# 5. DISCUSSION

## 5.1. SURVEY ON FERMENTED BEVERAGES

Common traditional fermented cereal-based beverages prepared and consumed by the different ethnic groups people in the Darjeeling hills and Sikkim are kodo ko jaanr, bhaati jaanr, makai ko jaanr and gahoon ko jaanr; and lesser-known alcoholic beverages are simal tarul ko jaanr, jao ko jaanr and faapar ko jaanr. Raksi, a wine distilled from fermented starchy materials is common alcoholic drink. Survey data indicated that in rural areas production of the traditional fermented beverages was mostly done at the individual household level for home consumption.

## 5.2. ETHNICAL IMPORTANCE

Traditional alcoholic beverages have strong ritual importance and are deep-rooted in the cultural heritage of the various ethnic groups of people in the Darjeeling hills and Sikkim. In these regions social activities require provision and consumption of appreciable quantities of alcoholic beverages by the 'matwali' castes meaning alcohol drinkers of the non-Brahmin Nepali community mostly Limboo, Rai, Gurung, Magar, Tamang, Sunwar, Newar and Sherpa; the Bhutia and the Lepcha tribes. Jaanr and raksi are essential to solemnize marriage ceremony of non-Brahmin Hindu Nepalis, the Buddhist tribes. Fermented beverages are offered to perform *the pitri puja* or *kul puja,* the religious practice to pray family Gods and Goddesses. Among the Lepcha, mong chee or kodo ko jaanr is essential to perform various cultural functions such as *lirum, sejum* and *namsung*. Mandokpenaa thea or kodo ko jaanr, filled in toongbaa and rice-made raksi are among the

important materials to perform the ritual practice of the Limboo called *tonsin mundhum*. In mourning, callers for condolences gathered to perform a memorial service are served traditional alcoholic beverages, mostly seen among the Sherpa and the Bhutia tribes. Spirit possession by the Limboo priests called *phedangma* and *bijuwa* need freshly distilled raksi.

## 5.3. MARCHA

Marcha is not a food but is the mixed dough inocula used as a starter culture for preparation of various indigenous alcoholic beverages. Marcha is produced at home in few villages in the Sikkim Himalayas. These marcha-making villages have been identified: Nor busty in Darjeeling (Bijanbari block), Kashyong and Mangzing in Kalimpong, Jhosing and Tibuk in North Sikkim, Chhejo and Lingchom in West Sikkim, Salghari and Barnyak in South Sikkim, Aho and Kopchey in East Sikkim. Marcha is exclusively prepared by the rural women belonging to the Limboo and Rai castes of the Nepali and the Lepcha tribes. This art of technology is protected as hereditary trade and passes from mother to daughters. Some local people are economically dependent upon this product. The marcha-making villages have linkages to nearby markets where marcha-makers can sell the products once or twice in a week. Earnings out of selling marcha supplement the domestic expenses. Among Rai, marital status is very strong determinant, only widows or spinsters are allowed to make marcha. Both Rai and Limboo consider marcha as an indispensable item for performing their ritual rites.

Unlike mixed culture starters of the other Asian countries, marcha is usually prepared by wrapping kneaded dough cakes in fern fronds with the fertile side touching them. This may be due to abundance of ferns locally called 'pire uneu' {*Glaphylopteriolopsis erubescens* (Wall ex Hook.) Ching} in the Sikkim Himalayas. Probably, germination of spores in sori helps to maintain the warmth of the fermenting mass in cold climates. Preparation of marcha is similar to those of other starter cultures of Asia. Marcha makers believe that addition of wild herbs give more sweetness to the product and they also believe that addition of chillies and ginger during marcha preparation is to get rid of devils that may spoil the product. This is actually to check growth of undesirable microorganisms that may inhibit growth of indigenous microflora of marcha. Studies of Soedarsono (1972) in ragi, an Indonesian rice-based starter culture, reveal that certain spices inhibit many undesirable microorganisms at the time of fermentation. Hesseltine (1983) has speculated that the spices, which are known to be inhibitory to many bacteria and moulds, are the agents that select the right population of microorganisms for fermentation. Marcha making technology reflects the traditional method of sub-culturing desirable inocula from previous batch to new culture using rice as base substrates. This technique preserves the microbial diversity essential for beverages production. Marcha retains its potency *in situ* for over a year or more.

Like other starter cultures of South-east Asia, marcha showed coexistence of mixed population of filamentous moulds, yeasts and lactic acid bacteria. The microbial analysis of marcha revealed that load of yeasts was higher than that of moulds and lactic acid bacteria. Out of 733 strains

of microorganisms isolated from sixty-six samples of marcha, 152 isolates were filamentous moulds, 321 were yeasts and 260 were lactic acid bacteria.

Following the taxonomical keys of Schipper (1976, 1984) and Hesseltine (1991), species of filamentous moulds were identified as *Mucor circinelloides* forma *circinelloides* van Tieghem, *Mucor* sp. close to *M. hiemalis* sensu lato, *Rhizopus chinensis* Saito, and *Rhizopus stolonifer* variety *lyococcus* (Ehrenb.) Stalp. & Schipper. Species of *Mucor* were more prevalent than species of *Rhizopus* in marcha samples anlaysed. *Aspergillus, Penicillium, Amylomyces* and *Actinomucor* were not recovered in marcha. According to Tanaka and Okazaki (1982), *Rhizopus* grows well on non-steamed grains, but its specific growth rate is decreased remarkably by steaming rice grain. The decrease is due to the heat denaturation of proteins in rice grain. *Rhizopus* and *Mucor* have also been reported in other similar rice-based cultures, such as ragi and chiuyueh (Hesseltine *et al.*, 1988), bubod (Tanimura *et al.*, 1977) and loogpang (Pichyangkura and Kulprecha, 1977).

Following the taxonomical keys described by Kreger-van Rij (1984) and Kurtzman and Fell (1998), four different genera of yeasts were isolated from marcha samples and identified as *Saccharomycopsis fibuligera* (Lindner) Klöcker, *Pichia anomala* (E.C. Hansen) Kurtzman, *Saccharomyces cerevisiae* Meyen ex Hansen and *Candida glabrata* (Anderson) Meyer et Yarrow. The nomenclature of *Endomyces fibuligera* Lindner (= *Endomyces lindneri* Saito) and *Hansenula anomala* (Hansen) Sydow and Sydow are now changed to *Saccharomycopsis fibuligera*

(Lindner) Klöcker and *Pichia anomala* (Hansen) Kurtzman, respectively (Hesseltine and Kurtzman, 1990). All strains of yeasts were present in marcha samples except *Candida glabrata* which showed only 45 % prevalence in sixty-six samples of marcha analysed. Among yeasts, *Saccharomycopsis fibuligera* was most dominant in marcha. *Saccharomycopsis fibuligera* is typically found growing on cereal products (Hesseltine and Kurtzman, 1990). *Saccharomycopsis malanga*, a phenotypically similar species that also produces amylase, was found in Chinese yeasts and ragi but not in bubod, probably due to geographical distribution of the species (Hesseltine and Kurtzman, 1990). In this finding, presence of *Saccharomyces cerevisiae* and *Candida glabrata* in marcha has been reported for the first time.

All isolates of lactic acid bacteria were Gram-positive, non-sporeforming, non-motile, catalase negative and facultative anaerobes and were grouped into coccii-tetrads and rods. Following the taxonomical keys of Sneath *et al.* (1986) and Wood and Holzapfel (1995), species of coccii-tetrads were identified as *Pediococcus pentosaceus* Mees. and heterofermentative rods were identified as *Lactobacillus bifermentans* Kandler, Schillinger and Weiss. Among lactic acid bacteria recovered *Pediococcus pentosaceus* was more predominant than *Lactobacillus bifermentans* in marcha. Hesseltine and Ray (1988) and Tamang and Sarkar (1995) reported only the presence of *Pediococcus pentosaceus* in marcha. Recovery of *Lactobacillus bifermentans* in marcha samples has been reported for the first time in this dissertation. The inability to utilize starch by these species indicates that they are not significant contributors to the

breakdown of starch of substrates during preparation of marcha itself or any beverage, probably their role is to give flavour and impart mild sour-taste, typical to jaanr. Role of lactic acid bacteria in the oriental starter cultures is likely to give flavour to the product with a pleasant taste (Hesseltine, 1983).

Marcha contains only 14 % moisture due to sun drying after the fermentation. The acidic nature (pH 5.58 with 0.01 % acidity) of marcha was due to the presence of high population of lactic acid bacteria. Due to low moisture content and acidic in nature, the shelf-life of marcha is long and can be stored at room temperature for a year or more.

The use of the API-zym technique has been reported (Arora *et al.*, 1990) as a rapid and simple means of evaluating and localizing 19 different hydrolases of microorganisms associated with dairy fermentations, mostly for selection of strains as potential starter cultures on the basis of superior enzyme profiles. We tried to use this API-zym technique to know the enzyme profiles of marcha isolates. Isolates of marcha showed relatively weak esterase and lipase activities (except by *Pichia anomala*) as compared with phosphatase activities. Yeasts strains mainly *Saccharomycopsis fibuligera* and *Pichia anomala* showed strong phosphatase and peptidase (leucine arylamidase) activities. Absence of proteinases (trypsin and chymotrypsin) and presence of high peptidase (leucine-arylamidase) and low esterase-lipase (C4 and C8) activities produced by the predominant organisms isolated from marcha are trades of desirable quality for their use in production of typical jaanr flavour.

Preliminary screenings of amylolytic activities of all isolates of marcha were tested in starch agar plates and strains were selected

accordingly. All strains of moulds and yeasts showed the amylolytic activities. None of the lactic acid bacteria showed amylolytic activity indicating they have no role in saccharification and liquefaction of substrates during jaanr fermentation. *Saccharomycopsis fibuligera* MS:YD4 showed highest liquefying activity (α-amylase) and *Rhizopus chinensis* MJ:Rh3 showed highest saccharifying activity (glucoamylase). It showed that *Rhizopus* played main roles in saccharification whereas *Saccharomycopsis fibuligera* played important role in liquefaction of substrate during jaanr fermentation. However, both of them played the main role in amylase production in starter cultures. Suprianto *et al.* (1989) reported the similar results in tapé that *Saccharomycopsis fibuligera* produced mainly α-amylase and *Rhizopus* sp. produced glucoamylase. Earlier reports showed that *Saccharomycopsis fibuligera* played the main roles in amylase production in starters of South-east Asia, and *Rhizopus* seemed to supplement the saccharification (Sukhumavasi *et al.*, 1975; Cronk *et al.*, 1977; Wei and Jong, 1983; Uchimura *et al.*, 1990; Yokotsuka, 1991). *Rhizopus* is known to produce good amount of glucomaylase (Ueda and Kano, 1975; Selvakumar *et al.*, 1996).

## 5.4. KODO KO JAANR

Kodo ko jaanr, prepared from dry seeds of finger millets, is one of the most popular fermented beverages consumed by 70 % of rural people in the Sikkim Himayalas. 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 a distinct feature of alcohol-drinking culture of the

mountain people. Consumption of fermented finger millets beverages in exclusively decorated bamboo or wood-made vessel called toongbaa is uncommon among other ethnic communities in Asia or elsewhere. This type of alcohol consumption or drinking is restricted only in the Himalayan regions. Guests are served with toongbaa along with fried meat or pickles. Description of alcohol-drinking custom in the Sikkim Himalayas was cited in some historical documents (Hooker, 1854, O'Malley, 1907; Risley, 1928). Kodo ko jaanr liquor is believed to be good tonic for ailing persons and post-natal women. After consumption, grits of kodo ko jaanr are used as fodder for pigs and cattle. This is a good example of total utilization of finger millet kodo as food beverage and fodder.

Population of yeasts was detected at the level of $10^7$ cfu/g whereas that of lactic acid bacteria was comparatively less (~$10^5$ cfu/g) in forty samples of kodo ko jaanr, collected from different places of the Darjeeling hills and Sikkim. Filamentous moulds were not recovered in any finish product of kodo ko jaanr indicating that moulds have roles only in the initial phase of fermentation mostly in saccharification of the substrates. Out of 161 strains of microorganisms isolated, 81 isolates were yeasts and 80 isolates were lactic acid bacteria. These representative strains of yeasts were identified as *Pichia anomala* (E.C. Hansen) Kurtzman, *Saccharomyces cerevisiae* Meyen ex Hansen and *Candida glabrata* (Anderson) Meyer et Yarrow. *Saccharomycopsis fibuligera* was not recovered in finish product. Since it showed high amylolytic activities on the initial stage of fermentation, breaking starch into glucose, and on later stage, the population declined and disappeared. *Pichia anomala* and

*Saccharomyces cerevisiae* were present in all samples whereas *Candida glabrata* were recovered only in 40 % of samples analysed. Out of 80 strains of lactic acid bacteria, isolated from forty samples of kodo ko jaanr, 44 strains were coccii-tetrads and 36 strains were non-sporeforming rods. Species of lactic acid bacteria were identified as *Pediococcus pentosaceus* Mees and *Lactobacillus bifermentans* Kandler, Schillinger and Weiss. Both of them were recovered in all samples of kodo ko jaanr. Marcha used as starter supplemented the dominant microorganisms in kodo ko jaanr fermentation.

Due to cooking prior to fermentation, moisture content of the product was slightly higher in fermented product. The pH, titratable acidity and alcohol content of the product was 4.1, 0.27 % and 4.8 %, respectively. Kodo ko jaanr is mild-alcoholic sweet-flavoured beverage. It is rich in crude fibre and is high calorie food beverage. Because of high calorie, ailing persons and post-natal women consume the extract of kodo ko jaanr to regain the strength. Samantray *et al.* (1989) reported that finger millet also called 'ragi' in South India, is a good source of iron, calcium, magnesium and phosphorus. Fermentation of finger millet to kodo ko jaanr enhanced bioenrichment of minerals such as Ca, Mg, Mn, Fe, K, P. Kodo ko jaanr thus contributes to the mineral intake in daily diet of the local people.

Kodo ko jaanr was prepared in the laboratory following the traditional method by using marcha, collected from Aho village, as mentioned in 3.3.6. Changes in microbial population, physico-chemical and enzymatic activities in fermenting finger millet seeds during kodo ko jaanr

fermentation were investigated. Mould population, which was originated from marcha, declined significantly ($P<0.05$) every day during fermentation and finally disappeared after 5 d. Mucoraceae fungi have roles in initial phase of fermentation mostly in saccharification of the substrates. Population of yeasts increased significantly ($P<0.05$) from $10^5$ cfu/g to $10^7$ cfu/g within 2 d indicating that yeasts played important roles in amylase production. Among the yeasts, population of *Saccharomycopsis fibuligera* was more dominant on second day than that of *Pichia anomala*, *Saccharomyces cerevisiae* and *Candida glabrata*. Subsequently, load of 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.

Temperature of fermenting finger millet increased significantly ($P<0.05$) from 26° C to 30° C within 2 days due to exponential growth activities of mixed population of microorganisms. However, after second day of fermentation, temperature of fermenting millet remained constant around the incubation temperature of 28° C. The cause of increase in acidity and consequent drop in pH during fermentation was likely due to utilization of free sugars of the substrate by yeasts and lactic acid bacteria. Since all the strains were able to ferment glucose. Alcohol content increased significantly ($P<0.05$) from 0.1 % to 6.9 % within 6 d. The result showed that reducing sugar content increased significantly ($P<0.05$) till 3 d followed by decrease in total sugar content. This is due to maximum break down of starch of substrates to reducing sugars by amylolytic enzymes produced by moulds and yeasts during fermentation. Maximum activities of

saccharification (glucoamylase) and liquefaction (∝-amylase) of finger millets were observed on second day of fermentation. Saccharifying activities were mostly shown by *Rhizopus* spp. and *Saccharomycopsis fibuligera* whereas liquefying activities were shown by *Saccharomycopsis fibuligera* and *Saccharomyces cerevisiae*. *Lactobacillus* and *Pediococcus* showed no amylolytic activities. The result indicated that *Saccharomycopsis fibuligera* and *Rhizopus* spp play the important role in saccharification process of *jaanr* fermentation, breaking starch of substrates into glucose for ethanol production. *Mucor* spp., *Pichia anomala* and *Candida glabrata* may supplement the saccharification.

Sterilised finger millet seeds were allowed to ferment with different combinations of strains of moulds, yeasts and lactic acid bacteria, previously isolated from marcha samples 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 on 6 d with slightly acidic pH than jaanr samples fermented by other strains. Sensory evaluation result showed that kodo ko jaanr prepared by a cell suspension mixture of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 had significantly ($P<0.05$) highest score in general acceptability and was significantly ($P<0.05$) acceptable to judges. As seen from Table 7, *Saccharomyces cerevisiae* strains posses strong tendency to ferment glucose, galactose, maltose, raffinose, sucrose and starch into ethanol. Kozaki and Uchimura (1990) observed that *Saccharomyces cerevisiae* plays main role in alcohol fermentation in tapuy,

rice wine of the Philippines. *Rhizopus chinensis* MJ:Rh3 had highest glucoamylase activities thus breaking the starch of cooked millet to glucose which was fermented by *Saccharomyces cerevisiae* into alcohol and also retained some sugar to give a sweet taste with desirable alcoholic flavour. Whereas jaanr prepared by a combination of *Rhizopus chinensis* MJ:Rh3 and *Saccharomycopsis fibuligera* MS:YDS2 had sweet-sour taste but due to low-alcohol content, the product had unpleasant odour, which could not be considered as good quality jaanr. Reddy and Basappa (1996) observed that *Saccharomycopsis fibuligera* produces high biomass during fermentation of cassava starch, which leads to lesser ethanol yield.

The consumers' preference trial showed that kodo ko jaanr prepared in the laboratory by cell suspension mixture of *Rhizopus chinensis* MJ:Rh3 and *Saccharomyces cerevisiae* MJ:YS2 as starter was more acceptable than the kodo ko jaanr prepared by conventional marcha. Sanchez and Kozaki (1984) stated that sensory evaluation in wine analysis is indispensable because even the accurate chemical analysis is not enough to guarantee the quality of a good wine. Laboratory-made kodo ko jaanr may have more advantages over the jaanr prepared by using marcha due to better quality in product, maintaining consistency and maximum utilization of substrates.

The results of the present studies demonstrate that to make a good quality jaanr, cell suspension mixture of a selected strain of mould *(Rhizopus)* and one amylolytic yeast (*Saccharomyces cerevisiae*) may be used as starter.

## 5.5. BHAATI JAANR

Bhaati jaanr is a soft, juicy and sweet-sour with mild-alcoholic beverage prepared from steamed glutinous rice and is consumed directly. Sometimes, yellowish-white supernatant liquor called nigaar was collected at the bottom of the earthenware crock, where bhaati jaanr was stored. It is a traditional diet for new mothers in villages who believe that it helps them to regain their strength.

Yeasts population was found higher than that of lactic acid bacteria in all twenty-four samples of bhaati jaanr samples. As in kodo ko jaanr, moulds were not recovered in any bhaati jaanr product analysed. Out of 127 strains of microorganisms isolated from bhaati jaanr samples, 69 isolates were yeasts and 58 isolates were lactic acid bacteria. Only two types of yeasts were recovered from bhaati jaanr samples and were identified as *Pichia anomala* (E.C. Hansen) Kurtzman and *Saccharomyces cerevisiae* Meyen ex Hansen. The association of *Saccharomyces cerevisiae* with bhaati jaanr fermentation can be attributed by its strong ability to ferment sugar as well as being ethanol tolerant and would be able to produce alcohol. Presence of *Pichia anomala* in bhaati jaanr samples may contribute ester odour to product. Cronk *et al.* (1979) reported that *Hansenula anomala* (now *Pichia anomala*) contributed ester odour to tapé which is similar to bhaati jaanr. *Pediococcus pentosaceus* and *Lactobacillus bifermentans* were found in bhaati jaanr samples. Probably the presence of lactic acid bacteria in bhaati jaanr contributes characteristic flavour to the product and making the product slightly acidic in nature.

The moisture content in bhaati jaanr was higher than unfermented steamed rice due to juicy nature of the fermented product. The pH, acidity and alcohol contents of the product were 3.5, 0.24 % and 5.9 %, respectively indicating bhaati jaanr as sour, acidic and mild-alcoholic beverage. Fat, protein and calorie contents remained same as the substrate. Considerable increase in calcium, manganese, iron, zinc, sodium, potassium and phosphorous was observed in bhaati jaanr over the substrate, which revealed that fermentation enhanced the bioenrichment of minerals in the product. Though bhaati jaanr is consumed as beverage, it can also be eaten as dissert or staple food.

Loads of moulds, decreased significantly ($P<0.05$) during fermentation and disappeared after the fifth day of fermentation. Population 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 in 10 d. Exponential increase in load of lactic acid bacteria was significant ($P<0.05$) till second day of fermentation, and then declined slowly.

Temperature of fermenting rice increased above the incubation temperature of 28° C due to growth of mixed microorganisms within 3 d and remained constant to that of incubation temperature. During fermentation, pH decreased and acidity increased significantly ($P<0.05$). The cause of increase in acidity and decreased in pH during fermentation was likely due to utilization of free sugars of the substrate by yeasts and lactic acid bacteria. Since all the strains were able to ferment glucose. Thus alcohol content increased significantly ($P<0.05$) during fermentation. The result showed that reducing sugar content increased significantly ($P<0.05$)

till 3 d followed by decrease in total sugar content. This is due to maximum break down of starch of substrates to reducing sugars by amylolytic enzymes produced by moulds and yeasts within 3 d during fermentation. Maximum activities of saccharification and liquefaction of glutinous rice were observed on the third day of fermentation. This result suggested that *Saccharomycopsis fibuligera* and *Rhizopus* spp contribute in saccharification and liquefaction of glutinous rice, breaking starch of substrates into glucose for alcohol production and also in aroma formation in bhaati jaanr preparation. Suprianto *et al.* (1989) reported that *Saccharomycopsis* is important in aroma formation in tapé preparation and *Rhizopus* sp. is important in liquefy and saccharify the glutinous rice.

## 5.6. MAKAI KO JAANR

Makai ko jaanr is viscous, slightly bitter, mild-alcoholic beverage, made from maize and the fermented mass extract is drunk directly. Africans consume varieties of fermented maize products, which include staples, gruel and beverages, and most of these are naturally fermented involving lactic acid bacteria or yeast or mixture of lactic acid bacteria-yeast (Niche, 1995). Unlike African fermented maize products, in the Sikkim Himalayas, maize is used only as mild-alcoholic beverage prepared by using starter.

Yeasts were found 10 times higher than that of lactic acid bacteria in makai ko jaanr samples. 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 identified as *Pichia anomala* (E.C. Hansen) Kurtzman and 24 strains were identified *Saccharomyces cerevisiae* Meyen ex Hansen. Out of 46 lactic acid bacteria strains, 28 were coccoid cells in tetrads (*Pediococcus pentosaceus* Mees) and 18 strains were non-sporeforming rods (*Lactobacillus bifermentans* Kandler, Schillinger and Weiss).

The pH and acidity of makai ko jaanr was 3.3. and 0.38 %, respectively indicating the products is slightly acidic in nature. Alcohol content of the product was 2.5 %, comparatively less than that of other cereal-based jaanr products. Makai ko jaanr is low-alcohol content food beverage. Increase in crude fibre content was observed in makai ko jaanr. Calorie value remained almost same in both unfermented and fermented maize. Remarkable increase in iron, potassium and phosphorous was observed in makai ko jaanr indicating that bioavailability of minerals is enhanced during fermentation.

Filamentous mould count declined significantly ($P<0.05$) during makai ko jaanr fermentation and disappeared after the fourth day, as seen in other jaanr fermentation, indicating that these organisms have roles only in the initial phase of fermentation, mainly in production of amylase. 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 of fermentation. This result indicated that maximum activities of saccharification and liquefaction of maize was shown by yeasts, mainly by *Saccharomycopsis fibuligera* within 2 d and, consequently the population

of these yeasts declined gradually towards the end of fermentation. This is the reason why *Saccharomycopsis fibuligera* was not recovered in any final product of makai ko jaanr. Lactic acid bacteria population increased significantly ($P<0.05$) from 0 day to 2 day and declined gradually till the end of fermentation. This may be due to availability of free sugars, after breaking starch of maize by moulds and yeasts during saccharfication, for the growth of lactic acid bacteria, originated from marcha. Ekundayo (1969) and Achi (1990) proposed that extracellular amylolytic enzymes of microorganisms help to break down the starch of maize and sorghum into beverage which are likely to stimulate the growth of lactic acid bacteria.

Temperature of fermenting maize remained relatively constant to incubation temperature throughout the fermentation. During fermentation, pH dropped significantly ($P<0.05$) within 10 d and acidity increased significantly ($P<0.05$) on each day. Reducing sugar increased significantly ($P<0.05$) within 1 d followed by maximum activities of saccharification by moulds and yeasts. However, liquefying activities was maximum till third day of fermentation. Saccharification and liquefaction of fermenting maize seeds were carried by *Saccharomycopsis fibuligera* and *Rhizopus* spp., thus increasing free sugars to produce alcohol. Alcohol content increased significantly ($P<0.05$) during fermentation of makai ko jaanr. *Mucor* spp. and *Pichia anomala* may supplement the saccharification. The decrease in total sugar content of maize could be attributed to the utilization of the sugars by fermenting microorganisms. Adegoke *et al.* (1995) observed that microorganisms are known to depend on reducing sugars for their metabolic processes.

## 5.7. GAHOON KO JAANR

Gahoon ko jaanr is wheat-based alcoholic beverage and is drunk directly by filtering the fermented grits. Gahoon ko jaanr is mostly used for distillation to get raksi.

Yeasts population was 10 times higher than that of lactic acid bacteria in ten samples of gahoon ko jaanr. Moulds were not recovered in the finished product. Out of 47 strains of microorganisms isolated from ten samples of gahoon ko jaanr, 25 isolates were yeasts {*Pichia anomala* (E.C. Hansen) Kurtzman and *Saccharomyces cerevisiae* Meyen ex Hansen} and 22 strains were lactic acid bacteria (*Pediococcus pentosaceus* Mees and *Lactobacillus bifermentans* Kandler, Schillinger and Weiss). The microbial composition was almost same as that of other jaanr products, since the microorganisms were originated from marcha, used for preparation of this alcoholic product.

The pH, acidity and alcohol content of the product was 3.9, 0.35 % and 3.1 %, respectively indicating that this product is acidic and mild alcoholic beverage. Fermentation resulted in increase in crude fibre content in gahoon ko jaanr. Minerals were enhanced due to fermentation. Gahoon ko jaanr is rich in iron, sodium, potassium and phosphorous.

## 5.8. RAKSI

Raksi is a clear, distilled wine with characteristic aroma prepared from fermented cereal beverages such as kodo ko jaanr, bhaati jaanr, makai ko jaanr, gahoon ko jaanr. It is drunk directly without addition of water. Unlike other cereal-based wine of Asian countries, where the fermented rice or other cereal is decanted or filtered, in the Sikkim Himalayas fermented cereals or starchy substrates are distilled to get high alcoholic drink, which can stored for long. Raksi preparation is done by alcoholic distillation method in traditional way by the rural women. The crude method is still operating in most of the villages, and some people have improvised the apparatus. The indigenous knowledge of alcohol distillation by mountain women is worth noting. Alcohol content of raksi is higher than the jaanr due to distillation. Raksi is slightly sour in taste. Raksi distilled from bhaati jaanr mixed with few petals of *Rhododendron* showed highest alcohol content (~ 27 %) comparable to raksi prepared from other fermented cereals.