6. SUMMARY

The people of Darjeeling hills and Sikkim consume a variety of traditional fermented foods including kinema (soya bean product), masayura (black gram product), gundruk (leafy vegetable product), sinki (radish tap root product), mesu (young bamboo shoot product), khalpi (cucumber pickle), shel roti (rice preparation), dahi (dairy product), kachcha churpi (dairy product), churpi (dairy product), dudh churpi (dairy product), sukako masu (meat product), murcha (starter culture, rice flour product), jnard (beverage) and raksi (beverage).

Kinema, a meat substitute to the majority of the people of these regions, is fried and made to a thick curry to use as a side dish of rice. Masayura, a ball-like or conical, hollow, black gram product, is used as a spicy condiment. Gundruk and sinki are dried sour vegetable products, eaten as soup or pickle. Mesu and khalpi are fermented pickles. Shel roti is a deep fried, ring-shaped confectionary bread, produced from fermented rice batter. Dahi is an acidic savory. Kachcha churpi, a soft mass product of cow's milk, is used as a condiment. Churpi and dudh churpi are hard products, prepared from cow's or yak's milk and used as masticatory. Sukako masu, prepared by smoking the strips of mutton, pork, beef or yak meat, is eaten as curry. Murcha, a spherical, flattened, solid cake, is a starter culture prepared from rice flour, wild herbs and spices, used to ferment finger millet seeds to produce a popular, mild alcoholic beverage known as jnard. Other starchy materials are also used to prepare jnard. Raksi is a distilled part of jnard, usually prepared from rice. All these foods are very important in that they are socio-culturally related. Majority

of the people of these regions consume all the foods mentioned above excepting mesu, khalpi and masayura, the consumption of which is confined to a few pockets of the hills.

Most of these fermented foods have not previously been investigated. In this report, the traditional methods of their preparation, mode of consumption and ethnic importance have been documented. Kinema, sinki, mesu and murcha were selected for microbial and biochemical studies.

Kinema contains a high amount of moisture. The moisture content of about 11% in raw soya beans increased to about 62% in kinema. This is a protein-rich product containing about 48% (on dry matter basis) protein. While the fat content in raw beans was about 22%, the same in kinema was about 17%. Since ash is usually added during preparation of kinema for marketing, the ash content of market samples showed a higher value, compared to that of the laboratory-made samples of kinema. Much higher values of pH, titratable acidity and free fatty acidity were observed in kinema than their corresponding values in soya beans. The energy values of soya beans and kinema were nearly the same (*ca* 2 MJ per 100 g dry matter).

A total of 502 bacterial and 198 yeast strains were isolated from 50 samples of kinema. All the sporeforming bacteria were tentatively identified as *Bacillus subtilis*. and the asporogenous cocci were identified as *Enterococcus faecium*. The yeasts were identified as *Candida parapsilosis* and *Geotrichum candidum*. The load of *B. subtilis*, the only organism recovered in raw soya beans at 8×10^5 cfu/g, was $3\text{-}5 \times 10^8$ cfu/g fresh kinema with 100%

prevalence in both the substrate and the fermented product. *Enterococcus faecium*, with a load of 5-9 x 10^7 cfu/g, occurred in 100% samples of kinema. The population of *C. parapsilosis* with 50-80% prevalence was 0.3-9 x 10^4 cfu/g, and that of *G. candidum*, recovered from 40-50% of the market samples only, was 0.8-4 x 10^4 cfu/g.

The traditional process parameters for the preparation of kinema were optimized at 10 min time for cooking soya beans at 0.7 kg/cm² steam pressure, and fermenting beans in perforated polythene bag, and at 37°C for 48 h. Studies on microbial changes during soya bean fermentation indicated *Bacillus subtilis* as the predominant microorganism which increased significantly ($P<0.05$) at every 8 h intervals till the end of fermentation. The population of *Enterococcus faecium* also increased at an approximately same rate as of *B. subtilis*. The load of *Candida parapsilosis*, although much less compared to the bacterial load, increased significantly ($P<0.05$) at every 8 h intervals till 32 h of fermentation. In laboratory preparations, *Geotrichum candidum* could not be detected. The biochemical changes during fermentation of soya beans revealed the initial decline in pH from 6.94 to 6.64 at 16 h after which there was a sharp increase in pH up to 8.51 at 40 h. The titratable and free fatty acid contents increased significantly ($P<0.05$) at every 8 h intervals during the entire course of fermentation. The moisture content remained relatively constant at about 62%. At the end of fermentation, the total nitrogen content increased significantly ($P<0.05$) over the substrate at 0 h of fermentation. Due to proteolytic activities of *B. subtilis*, there were remarkable

changes in protein, non-protein and soluble nitrogen during the fermentation. While the protein nitrogen declined, the non-protein and soluble nitrogen contents increased significantly ($P<0.05$) at almost every 8 h intervals till the end of fermentation.

Bacillus subtilis DK-W1, isolated from kinema of Darjeeling market, was selected as the best proven strain for production of kinema. Attempts were made to improve the general acceptibility of kinema by inoculating sterile beans with that strain and fermenting at 45°C for 18 h, optimized earlier. The studies revealed that *B. subtilis* DK-W1 could effectively be used for desirable fermentation within a much shorter period and with excellent product development comparable to the natural fermentation of kinema. The changes in proximate composition of the fermenting beans were similar in monoculture and natural fermentations.

The market sinki had about 21% moisture, while the freshly prepared laboratory-made samples had 93.5% moisture. Regarding the contents of protein, fat and ash, no change was observed between raw radish tap root and sinki. There was a remarkable decrease in pH and increase in acidity in sinki from its substrate. A total of 453 strains of lactic acid bacteria were isolated from 40 samples of sinki. Two species of *Lactobacillus*, *L. plantarum* and *L. brevis* were isolated from sinki. In addition to these two, *L. fermentum* was present in the substrate. In sinki. *L. plantarum* was dominant (6×10^8 cfu/g) followed by *L. brevis* ($6\text{-}7 \times 10^3$ cfu/g) with their prevalence in 100% of the samples analysed.

The traditional process parameters for the production of sinki were optimized at fermenting radish tap root in glass jar at 30°C for 12 days. The fermentation was initiated by hetero-fermentative *Lactobacillus fermentum*, followed by another hetero-fermentative *L. brevis*, both dominated in the early stages of fermentation, and succeeded later on by more acid-producing homofermentative *L. plantarum* till the end of fermentation, when *L. fermentum*, which initiated the process, disappeared. During fermentation, the drop in pH and rise in titratable acidity were 6.72 to 3.30 and 0.04 to 1.28%, respectively. The moisture and total nitrogen contents remained relatively constant throughout fermentation.

The contents of moisture, protein, fat and ash were same in both bamboo shoot and mesu. The remarkable decrease in pH and increase in acidity in mesu over its substrate were observed. A total of 327 strains of lactic acid bacteria representing *Lactobacillus plantarum*, *L. brevis* and *Pediococcus pentosaceus* were isolated from 30 samples of mesu. All these lactics were present in 100% samples of the raw bamboo shoots. In mesu, *L. plantarum* was dominant (3×10^8 cfu/g) followed by *L. brevis* ($4\text{-}5 \times 10^3$ cfu/g) with 100% prevalence in both of them. *Pediococcus pentosaceus* was least populated (20-30 cfu/g), and recovered from 40-50% of the market samples.

The traditional process parameters for the production of mesu were optimized at fermenting bamboo shoots in glass jar at 30°C for 10 days. During fermentation, initially, the homofermentative *P. pentosaceus* comprised the most dominant microflora. Its

dominance was soon overtaken by the heterofermentative *Lactobacillus brevis* which attained its peak at the 4th day of fermentation. This was finally succeeded by the more acid-producing homofermentative *L. plantarum* which became the dominant organism at the end. The fermentation caused the decline in pH from 6.35 to 3.84 and increase in acidity from 0.04 to 0.95%. Moisture and total nitrogen contents remained the same.

Murcha contains 13% moisture and 0.7% ash (dry matter basis), having pH 5.1-5.2. A total of 194 bacterial, 190 yeast and 58 mould strains were isolated from 30 market samples of murcha. The bacterial strains were identified as *Pediococcus pentosaceus*, the yeasts as *Saccharomycopsis fibuligera* and *Pichia anomala*, and the moulds as *Mucor circinelloides*. The samples contained (cfu/g fresh weight): 1.9-2.1 x 10^8 *P. pentosaceus*, 3.6-3.9 x 10^8 *S. fibuligera*, 2.8-3.0 x 10^7 *P. anomala* and 2.0-2.3 x 10^7 *M. circinelloides*. All these organisms were present in 100% of the samples analysed.