha Hill Council. Topographically, culturally and ethnically the Darjeeling hills and Sikkim have similarities. The Darjeeling hills and Sikkim are located in the Eastern Himalayan regions and are ecologically grouped as the Sikkim Himalaya. Three major ethnic communities of the Sikkim Himalaya are the Nepalis, the Bhutias and the Lepchas.

Traditional fermented foods and beverages constitute an important part of the local diet in the Sikkim Himalaya (Tamang, 2005a). Traditional foods in the Darjeeling hills and Sikkim are closely associated with socio-economical development, ethnic importance, religious and cultural practices, and has been evolved as the result of tradition and empirical experiences of generations over a period of time (Tamang *et al.*, 1988). Daily per capita consumption of fermented foods and beverages in the Sikkim Himalaya is 87.6 g representing 10 % of the total daily food consumed in the local diet (Yonzan and Tamang, 1998). Depending on the altitudinal variation, various types of cereals crops such as rice (*Oryza sativa* L.), maize (*Zea mays* L.), finger millet (*Eleusine coracana* Gaertn.), wheat (*Triticum aestivum* L.),

barley (*Hordeum vulgare* L.) and buckwheat (*Fagopyrum esculentum* Moench.) are cultivated and eaten as staple food items in the Sikkim Himalaya. Annual production of cereals in Sikkim during 2004-05 was 94.73 tonnes with annual yield as 1439.68 kg/hectare (Annual Progress Report, 2005).

Varieties of fermented foods and beverages are traditionally prepared and consumed, and even marketed locally in North East India (Tamang, 2001). Some of the ethnic fermented foods and beverages of North East India were extensively studied (Tamang, 2005b), however, several less familiar fermented foods are yet to be investigated scientifically. *Selroti* is a popular fermented rice-based food which is ring-shaped, spongy, pretzel-like, deep fried and commonly consumed in Sikkim and the Darjeeling hills in India, Nepal and Bhutan. To the best of our knowledge, no microbiological and biochemical aspects of *Selroti* of the Sikkim Himalaya have been studied. The present dissertation will focus on the microbiological and biochemical aspects of *Selroti* batter fermentation, and also on optimization of the traditional processing method using starter cultures in order to minimize the production time, maximize the substrate utilization and improve the quality of the product.

Objectives

The present thesis was aimed to document the indigenous knowledge of traditional processing of *Selroti* batter preparation in the Sikkim Himalaya; and to isolate, characterise and identify the predominant microorganisms in fermented batters collected from different sources. The objective of the thesis was also to study the effect of seasonal variation on microbial load, technological properties of the identified strains of functional lactic acid bacteria and yeasts such as acidifying capacity, enzymatic profiles, and their antimicrobial activities against pathogenic bacteria. Microbial population dynamics of lactic acid bacteria and yeasts during *in situ* fermentation were also examined. The strains of lactic acid bacteria and yeasts were selected and tested for production of fermented batters under laboratory conditions, and subjected to sensory evaluation. Proximate composition of raw materials and *Selroti* batters were also calculated.