

6. SUMMARY

Traditional fermented beverages constitute an integral part of dietary culture and have strong ritual importance among the ethnic people in the Darjeeling hills and Sikkim of the Sikkim Himalayas in India. Common indigenous fermented beverages are kodo ko jaanr, bhaati jaanr, makai ko jaanr and gahoon ko jaanr. Raksi is distilled liquor prepared from fermented starchy materials. About 57.6 % and 76.7 % of rural people prepared fermented beverages for home consumption in the Darjeeling hills and in Sikkim, respectively.

Marcha, a dry, spherical or flattened, solid ball-like starter used for production of indigenous alcoholic beverages. It is traditionally prepared from rice mixed with wild herbs and spices. Sixty-six samples of marcha, collected from eleven different important marcha-making villages of the region, were analysed and microbial load of filamentous moulds, yeasts, lactic acid bacteria and total viable counts were found at the level of 10^6 cfu/g, 10^8 cfu/g, 10^7 cfu/g, and 10^8 cfu/g, respectively. Out of 733 strains of microorganisms, 152 were filamentous moulds, 321 strains were yeasts and 260 were lactic acid bacteria. Filamentous moulds were identified as *Mucor circinelloides* forma *circinelloides*, *Mucor* sp. (close to *M. hiemalis*), *Rhizopus chinensis* and *Rhizopus stolonifer* variety *lyococcus*. Species of *Mucor* were more prevalent than species of *Rhizopus* in marcha samples analysed. Yeasts were identified as *Saccharomycopsis fibuligera*, *Pichia anomala*, *Saccharomyces cerevisiae* and *Candida glabrata*. Among yeasts, *Saccharomycopsis fibuligera* was most dominant in marcha. Lactic acid bacteria *Pediococcus pentosaceus* and *Lactobacillus bif fermentans* were isolated from marcha. *Pediococcus pentosaceus* was more predominant

than *Lactobacillus bifermentans* in marcha. Marcha contained 14 % moisture, pH 5.58, 0.01 % acidity and 1.4 % ash.

Saccharomycopsis fibuligera and *Pichia anomala* showed strong phosphatase and peptidase activities in API-zym system applied. All isolates showed relatively weak esterase and lipase activities except by *Pichia anomala* as compared with phosphatase activities. Strains of moulds and yeasts showed the amylolytic activities whereas none of the lactic acid bacteria showed amylolytic activity. *Saccharomycopsis fibuligera* MS:YD4 showed highest α -amylase activity and *Rhizopus chinensis* MJ:Rh3 showed highest glucoamylase activity.

Kodo ko jaanr, prepared from finger millets, is common fermented beverage in the Sikkim Himalayas. Milky white extract of kodo ko jaanr is sipped through a narrow bamboo straw called pipsing in a bamboo-or wood-made vessel called toongbaa, which is unique food culture in the region. Population of yeasts was detected at the level of 10^7 cfu/g whereas that of lactic acid bacteria was comparatively less ($\sim 10^5$ cfu/g) in forty samples of kodo ko jaanr. Filamentous moulds were not recovered in any finish product. Out of 161 strains of microorganisms, 81 isolates were yeasts *Pichia anomala*, *Saccharomyces cerevisiae* and *Candida glabrata* and 80 isolates were lactic acid bacteria *Pediococcus pentosaceus* and *Lactobacillus bifermentans*. *Pichia anomala* and *Saccharomyces cerevisiae* were present in all samples whereas *Candida glabrata* were present only in 40 % of samples analysed. Lactic acid bacteria were recovered in all samples. The proximate composition of kodo ko jaanr sample was analysed. The pH, moisture, acidity and alcohol content of the product was 4.1, 69.7

%, 0.27 % and 4.8 %, respectively. Kodo ko jaanr is rich in crude fibre. Ash, fat and protein content remained the same as that of substrate. Fermentation of finger millet enhanced bio-enrichment of minerals.

Kodo ko jaanr was prepared in the laboratory following the traditional method by using marcha. Changes in microbial population, physico-chemical and enzymatic activities in fermenting finger millet seeds during kodo ko jaanr fermentation were investigated. Mould population declined significantly ($P<0.05$) every day during fermentation and finally disappeared after 5 d. Population of yeasts increased significantly ($P<0.05$) from 10^5 cfu/g to 10^7 cfu/g within 2 d. Lactic acid bacteria also increased significantly ($P<0.05$) from 10^6 cfu/g to 10^8 cfu/g in first day and decreased significantly ($P<0.05$) to a level of 10^3 cfu/g at the end. Total viable count increased significantly ($P<0.05$) within 1 d and decreased. Temperature of fermenting finger millet remained between 26° C to 30° C during fermentation. The mean pH decreased and acidity increased during fermentation. Alcohol content increased significantly ($P<0.05$) from 0.1 % to 6.9 % within 6 d. Reducing sugar content increased significantly ($P<0.05$) till 3 d and decreased, followed by decrease in total sugar content. Maximum activities of saccharification and liquefaction of finger millets were observed on second day of fermentation.

Different combinations of moulds, yeasts and lactic acid bacteria isolates, previously isolated from marcha, were inoculated aseptically on sterilized finger millets to test the ability of isolates to produce kodo ko jaanr under controlled conditions. Jaanr prepared by a combination of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 showed

significantly ($P<0.05$) high reducing sugar and alcohol contents than jaanr fermented by other strains. Sensory evaluation result also showed that kodo ko jaanr prepared by this combination had significantly ($P<0.05$) highest score in general acceptability. The consumers' preference trial was carried out. Kodo ko jaanr prepared in the laboratory by mixture of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 as starter was more acceptable than product prepared by conventional marcha.

Bhaati jaanr is a soft, juicy and sweet-sour with mild-alcoholic beverage prepared from steamed glutinous rice and is consumed directly. Yeasts population was found higher than that of lactic acid bacteria in all twenty-four samples of bhaati jaanr samples. Moulds were not recovered in any final product analysed. Out of 127 strains of microorganisms isolated, 69 isolates were yeasts *Pichia anomala* and *Saccharomyces cerevisiae*, and 58 isolates were lactic acid bacteria *Pediococcus pentosaceus* and *Lactobacillus bif fermentans*. The pH, moisture, acidity and alcohol contents of the product were 3.5, 83.4%, 0.24 % and 5.9 %, respectively. Ash content was slightly higher than the substrate. Fat, protein and calorie contents remained same as the substrate. Mineral contents increased in bhaati jaanr over the substrate.

Moulds decreased significantly ($P<0.05$) during fermentation and disappeared after the fifth day of fermentation. Loads of yeasts increased significantly ($P<0.05$) from 10^5 cfu/g to 10^8 cfu/g within 2 d, and decreased to a level of 10^5 cfu/g at the end. Lactic acid bacteria increased significantly ($P<0.05$) till second day of fermentation, and then declined slowly. Temperature of fermenting rice remained between 28° C to 30° C. During

fermentation, pH decreased, and acidity and alcohol content increased significantly ($P<0.05$). Reducing sugar content increased significantly ($P<0.05$) till 3 d, and decreased followed by decrease in total sugar content. Maximum activities of saccharification and liquefaction of glutinous rice were observed on the third day of fermentation.

Makai ko jaanr is a viscous, mild-alcoholic beverage, made from maize and is drunk directly. Yeasts were found 10 times higher than that of lactic acid bacteria. Out of 100 strains of microorganisms isolated from twelve samples of makai ko jaanr, 54 isolates were yeasts and 46 isolates were lactic acid bacteria. Filamentous moulds were not recovered in the final product. Among the yeasts, 30 strains were *Pichia anomala* and 24 strains were *Saccharomyces cerevisiae*. Out of 46 lactic acid bacteria strains, 28 were *Pediococcus pentosaceus* and 18 strains were *Lactobacillus bifementans*. The pH, moisture and acidity of makai ko jaanr were 3.3, 81.9 % and 0.38 %, respectively. Alcohol content of the product was 2.5 %, comparatively less than that of other cereal-based jaanr products. Ash content was slightly higher in the product. Fat, protein and calorie value remained same. Crude fibre content increased in makai ko jaanr. Mineral contents increased in makai ko jaanr.

Mould count declined significantly ($P<0.05$) during makai ko jaanr fermentation and disappeared after the fourth day. The load of yeasts increased significantly ($P<0.05$) within 2 d and remained constant till eighth day, and decreased significantly ($P<0.05$) till an end. Lactic acid bacteria population increased significantly ($P<0.05$) from 0 day to 2 day and declined gradually. Temperature of fermenting maize remained relatively

constant to incubation temperature. During fermentation, pH dropped significantly ($P<0.05$) whereas acidity and alcohol increased significantly ($P<0.05$). Reducing sugar increased significantly ($P<0.05$) within 1 d followed by decrease in total sugar. Maximum activities of α -amylase and glucoamylase were observed on third and first day, respectively.

Gáhoon ko jaanr is a fermented wheat-based, mild-alcoholic beverage and is drunk directly by filtering the fermented grits. It is mostly used for distillation to get raksi. Yeasts population was 10 times higher than that of lactic acid bacteria in ten samples. Moulds were not recovered in the finished product. Out of 47 strains of microorganisms, 25 isolates were yeasts *Pichia anomala* and *Saccharomyces cerevisiae*, and 22 strains were lactic acid bacteria *Pediococcus pentosaceus* and *Lactobacillus bifementans*. The pH, moisture, acidity and alcohol content of the product was 3.9, 73.7%, 0.35 % and 3.1 %, respectively. Ash content was slightly higher whereas, fat and protein remained the same. Fermentation resulted in increase in crude fibre content. Minerals were enhanced due to fermentation.

Raksi is a clear, distilled wine with characteristic aroma prepared from fermented cereal beverages and is drunk daily. Raksi is prepared using traditional distillation apparatus, used by the rural women. The pH, acidity and alcohol content of raksi were 3.6, 0.06% and 22.9% respectively.

Outlines of findings are

- ❖ Traditional method of sub-culturing of mixed inocula using rice as base substrates for preparation of starter called marcha was investigated.
- ❖ Indigenous knowledge on beverages making technology practiced by rural women belonging to ethnic communities of the Darjeeling hills and Sikkim was documented.
- ❖ Microorganisms ranging from filamentous moulds (*Mucor circinelloides*, *Mucor* sp. (close to *Mucor hiemalis*), *Rhizopus chinensis*, *Rhizopus stolonifer* variety *lyococcus*;) to amylolytic and alcohol-producing yeasts (*Saccharomycopsis fibuligera*, *Pichia anomala*, *Saccharomyces cerevisiae* and *Candida glabrata*) and acid producing bacteria (*Pediococcus pentosaceus* and *Lactobacillus bif fermentans*) associated with marcha and various types of jaanr products were isolated, characterised, identified, indexed and preserved.
- ❖ Proximate composition of these indigenous fermented beverages was determined to know their food value.
- ❖ Attempt was made to upgrade the traditional processing of kodo ko jaanr using selected strains instead of conventional marcha.